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Knowledge, attitude, practice (KAP) and dietary intake of young university athletes following sports nutrition education

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ABSTRACT

Introduction: Nutrition plays a vital role in sports. Athletes must understand the importance of diet and ensure that they meet the nutrient requirements to enhance sports performance. The lack of understanding in sports nutrition will lead to poor dietary practices that can cause detrimental effects on athletic achievements. This study aims to evaluate the effects of knowledge, attitude, and practice (KAP) regarding sports nutrition and dietary intake among young university athletes. **Methods:** Twenty-one local university athletes (23.8±3.4 years) were recruited, and their anthropometric and socio-demographic data were assessed. All participants attended a 1-day sports nutrition class. The KAP-Sports nutrition questionnaire was administered. Three days of dietary intake were also recorded at the same timepoints among the participants. **Results:** There was a significant increment (p<0.05) in the mean scores for KAP among the participants. Total energy and total carbohydrate intakes per day were significantly increased (p<0.05). However, overall protein and fat intakes did not improve as the readings were higher than the recommended values. **Conclusion:** In this study, sports nutrition education improved participants' KAP, but not the actual dietary intake. Changes in habit require more effort, with extra attention on protein and fat intakes.

Keywords: knowledge, attitudes, and practice (KAP), dietary intake, sports nutrition education, athletes

INTRODUCTION

Appropriate nutrition intake is crucial for optimal athletic performance as it supplies fuel for biological work and energy for movement. It is also important for building and synthesising new tissues, repairing the cells, balancing fluid and electrolyte levels, and regulating all metabolic processes (McArdle, Katch & Katch, 2013). Previous study has reported that proper and optimal nutrient intake is associated with peak performance, while nutrient deficiencies may diminish the athletes' performance (Hornstrom *et al.*, 2011). Athletes are aware that the right nutrition is important to optimise their performance (Kiens, Ivy & Burke, 2004). However, they still lack knowledge, and have poor attitudes and practice towards sports nutrition (Jacobson, Sobonya & Ransone, 2001).

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Energy balance is vital for athletes to improve their exercise capacity. The optimal food intake must be equivalent to the demand for energy usage during training or competition. If the energy is not well distributed, it may lead to unfavourable alterations in the athletes' body function, as well as their body systems, thus reducing the capacity to exercise (Arieli & Constantini, 2012). Some athletes and coaches in aesthetic sports like diving or gymnastics believe that a reduction in weight or body fat by controlling energy balance will improve the performance of the athletes (Deutz *et al.*, 2000). However, controlling food intake without proper knowledge in sports nutrition might lead to problems such as undernutrition. Therefore, giving the right nutrition knowledge to athletes especially on how to eat according to their body needs, daily calorie intake, and proper foods and supplement selection is vital to maintain their health and optimise their sports performance, as well as to speed up recovery process and to prevent injuries (Fink & Mikesky, 2018).

Athletes' knowledge on sports nutrition needs to be assessed in order to ensure correct practices, and at the same time, to avoid wrong information, myths, and misconceptions (Paugh, 2005). Assessing athletes' dietary intake will provide an overview of their actual dietary practices and basic understanding of nutrition knowledge. Therefore, it is good to assess both dietary intake and nutrition knowledge as these may help to develop a better nutritional plan besides providing information about desirable eating behaviours (Paugh, 2005). Furthermore, this information may be useful in providing nutrition counselling for athletes if they have issues with nutrition intakes (Paugh, 2005).

Studies have reported that athletes always have difficulty in achieving optimal nutrition due to a lack of understanding of basic nutrition concepts (Dunn, Turner & Denny, 2007). The requirement of nutritional intake among athletes is different from the general population and also different according to their type of sport. Thus, athletes need to be educated on the requirement of food intake based on their sports category (Trabucco, Nikoic & Mirkovic, 2013). To date, information regarding the level of knowledge, attitude, and practice (KAP) on sports nutrition among young university athletes are scarce. Thus, this study aims to determine the KAP level, as well as to evaluate the effectiveness of sports nutrition education on KAP and dietary intake among young university athletes.

MATERIALS AND METHODS

Study design

This was an intervention study with pre- and post-test measurements. This study declares no conflict of interest, and ethical approval was obtained from the Human Research Ethics Committee of Universiti Sains Malaysia (USM) (USM/JEPeM/18120796).

Participants and sample size calculation

Participants were recruited voluntarily among the Universiti Sains Malaysia (USM) students via purposive sampling approach. The inclusion criteria were university athletes who had participated in any competition at least at the university level, healthy, aged between 19 to 30 years old, and did not have any formal or informal nutrition classes. The exclusion criteria were those with health problems, took part in other research interventions, and on a special diet as per doctor's recommendation. The sample size calculation was carried out based on a previous study (Siti Soraya *et al.*, 2018) by using PS Power and Sample Size Calculation Version 3.1.2. Twenty-

one participants were recruited for the study with the consideration of a 10% drop-out.

Study procedures

At the beginning of the study, the objectives and procedures of the study were explained to the participants. Their informed consent were obtained to indicate their willingness to participate in the study. Pre-test measurements were then carried out on each participant. The pre-test assessments included anthropometry (height and weight measurements), socio-demographic data, administration of the KAP-Sports nutrition questionnaire, and recording of dietary intake for three days (two weekdays and one day on the weekend). After pre-test, the participants attended a one-day sports nutrition class conducted by the sports nutrition lecturers from USM. Four weeks after attending the class, post-test measurements were conducted, which included administration of the KAP-Sports nutrition questionnaire and recording of dietary intake for three days.

KAP-Sports nutrition questionnaire

A validated questionnaire developed by Siti Soraya *et al.* (2018) and Hornstrom *et al.* (2011) was used in this study. The Cronbach's alpha value was 0.79, and the questionnaire was divided into three sections. These sections were knowledge that consisted of 25 questions, attitude with 20 questions, and practice with 12 questions. The total score for the knowledge section was obtained by summing up the points for all questions. One point was given for each correct answer, while zero point was given for each incorrect answer. The maximum score for the knowledge section was 25 points, where 20 to 25 points was categorised as high level of knowledge, 15 to 19 points as moderate level, and 0 to 14 points as low level.

The total score for the attitude section was also calculated by adding the points obtained for all questions. The maximum score for the attitude section was 80 points with 65 to 80 points categorised as positive attitude, 48 to 64 points categorised as neutral, and 0 to 47 points categorised as negative attitude. Similarly, for the practice section, the total score was calculated by adding the points obtained for all 12 questions. The maximum score for the practice section was 48 points. The total practice scores were categorised into three groups: good (40 – 48 points), fair (20 – 39 points), and poor (0 –19 points).

Anthropometric measurements

Participant's body weight was measured to the nearest 0.1kg using a digital weighing scale (Tanita, UM-076, Japan), whereas height was measured using a portable stadiometer (SECA 217, SECA Corp., Germany) to the nearest 0.1cm. Body mass index (BMI) was calculated in kg/m^2 and categorised into four categories based on the World Health Organization (WHO) reference of underweight, normal, overweight, and obese (WHO, 2004).

Dietary assessment

The three-day dietary intake record was self-administered by the participants using a food diary. The participants were required to record the types and amount of their entire food and beverage intakes, weight information, brand name, portion sizes, and food preparation for three days - two weekdays and one day on the weekend. A photo album consisting of utensils and food designs in three common sizes (small, medium, and large) was provided as a resource to assist the participants in recording their dietary intake. To ensure the accuracy of recording, the participants were briefed by the researchers at pre-test on how to record their dietary intake

in the food diary. After post-test, total energy and macronutrient intakes of the participants were examined by the researchers with the help from a dietitian by using the Nutritionist Pro software. Expert judgement was used in analysing food items that were not in the food composition tables (e.g. purchased and pre-cooked foods, supplements, etc.).

Sports nutrition class

All participants attended a one-day sports nutrition class conducted by the sports nutrition lecturers from USM who were not part of the research team. The topics covered during the sports nutrition class are listed in Box 1. The lessons were conducted in the form of three separate lectures and discussions, which began from 8:00 a.m. and ended at 5:30 p.m. Each lecture and discussion session covered 2-3 topics and was conducted for about 2.5 – 3 hours with a short break in between each session (Box 1).

Statistical analysis

The completed questionnaires were compiled and statistically analysed using the SPSS software Version 24. Descriptive analysis and paired t-test were used to analyse the data. Data were checked for normality by using the histogram, and $p<0.05$ was used to indicate a statistical significance. Results were reported as mean±standard deviation (*SD*).

RESULTS

Participants' characteristics and demographic data

The demographic data of the participants are presented in Table 1. A total of 21 participants completed the pre- and

Box 1. Topics covered during the sports nutrition class

Lecture & Discussion 1

1. Food and healthy nutrition
   - 1.1. Food pyramid, food plate, food exchange, serving size
2. Macronutrients
   - 1.1. Recommended Nutrient Intakes (RNI) for CHO, protein, fat and fibre
   - 1.2. Role of the macronutrients, types and quantity
3. Micronutrient
   - 1.1. Determine recommended nutrient intakes of several important minerals and vitamins
   - 1.2. Explain roles of the minerals and vitamins in metabolic pathways and exercise

Lecture & Discussion 2

4. Fluid and hydration
   - 4.1. Understand functions and importance of water and electrolytes for human body
   - 4.2. Describe fluid intake requirement at pre-, during and after exercise/competition
5. Nutrition before, during and after training or competition
   - 5.1. Describe and plan pre-training, during training and post-training meals

Lecture & Discussion 3

6. Dietary supplement
   - 6.1. Discuss current/popular supplements (protein powder, caffeine)
   - 6.2. Discuss banned supplements
7. Energy balance and weight management
   - 7.1. Understand importance of energy balance and weight management
   - 7.2. Understand how to calculate energy balance
   - 7.3. Discuss weight management strategies

post-test. The participants were USM student athletes who were actively involved in various types of sports and had participated in competition at least at the university level. The participants comprised 17 Malays, one Chinese, and three from other ethnicities. The distribution of male and female participants was 52.4% and 47.6%, respectively. Mean age of male participants was 22.8±1.8 years, whereas mean age of female participants was 24.6±4.6 years. The education level of the participants varied, ranging from diploma to PhD level, with most of them being undergraduate students. Participant's BMI was calculated based on the participant's weight and height measured at pre-test and the results showed that all participants had normal BMI, which is between 18.5 and 24.9 kg/m^2 (WHO, 2004). Majority of male (33.3%) and female (28.5%) participants were football players and aerobic dancers, respectively.

Knowledge

At pre-test, results in Table 2 show that majority of the participants (57.1%) had a moderate level of knowledge on sports nutrition. None of them were categorised in the low level. After attending the sports nutrition class, post-test results showed that there was an increment in the knowledge level of the participants, where a majority (76.2%) of them were categorised as having a high level of knowledge on sports nutrition. Table 3 shows the results of paired *t*-test analysis, which compared the mean scores of the participants' knowledge between pre- and post-test for male and female participants. Mean score for male participants were significantly increased (p=0.022) from 19.0±2.2 (moderate level) at pre-test to 21.0±1.8 (high level)

Table 1. Characteristics and socio-demography of participants (*N = 21*)

Variables	*Male*	*Female*	*Overall*
Age (years), Mean±*SD*	22.8±1.8	24.6±4.6	23.8±3.4
Gender, *n* (%)	11 (52.4)	10 (47.6)	21 (100.0)
BMI (kg/m^2), Mean±*SD*	23.5±3.7	21.9±2.4	22.3±3.1
Ethnicity, *n* (%)			
Malay	10 (90.9)	7 (70.0)	17 (81.0)
Chinese	1 (9.1)	0 (0.0)	1 (4.8)
Others	0 (0.0)	3 (30.0)	3 (14.2)
Types of sports, *n* (%)			
Badminton	3 (27.3)	0 (0.0)	3 (14.2)
Basketball	0 (0.0)	1 (10.0)	1 (4.8)
Football	7 (63.6)	0 (0.0)	7 (33.3)
Frisbee	0 (0.0)	1 (10.0)	1 (4.8)
Aerobic dance	0 (0.0)	6 (60.0)	6 (28.5)
Taekwondo	1 (9.1)	0 (0.0)	1 (4.8)
Track & field	0 (0.0)	1 (10.0)	1 (4.8)
Volleyball	0 (0.0)	1 (10.0)	1 (4.8)
Education level, *n* (%)			
Diploma	1 (9.1)	0 (0.0)	1 (4.8)
Degree	7 (63.6)	7 (70.0)	14 (66.7)
Master	3 (27.3)	2 (20.0)	5 (23.7)
Ph.D.	0 (0.0)	1 (10.0)	1 (4.8)

at post-test. Female participants also showed significant increment (p=0.007) in their knowledge score from 19.0±2.4 (moderate level) at pre-test to 21.0±1.4 (high level) at post-test.

Attitude

As shown in Table 2, at pre-test, 57.1% of the participants had a neutral attitude towards sports nutrition. Majority of the male participants (63.6%) showed a positive attitude towards sports nutrition, while majority of the female participants (80.0%) showed neutral attitude. None of the male and female participants scored below 48 points (negative attitude). At post-test, the level of attitude towards sports nutrition was increased in both genders, with majority of the participants (61.9%) having a positive attitude. In Table 3, the mean score of attitude for male participants increased from 65.0±7.1 (positive attitude) at pre-test to 70.0±6.9 (positive attitude) at post-test; however, this change was not significantly different (p=0.090). Female participants showed significant increment (p=0.035) in attitude score from moderate (61.0±4.5) at pre-test to positive (65.0±6.1) at post-test.

Practice

Table 2 also shows the dietary practice scores among the participants at pre- and post-test. Overall, 72.7% and 70.0% of male and female participants, respectively, showed poor level of practice in nutrition. Four weeks after attending the sports nutrition class (post-test), majority of the participants (71.4%) showed fair level of practice in nutrition. As shown in Table 3, the mean score of practice for male participants was significantly increased (p=0.012) from 27.0±5.0 (poor level) at pre-test to 32.0±4.5 (fair level) at post-test. Female participants also showed significant

Table 2. Knowledge, attitude, and practice on sports nutrition among participants

Variables	*Pre-test, n (%)*			*Post-test, n (%)*		
	Male	*Female*	*Overall*	*Male*	*Female*	*Overall*
Knowledge						
High (20 – 25 points)	5 (45.5)	4 (40.0)	9 (42.9)	8 (72.7)	8 (80.0)	16 (76.2)
Moderate (15 – 19 points)	6 (54.5)	6 (60.0)	12 (57.1)	3 (27.3)	2 (20.0)	5 (23.8)
Low (0 – 14 points)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Attitude						
Positive (65 – 80 points)	7 (63.6)	2 (20.0)	9 (42.9)	8 (72.7)	5 (50.0)	13 (61.9)
Neutral (48 – 64 points)	4 (36.4)	8 (80.0)	12 (57.1)	3 (27.3)	5 (50.0)	8 (38.1)
Negative (0 – 47 points)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Practice						
Good (40 – 48 points)	0 (0.0)	1 (10.0)	1 (4.8)	1 (9.1)	0 (0.0)	1 (4.8)
Fair (29 – 39 points)	3 (27.3)	2 (20.0)	5 (23.8)	8 (72.7)	7 (70.0)	15 (71.4)
Poor (0 – 28 points)	8 (72.7)	7 (70.0)	15 (71.4)	2 (18.2)	3 (30.0)	5 (23.8)

Table 3. Comparison of KAP's mean scores between pre- and post-test

Variables	*Pre-test*	*Post-test*	*p-value*
	Mean±SD	*Mean±SD*	
Knowledge			
Male	19.0±2.2	21.0±1.8	0.022*
Female	19.0±2.4	21.0±1.4	0.007*
Overall	19.0±2.2	21.0±1.7	0.001*
Attitude			
Male	65.0±7.1	70.0±6.9	0.090
Female	61.0±4.5	65.0±6.1	0.035*
Overall	63.0±6.2	67.0±6.9	0.009*
Practice			
Male	27.0±5.0	32.0±4.5	0.012*
Female	27.0±3.8	31.0±4.5	0.042*
Overall	27.0±4.4	31.0±4.5	0.001*

*significantly different at $p<0.05$; $N=21$

increment (p=0.042) in their mean score of practice from 27.0±3.8 (poor level) at pre-test to 31.0±4.5 (fair level) at post-test.

Dietary intake

Mean total energy and macronutrient intakes among the participants are shown in Table 4. The mean total energy intake for both male (1389±190kcal at pre-test; 2172±267 kcal at post-test) and female (1227±202 kcal at pre-test; 1962±389 kcal at post-test) participants were significantly increased (p=0.001) from pre- to post-test. Although both genders showed an increment in total energy intake, the values were still below the recommended RNI values, which are between 2520-2740 kcal for males and 2080-2370 kcal for females.

The average carbohydrate intake per day had also significantly increased (p=0.002) from 168.4±31.2g at pre-test to 253.7±65.2g at post-test for male participants, and it was significantly increased (p=0.001) from 152.5±30.9g at pre-test to 235.9±52.5g at post-test for female participants. The pre-test average carbohydrate intake per day for both genders was less than the recommended nutrient intake (RNI) values, but after attending the sports nutrition class, the values were increased and fell within the recommended RNI values; 200-330g for males and 180-230 g for females. However, the percentage of carbohydrate intake between pre- and post-test in both genders were not significantly different (p>0.05). It was less than the recommended percentage by RNI, which is between 55-70%.

Table 4 also shows that total protein intake per day for both male (54.6±13.3 g) and female (49.2±13.2 g) participants at pre-test was less than RNI. However, total protein intake was significantly increased (p<0.05) at post-test and was more than RNI. As for protein intake percentage, its value in both genders at pre- and post-test was within the recommended RNI value (15-20%) and not significantly different (p>0.05).

Total fat intake per day in male participants at pre-test (91.4±121.7g) was higher than the RNI (62-75g), whereby the value was not significantly different (p=0.959) compared to post-test (89.5±16.6g). On the other hand, total fat intake per day in female participants (46.9±9.6g) was less than RNI at pre-test but had significantly increased (p=0.001) beyond the recommendation at post-test (79.4±18.5g). In both genders, the percentage of fat intake was higher (>30%) than the recommended RNI value (25-30%). There was no significant difference in the percentage between pre- and post-test in both genders (p>0.05).

DISCUSSION

This study was conducted to examine the effects of sports nutrition class on knowledge, attitude, and practice (KAP) regarding sports nutrition, as well as dietary intake of young university athletes. All participants attended a one-day class on sports nutrition as an intervention in this study. The topics covered in this class are listed in Table

Table 4. Comparison of total energy and macronutrient intakes per day between pre- and post-test

Variables	*Pre-test*	*Post-test*	*p-value*	*Recommended RNI value*
	Mean±SD	*Mean±SD*		
Total energy (kcal)				
Male	1389±190	2172±267	0.001*	2520-2740
Female	1227±202	1962±389	0.001*	2080-2370
Total carbohydrate intake (g)				
Male	168.4±31.2	253.7±65.2	0.002*	200-330
Female	152.5±30.9	235.9±52.5	0.001*	180-230
Carbohydrate intake (%)				
Male	48.7±7.3	46.6±9.5	0.404	55-70
Female	49.6±5.0	48.4±5.6	0.582	55-70
Total protein intake (g)				
Male	54.7±13.3	88.4±33.7	0.006*	61-62
Female	49.2±13.2	76.1±25.8	0.009*	52-53
Protein intake (%)				
Male	16.3±2.5	16.2±5.7	0.926	15-20
Female	16.0±3.5	15.2±2.8	0.596	15-20
Total fat intake (g)				
Male	91.4±121.7	89.5±16.6	0.959	62-75
Female	46.9±9.6	79.4±18.5	0.001*	51-61
Fat intake (%)				
Male	35.0±6.5	37.3±6.1	0.327	25-30
Female	34.4±4.7	36.4±6.1	0.418	25-30

*significantly different between pre- and post-test at $p<0.05$; $N=21$

1. Instead of separating all these topics into individual sessions per week across several weeks, the present study decided to cover all the topics in a one-day class with three separate lectures, separated by lunch or tea breaks in between. To date, no method of topic distribution (either in one day or one session per week for several weeks) has proven to be effective in changing an individual's habit. However, exposure to all these topics, regardless of the method, is the major concern. Hence, this study chose to conduct the class in one day due to time constraint. The participants were university students who were used to a full day class. Hence, having a one-day class on sports nutrition with substantial breaks in between lectures was deemed tolerable.

Overall, sports nutrition knowledge among the participants had improved after attending the sports nutrition class, with majority of the participants categorised under high level of knowledge at post-test (Table 2), whereby the increment from moderate score to high score was significant in both genders (Table 3). This indicates that education has a positive impact on increasing the athletes' knowledge level. The finding of this study is consistent with the result findings from previous studies that focused on the implementation of nutrition education as an intervention (Siti Soraya *et al.*, 2018; Rossi *et al.*, 2017). In a previous research (Siti Soraya *et al.*, 2018), 105 male team sports athletes were divided into experimental and control groups, where the experimental

group received seven weeks of education intervention programme, while the control group did not. As a result, mean score for knowledge increased in the intervention group (6.21±2.95) and decreased in the control group (-2.15±1.45), providing further evidence that education is an effective tool for increasing an individual's knowledge level. A similar study was also conducted among adolescent cricket players, where both female and male athletes had poor understanding of sports nutrition (Sobana, 2016). Moreover, this previous study also found that female athletes showed better mean score in knowledge than male athletes.

Several factors contributed to the increase in nutrition knowledge among the participants during intervention. These factors included the content of the education, duration of the intervention programme, and the teaching techniques (Bratianu, 2015). To ensure maximum commitment from the participants, the content of teaching should be relevant and focused on the target audience, while the learning duration should not be too long or too frequent. Besides, to capture the participants' attention, they should be actively engaged during the learning process, thus a variety of teaching techniques should be implemented. In the present study, the sports nutrition class was held for one day with several breaks in between, and the teaching techniques included lectures, group discussions, and some group activities. Education background could be a determinant of the level of knowledge of an individual. According to a previous study, a population with a low educational level showed less correct answers concerning knowledge about the subject matter. Consequently, this might lead to low scores in attitude and practice (Diaz-Quijano *et al.*, 2018). Participants in this study were recruited among university students who had not received any prior formal sports nutrition education.

The participants' attitudes on sports nutrition were also measured at pre- and post-test. There was improvement in attitude scores among participants from pre- to post-test in both genders, but it was only significant in female participants (Table 3). None of the participants showed negative attitude since the beginning of the study. This attitude is a strong predictor (Backman *et al.*, 2002) and a vital one for athletes to practise eating a healthy diet (Shifflett, Timm & Kahanov, 2002). In several previous studies, attitude scores have been significantly improved in primary school children after attending a nutrition education programme (Zalilah *et al.*, 2008; Ruzita, Wan Azdie & Ismail, 2007). A positive relationship between knowledge and attitude was reported among softball players, and higher nutrition knowledge led to a better attitude towards nutrition (Hornstrom *et al.*, 2011). This can be attributed to an increase in awareness among individuals after receiving proper education. In this study, no participants showed a negative attitude towards sports nutrition. This might be due to their education background that makes them aware about the importance of correct nutrition. Furthermore, environmental factors, such as friends, family, and culture, might also influence their attitudes towards nutrition intake.

The mean score for dietary practice was significantly improved from poor to fair in both genders after attending the sports nutrition class (Tables 2 and 3). This implies that the sports nutrition class conducted was successful in improving participants' eating practices. This finding is supported by previous studies (Siti Soraya *et al.*, 2018; Zalilah *et al.*, 2008), which also reported increased mean score for practice following an

education intervention. There are several factors affecting dietary practices and food choices among athletes. Food availability in their surroundings, knowledge about food, and family beliefs are among the major factors (Othman *et al.*, 2013). Food choices by athletes are also likely to be influenced by their coaches. Some athletes are concerned about their weight and body image, particularly for weight-category sports or aesthetic sports, which contribute added pressure for them in food choices and the need to practise a healthy diet. Furthermore, the impact of media and social influences are also significant in food choices and dietary practices among athletes, aside from the general population (Birkenhead & Slater, 2015).

The priority for athletes with regards to nutrition is meeting their energy needs. Athletes must consume foods that provide enough energy to maintain body weight and body composition, especially during training. The present study found a significant increase in mean total energy intake at post-test in both male and female participants (Table 4). Similarly, it was previously reported that total calorie intake improved among team sports athletes after receiving education intervention (Siti Soraya *et al.*, 2018). Another study also reported similar findings; improvement in the overall energy intake as a result of sports nutrition education (Molina-Lopez *et al.*, 2013). According to the Malaysian RNI, several factors should be considered while determining the energy expenditure for an individual. These include socio-demography, body composition and body size, presence of any medical condition, level of physical activity, as well as surrounding environments (NCCFN, 2017; WHO, 2010).

As suggested by the RNI, total energy intake for non-athlete adult men aged 19 to 30 years old is between 2520 to 2740 kcal per day, and for non-athlete adult women of similar age, the range is between 2080 to 2370 kcal per day (NCCFN, 2017). However, the total energy intake needed for athlete population is slightly higher, depending on the types of sports and training load. Based on the result of this study, although mean total energy intake had increased, the amount for both male and female participants did not meet the recommended values as suggested by the RNI (Table 4). Similarly, previous studies also discovered that the energy intake of the participants was below the recommended value even after receiving sports nutrition education (Molina-Lopez *et al.*, 2013; Cholewa *et al.*, 2015). Many factors could have contributed to this finding - concern about body weight, inaccurate reporting of food intake, or even under- or overestimation of the participants' calories. Athletes that are involved in gymnastics, wrestling, bodybuilding, and long-distance running, which focus too much on having a lean body are at higher risk of poor nutrient intakes (Williams, 2017). In this study, majority of the participants were final year undergraduate students with a very tight schedule. At the same time, they were actively involved in training and sports. Hence, having a busy lifestyle might be one of the reasons they were taking slightly less calories than what their requirement. It is most unlikely for them to under-report their dietary intake since they have been taught properly about the right way to report dietary intakes and the importance of reporting it with honesty.

The mean total carbohydrate intake per day had significantly increased from pre- to post-test, where the value at post-test was within the range recommended by RNI (Table 4). However, the percentage of carbohydrate intake at post-test was still below the RNI recommendation for carbohydrate intake, which is between

55% and 70% of total energy intake. This is due to the significant increase in protein and fat intakes at post-test by the participants. Total protein intake per day significantly increased beyond the recommended value by RNI, whereas for fat, its total intake was already high at pre-test and continued to be high even four weeks after attending the sports nutrition class.

These findings on dietary intake contradicted the KAP mean scores of the participants. Although the mean scores for knowledge, attitude, and practice among participants had increased, their actual dietary intake had not improved. Protein is responsible for rebuilding muscle tissues after exercise, and fat is a source of energy. Yet, diets high in protein and fat have not been shown to be beneficial for athletic performance, especially in the presence of inadequate carbohydrate ingestion (Loucks, 2004). Thus, meeting the recommended guidelines is very crucial, especially for the young athlete population.

CONCLUSION

This study revealed that a one-day sports nutrition class significantly increased the participants' knowledge, attitude, and practice scores in sports nutrition. However, the participants' dietary intake did not improve. The total intake per day of protein and fat were higher than the RNI even after attending the sports nutrition class. Future studies should give extra attention on protein and fat intakes as well as address all the limitations highlighted in this study.

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Authors' contributions

NNZKZ, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; ASM, advised on data analysis and interpretation, and reviewed the manuscript; MRCJ, advised on data analysis and interpretation, and reviewed the manuscript.

Conflict of interest

The authors declare no conflict of interests.

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SHORT COMMUNICATION

Recommendations to promote breast milk feeding and enhance nutritional care for preterm infants in the Asia-Pacific region: highlights from a roundtable discussion of key opinion leaders

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ABSTRACT

Introduction: Preterm infants are vulnerable to nutritional deficiencies, thus optimal nutrition is crucial in promoting growth among these infants. However, socio-cultural complexities and limited resources in the Asia-Pacific demands a judicious approach in implementing nutritional care that is pragmatic to align with current evidence-based recommendations. **Methods:** A roundtable meeting was held in Jakarta in 2017 for key opinion leaders in neonatology from the Asia-Pacific to discuss issues when delivering nutritional care in this region and the unique circumstances encountered. **Results:** Priority areas discussed include: (i) breast milk feeding, (ii) donor milk bank/sharing, (iii) human milk fortification, and (iv) nutrient-enriched breast milk substitutes. Socio-cultural practices impeding breastfeeding, insufficient maternity leave, the religious issue of milk kinship, and limited availability of specialty nutritional care products were among the most challenging factors. **Conclusion:** The group proposed recommendations to enhance breastfeeding uptake, accessibility to a complete portfolio of specialty nutritional care products, and encouraging more active collaborations to engage policy makers in addressing these contemporary issues.

Keywords: breastfeeding, donor human milk, human milk fortifier, milk kinship, preterm infant

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INTRODUCTION

Preterm birth, defined as any live birth before 37 completed weeks of pregnancy, is one of the primary contributors to increased neonatal morbidity and mortality. Globally, it is estimated that one in ten infants are born preterm and more than half are in Asia (Chawanpaiboon *et al.*, 2019). Being cared for outside the womb, preterm infants are vulnerable to nutritional deficiencies and imbalance, which impact on their growth. There is substantial evidence that growth deficits amongst preterm infants are associated with poor neurodevelopmental outcomes later in life (Meyers *et al.*, 2018). Curbing postnatal growth failure is, therefore, an important agenda for improving neonatal care in this region.

METHODS

Given the socio-cultural complexity in the Asia-Pacific population, it is necessary for key opinion leaders (KOLs) in this region to meet and review challenges faced when providing nutritional care to preterm infants and to put forward a proposal to overcome these obstacles. A meeting was organised in Jakarta in 2017 to render a platform for neonatologists to exchange views on these issues. Eight experts in the field of neonatology from Australia, India, Cambodia, Indonesia, and Malaysia evaluated present feeding practices in this region and discussed the strengths and weaknesses in conforming to the current recommendations in feeding preterm infants.

RESULTS AND DISCUSSION

The roundtable discussion (RTD) highlighted four key areas in implementing quality nutritional care among preterm infants, namely (i) breastfeeding, (ii) donor milk bank/ sharing, (iii) human milk fortification, and (iv) the use of nutrient-enriched breast milk substitutes (BMS).

(i) Breastfeeding

The KOLs agreed on the use of human milk or breastfeeding as the first feeding option for preterm infants. Breastfeeding confers both short- and long-term advantages to infants and mothers. Human milk contains biologically active substances that promote gut development and foster immunity to fight against infections.

Despite the benefits of breastfeeding, it is not always feasible to breastfeed preterm infants due to several factors. Since preterm labour is a stressful event, a high level of stress hormone disturbs the lactational pathways, reducing the amount of breast milk being produced. The lack of nipple stimulation due to the inability of preterm infants to suck at the breast compromises breast milk production and the letdown process. Moreover, cultural practices in the postpartum confinement period, where women who just gave birth recuperate at home, separates the preterm infants in hospital from their mothers. Besides, large family units exist in this region, causing mothers to have difficulty to breastfeed their infants at the hospital as they have other children to care for at home. Short maternity leave is also associated with a shorter duration of breastfeeding (Seah & Cheah, 2017).

Promoting breastfeeding optimises the nutritional support of preterm infants and reduces the dependency on financial resources to buy BMS. Regarding this, more lactation consultants (nursing professionals who are trained to assist new mothers in breastfeeding) are needed to intensify breastfeeding support for mothers. Experts in this meeting acknowledged that socio-cultural practices, such as postpartum confinement, may have a negative impact on breastfeeding

preterm infants, but it may be culturally insensitive and practically impossible to do away with these rituals. The mobilisation of community nurses during home visits for mothers who just gave birth to educate on the necessary skills and facilitate the transport of expressed milk to hospital so that their preterm infants in hospital can receive the benefits of mother's own milk (MOM) may partly help in addressing this issue. Interestingly, in Indonesia, the engagement of courier services by mothers who returned to work early or who are still in confinement could be one way to facilitate the transfer of MOM to the hospital. However, strict quality assurance monitoring should be enforced to ensure the safety and quality of MOM especially in the tropical heat and with the significant delays from congested road traffic. The provision of more rooming-in facilities and private spaces for kangaroo mother care (KMC, the practice of skin-to-skin contact nursing for the baby by a parent) prior to discharge from hospital is likely to promote breastfeeding uptake that persists for a longer duration. If not possible, mothers should be encouraged to visit and stay for as long as possible in the neonatal intensive care unit (NICU) to partake in the care of their preterm infants and indulge in as many KMC sessions as possible to increase bonding and breastfeeding. At home, when mothers are not with their infants, they should be reminded to express their breast milk at least six to eight times a day, as if the infants were breastfeeding regularly.

The matter of short maternity leave was raised by several KOLs in this meeting. It was suggested that more efforts were required to engage with law-makers in enacting legislation to extend paid maternity leave to 18 weeks as per recommendations by the International Labour Organisation (Addati, Cassirer & Ghilcrist, 2014), in order to promote exclusive breastfeeding for at least six months after birth. Leaders in the community, non-governmental organisations, and celebrity personalities were some of the proposed personnels that could help to move this change. In India, the government has mandated paid maternity leave for six months to enhance the rate of continued exclusive breastfeeding in the country.

(ii) Donor milk bank/sharing

When there is inadequate MOM, donor human milk (DHM) is the next best option as recommended by the World Health Organization (WHO) (WHO, 2006). There are two primary approaches in obtaining DHM, either from the human milk bank or one-to-one breast milk sharing. In this meeting, only the KOL from Australia maintained that a milk bank is being established in Australia, but not in the other countries represented. The human milk bank is a centralised institution that collects and pools breast milk from multiple pre-screened mothers. The collected milk is pasteurised to reduce pathogenic contamination before being used to feed infants. This concept of distributing pooled DHM from a centralised institution, however, may not be acceptable in certain Southeast Asian countries with a predominantly Muslim population due to the issue of milk kinship (Seah & Cheah, 2017). Milk kinship in the Islamic context is deemed established when a baby is breastfed at least five times by a donor mother, which results in the recipient baby and donor's children becoming siblings. Although they are not biologically-related, marriage is prohibited between them in this religious perspective. The lack of expertise, shortage of funds, and limited infrastructure further hinder the establishment of human milk banks in most parts of this region.

Another approach is through one-to-one milk sharing. This remains as the current mainstream approach in the region's predominantly Muslim nations. Even so, the social, medical and legal implications of this practice need to be addressed more thoroughly as most hospitals do not have a formalised protocol to supervise and coordinate the milk sharing process.

With the above considerations, the involvement of religious leaders in human milk banking initiatives to address the issue of milk kinship is crucial to establish a permissible or "halal" milk bank in the religious context. Various safeguarding measures have been proposed when establishing a human milk bank in Muslim countries (Seah & Cheah, 2017). In addition, international collaborations may further facilitate the alignment of human milk banks globally with countries in the Asia-Pacific region. Currently, one-to-one milk sharing is practised sporadically and regarded as a feasible alternative. Nevertheless, the entire sharing process is preferably institutionalised, taking into account the safety aspects and protection of individuals, with pre-screening of the donors and pasteurisation of the donor's milk. It is emphasised that the commercialisation of milk sharing and informal sharing via social media should be strongly discouraged (European Milk Bank Association, 2020).

(iii) Human milk fortification

Next, fortification of breast milk refers to the addition of a milk-based supplement to boost the nutrient content of human milk. It is especially important to preterm infants, who require more energy and protein for growth and micronutrients for bone mineralisation when compared with term normal infants (Table 1). Despite its importance, human milk fortifiers were not accessible in some countries like Cambodia.

Table 1. Comparison of nutrient contents in unfortified, fortified breast milk, preterm and post-discharge formula in 100mL against the recommended intake (per kg/day) for preterm infants

Nutrient content	*Breast milk (unfortified)*	*Breast milk added with human milk fortifier (fortified)*	*Preterm formula*	*Post-discharge formula*	*Recommended intake*[†]
Energy (kcal)	49-73	63-87	82	74	110-135
Protein (g)	1.1-2.7	2.1-3.7	2.2	1.9	3.5-4.5
Carbohydrate (g)	5.1-6.2	6.9-8	8.4	7.7	11.6-13.2
Lipid (g)	2.2-3.3	2.5-3.6	4.4	4.1	4.8-6.6
Calcium (mg)	25-29	142-146	101	78	120-140
Phosphorus (mg)	9.5-12.8	76.5-79.8	61	46	60-90

[†]Recommended intake by the European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition (Agostoni, Carnelli & Buonocore, 2010)
Note: The energy, macronutrient and micronutrient contents of breast milk (unfortified) (Gidrewicz & Fenton, 2014) are relatively lower than the ESPGHAN recommendation. However, the addition of human milk fortifier to breast milk increases the nutritional value to meet the recommended requirement and is comparable to the nutrient-enriched formula. Values are adapted with reference to the use of a typical human milk fortifier, preterm formula or post-discharge formula (Cheah, 2017).

(iv) Nutrient-enriched breast milk substitutes

Preterm infants who do not receive breast milk because of ongoing maternal illnesses require the use of specialty BMS, which is vital to promote growth. The BMS, previously called infant formula, is predominantly a cow's milk-based, nutrient-enriched formula, compounded to meet most of the infants' nutritional and growth requirement. The preterm infant BMS is a special category of nutrient-enriched BMS that supplies higher energy content to these infants, while post-discharge formula (PDF) is formulated with some extra energy and micronutrients for feeding preterm infants after hospital discharge until satisfactory "catch-up" growth is attained, while the standard term BMS is for normal term infants (Table 1).

The BMS for preterm infants is available in powder or ready-to-feed (RTF) liquid form. The RTF liquid formulation is made sterile with no risk of bacterial contamination. Less manpower is required compared with milk prepared from powder form and it may prove to be more cost-effective in the long run (Marino, Meyer & Cooke, 2013). From the safety perspective, it is generally recommended that the use of RTF is the preferred option over powdered milk formula for preterm infants in the neonatal intensive care unit. Regrettably, majority of the KOLs (except Malaysia and Australia) revealed that many countries in this region still have no access to RTF preterm formula.

At the time of hospital discharge, more than 80% of preterm infants are in some state of suboptimal growth in two countries in Asia (Lee *et al.*, 2019). In this regard, the nutrient-enriched PDF could promote some "catch-up" to attain a more satisfactory growth trajectory in infants who are especially not being breastfed at home. The use of PDF is associated with better weight gain and improved linear growth, likely from increased bone mineralisation compared to the standard term formula, particularly among preterm infants of very low birth weight (<1500 grams at birth) (Teller *et al.*, 2016). Nevertheless, PDF was considered expensive and still inaccessible in many resource-limited countries.

With regards to the limited and inconsistent availability of HMF and specialty BMS, a persuasive call is necessarily made to the respective governments, industries and related agencies to work together to actively promote breastfeeding and relax some regulatory processes such as reducing the price of human milk fortifiers and specialty preterm BMS. Furthermore, the expansion of health insurance benefits to subsidise the cost of special medical purpose formulas for infants, as ongoing in Victoria, Australia could promote affordable unlimited access to quality nutritional care for preterm infants. The key highlights of this RTD are summarised in Table 2.

CONCLUSION

In conclusion, with a socio-culturally diverse population in Asia-Pacific, the KOLs in this RTD canvassed all countries in the Asia Pacific region to collaborate in conducting multi-centre research projects to improve the uptake of breast milk, such as donor milk sharing or banking, and to promote ways to enhance the portfolio of nutritional care in the feeding of preterm infants. It is hoped that this research output will empower healthcare providers in putting forward an agenda to convince policy- and law-makers to create changes to overcome the various impediments to optimal nutrition for preterm infants in the Asia-Pacific.

Table 2. Key highlights from a Roundtable Discussion on Challenges and Potential Solutions in Feeding Preterm Infants in the Asia-Pacific

	Identified challenges	*Proposed solutions*
Mother's own milk	• Lacking in many cases because of maternal illness or preterm birth.	• Increase lactation consultants and community nurses for home visit to support mothers of preterm infants. • Provide quality and regulated courier services for transporting breast milk from home to hospital. • Offer rooming-in and kangaroo mother care support facilities. • Promote legislated longer and fully paid maternity leave.
Donor human milk	• The religious issue of milk kinship. • Lack of milk banking resources. • Lack of formal protocol for milk sharing. • Screening of the donor. • Safety issues.	• Continuous engagement of religious leaders' participation in dealing with issues on the use of donor milk. • Intensify sharing of expertise between countries in establishing human milk banking. • Formalise breast milk sharing procedures. • Develop a system that streamlines processes to address milk kinship.
Human milk fortifier (HMF)	• Relatively expensive. • Limited availability globally.	• Reduce price or subsidise. • Increase availability and distribution. • Innovative measures needed so as to not interrupt direct breastfeeding when HMF is added to breast milk.
Breast milk substitute (BMS)	• Limited range of nutrient-enriched BMS. • Limited availability and distribution of ready-to-feed (RTF) formulation. • Relatively expensive per unit of RTF item.	• Reduce price or subsidise. • Increase availability and distribution. • Regulatory bodies to refine policies on the use of specialty formula for preterm infants. • Balanced perspective on the use of BMS for preterm infants to avoid conflicting with the BFHI†.

†BFHI: Baby-Friendly Hospital Initiative – An effort to ensure that all hospitals, maternity facilities and communities become centres of breastfeeding support launched by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) since 1991.

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Authors' contributions

CFC, conceptualised and drafted the framework of this manuscript, represented Malaysia, and contributed in the writing and reviewed the manuscript; TTL, prepared the draft of the manuscript, reviewed the literature and compiled the references; HU and DC, coordinated the

project and collated all the data obtained during the roundtable meeting; ML, led and moderated the discussion of the group and summarised the highlights of the meeting; DG, represented the Australasian region and contributed in the writing and reviewed the manuscript; RR, represented Indonesia and contributed in the writing and reviewed the manuscript; SS, represented India and contributed in the writing and reviewed the manuscript; VERM, chaired the discussion and contributed in reviewing the manuscript.

Conflict of interest

The KOLs received travel and lodging financial support to attend this roundtable discussion in Jakarta from the Danone-Nutricia establishment in Indonesia. Staffs employed by Danone-Nutricia participated in the meeting to provide support but were not involved in any way in presenting the viewpoints raised at this roundtable discussion as reported in this manuscript.

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Dietary diversity and nutritional status of 2 to 5 years old children in households with and without home gardens in selected districts in Siem Reap province, Cambodia

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ABSTRACT

Introduction: There is a high prevalence of malnutrition in Cambodia during the past 10 years. One of the programmes implemented to secure household food security in order to alleviate malnutrition was home gardening. This study aimed to compare the nutritional status of 2 to 5 years old children in households with and without home gardens. **Methods:** Twenty-five households with home gardens and 60 households without home gardens were sampled. **Results:** The households sampled had a mixed type of home garden with three to five crop varieties occupying 20 to 200 square meters area. The purpose of the garden was to increase vegetable consumption and produce additional income. Results showed that 72% and 65% of the children resided in households with and without home gardens, respectively. Majority had medium dietary diversity score, i.e. consumed four to five different food groups per day. Except for stunting, higher prevalences of undernutrition (32%) and wasting (8%) were observed among children residing in households with home gardens compared to children in households without home gardens. **Conclusion:** There were no associations for dietary diversity score and nutritional status of children from households with and without home gardens. Among the socio-demographic and economic factors, age, sex, family size, and vegetable consumption were significantly associated with dietary diversity score and nutritional status of the children.

Keywords: home garden, dietary diversity score, nutritional status

INTRODUCTION

Malnutrition (stunting and wasting) in Cambodia remains a critical issue, with significant numbers of undernourished children and women from all levels of income, especially in the rural areas (National Institute of Statistics, 2014). This problem is reported to be caused by factors such as limited social protection for the poor and vulnerable families, the inability of households to secure sufficient and nutritious foods due to high rural poverty, and the occurrence of natural disasters such as flooding and droughts (Chaparro, 2014). Approximately 32% and 9% of

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children under 5 years were assessed to be stunted and severely stunted, respectively. High prevalence of stunting (37%) was also observed in children with less than 24 months of birth interval. Wasted and severely wasted children under 5 years of age were reported at 10% and 2%, respectively. The prevalence of wasting among children of underweight mothers was noted to be more than twice of those children with mothers of either normal or overweight nutritional status. Underweight in children under age 5 was 24%, with 5% being severely underweight. Children who were born from mothers with the lowest wealth quintile were more likely to be underweight compared to children born from mothers in the highest wealth quintile. A total of 2% of children below age 5 were reported to be overweight. It was also reported that overnutrition among children tends to decrease with increasing age (National Institute of Statistics, 2014). It was assumed that overweight in young children was greater than in adults (National Institute of Statistics, 2014).

Approaches to reduce malnutrition in Cambodia include multi-sectoral efforts that cover the following: improving access to safe water supply, sanitation, and hygiene; increasing access to more diverse foods for women and children; addressing suboptimal infant and young child feeding (IYCF) practices; and supporting livelihood and social safety net (National Institute of Statistics, 2010). Since 1996, both government and non-government agencies in Cambodia have proposed different strategies, plans, and policies to reduce malnutrition among young children, majority of which had been funded by the United States Agency for International Development (USAID) (USAID, 2014). Home gardening was one of the proposed nutrition and food security projects in Cambodia, implemented mostly in the rural areas. A home garden is an area where vegetables, annual and perennial plants, spices, herbs, shrubs, and fruits are grown seasonally or throughout the year to meet family requirements (Van der Stege, 2009; Nordin, 2005; Gari, 2004; Helen Keller International, 2003; Midmore *et al.*, 1991). Around 40 organisations are involved in vegetable production or home gardening activities. The Food and Agriculture Organization (FAO) recognises the important benefits of a home garden beyond the access to nutritional foods, which includes fuel for cooking, wood for building, medicinal plants, and additional income. In 2013, the home gardening project, funded by the USAID, was initiated in Siem Reap, and was also used as a tool to advocate conservation agriculture with women in Siem Reap. Initially, 15 farmers from three different districts participated in the Sustainable Agricultural and Natural Resource Management (SANREM) programme, in partnership with the Agricultural Development Denmark Asia (ADDA), which focused on conservation agriculture and irrigation technologies. This initial project by the University of California Davis (UC Davis) Horticulture Innovation Lab ended in late 2014, but USAID Center of Excellence of Sustainable Agriculture Intensification and Nutrition (CESAIN) continued it in 2015 with the previous 15 farmers, as well as an additional 40 selected farmers. The project aims to secure agricultural sustainability through conservation agriculture and to improve food security, dietary diversity, as well as income. With that, this study aimed to compare the dietary diversity and nutritional status of children aged 2 to 5 years in households with and without home gardens in Siem Reap province, Cambodia.

MATERIALS AND METHODS

Study area

Siem Reap province was selected as the study area as the home gardening project was implemented in different

districts of Siem Reap since 2013. Based on the study of LEAP & DIME Project (2011), Siem Reap is the poorest province in Cambodia with 28.6% of the total households belonging to Poor 1 and Poor 2 levels, characterised by a shortage of properties, lack of investment and productivity, and inaccessibility to the market. Siem Reap province is located in the northwestern part of Cambodia, surrounded by Battambong, Bantey Meanchey, Kompong Thom, Preah Vihear, and Oddor Meanchey provinces. Siem Reap has a total land area of 10,299 km^2, which is divided into 12 districts, 100 communes, and 924 villages (GPCC, 2008). The total population of the province is 139,458 (World Population Review, 2018). The nutritional status of young children in Siem Reap has been a concern. The 2014 Cambodia Demographic Health Survey (CDHS) (National Institute of Statistics, 2014) reported that the prevalence of stunting is significantly higher among children living in poor households (42%) than children living in moderate and rich households (19%). Based on FAO's conceptual framework (FAO, 1999), poverty may strongly affect the nutritional status and food security in a family.

Research design

Case-control study was utilised for this research. The respondents of the study composed of households with and without home gardens, and with 2 to 5 years old children. Twenty-five households that have been implementing the home gardening project since 2013 were sampled from three districts, namely Prasat Bakong, Pouk, and Sot Nikom in the Siem Reap province. A complete enumeration of these 25 households with home gardens and 2 to 5 years old children was done. A total of 60 households without home gardens were also randomly selected from these three districts. This sampling number was computed based on the set value of 95% level of confidence, 8% sampling error, and the proportion of stunting at 32%.

Data collection

Demographic and socio-economic profile of caregiver

The questionnaire format was patterned from the CDHS. The questions included the date of birth, sex of the child, civil status, educational attainment of the caregiver and household head, child's caregiver, occupation, living standard, source of income, expenditure, and household size. Living standards of the household were categorised into three categories: poor 1, poor 2, and moderate. Poor 1 and poor 2 levels were focused on families that had the poverty identification card provided by the government based on unavailability of properties, shortage of investment and productivity, and unreachability to the market. The mothers or caregivers of children were interviewed about the demographic and socio-economic status of the family, home garden information, and dietary intake for the last 24 hours before the interview. The children were measured for their anthropometric data (weight and height).

Characteristics of the family's home garden

Home gardening is an agro-ecosystem and a traditional land use practice carried out around a homestead consisting of mixed planting of herbs and species, leafy vegetables, roots, fruits, shrubs, grasses in a small plot to provide food, spices, medicines, and construction materials that are grown and maintained by the family members. The households in the project were interviewed for information related to home gardening. Size, variety of vegetables, frequency of vegetable consumption from home

garden, vegetable income, and budget expenditure on foods were included in the questionnaire.

Assessment of dietary intake

Three days (2 weekdays, 1 weekend) of 24-hour food recalls were collected to determine dietary intake. The dietary diversity score questionnaire was used for data collection. The eight food groups considered for dietary diversity score included: 1) grains, roots or tubers; 2) vitamin A-rich plant foods; 3) other fruits or vegetables; 4) meat, poultry, fish, seafood; 5) egg; 6) pulse, legumes, nut, and seed; 7) milk and milk products; 8) foods cooked in oils or fats. The questionnaire used was based on the FAO guidelines (FAO 2010). The qualitative interpretation of dietary diversity was done by three classes: low dietary diversity (≤3 food groups), medium dietary diversity (4 to 5 food groups), and high dietary diversity (≥6 food groups).

Anthropometric measurements

The weight and height of 2 to 5 years old children were measured. Weight measurements were collected using a balance weighing scale provided by the village coordinator, supported by the Ministry of Health. Weight was obtained following protocols such as minimal clothing and emptying of pockets. Height measurements were carried out using a measuring board, following guidelines by the United Nations Children's Fund (UNICEF), with children measured in a standing position. Protocols such as heels, buttocks, scapula and occiput of the head in a straight line, with measuring board touching the head in Frankfort position, hand relaxed on the sides were strictly observed. Recording of weight was done to the nearest 100g, while height was measured to the nearest 0.1mm. Three nutritional indices were calculated using the children's age, height, and weight, which were then converted to sex-specific z-scores using the World Health Organization (WHO) AnthroPlus software. Children with weight-for-age, height-for-age, and weight-for-height between -2 standard deviation (*SD*) to +2*SD* were classified as normal, while those with greater than +2*SD* were regarded as overweight, tall, and obese, and those with z-scores between -3*SD* to -2*SD* were classified as underweight, stunted and wasted (WHO, 1995).

Statistical analysis

Statistical Package for Social Sciences (SPSS) for Windows version 20 and Microsoft Excel were used for data entry and data analysis. Frequencies and percentages were calculated for categorical variables, while means and standard deviations were used for continuous variables. Spearman's test was used to correlate dietary diversity and nutritional status with age, respondent's education level, family size, monthly income factors, and vegetable consumption. On the other hand, Cramer's V was used to correlate dietary diversity and nutritional status with sex, child's caregiver, and respondent's occupation. The level of statistical significance for all analyses was set at $p<0.05$.

RESULTS

Respondents' socio-demographic and economic characteristics

Results of the study (Table 1) showed that majority of respondents from households with and without home gardens were young adult females, married, and had primary education. Respondents' age ranked from 20-69 years old, with an average of 35 years old. The average household size was five. Mother was a child's main caregiver. Eighty-four and 78 percent of the households with and

Table 1. Socio-demographic and economic characteristics of respondents (*n*=85)

Variable	*With*		*Without*		*Total*	
	n	*%*	*n*	*%*	*n*	*%*
Sex						
Male	4	16	4	7	8	9
Female	21	84	56	93	77	91
Age (years)						
20-39 (young adult)	17	68	44	74	61	72
40-59 (adult)	8	32	13	22	21	24
60-69 (old)	0	0	3	5	3	4
Civil status						
Married	25	100	57	95	82	96
Widow/er	0	0	3	5	3	4
Educational attainment						
No education	2	8	8	13	10	12
Primary school (Grades 1 to 6)	11	44	33	55	44	52
Secondary school (Grades 7 to 9)	10	40	14	23	24	28
High school (Grades 10 to 12)	2	8	4	7	6	7
University	0	0	1	2	1	1
Household size						
3 - 5	17	68	50	83	67	79
6 - 9	8	32	10	17	18	21
Living standard of household						
Poor 1	2	8	8	13	10	12
Poor 2	2	8	5	8	7	8
Moderate	21	84	47	78	68	80
Child's caregiver						
Mother	16	64	43	72	59	69
Father	2	8	1	2	3	4
Sibling	0	0	1	2	1	1
Aunt	1	4	2	3	3	4
Grandparents	6	24	13	22	19	22
Occupation						
Farmer	20	80	39	65	59	69
Small business owner	3	12	7	12	10	12
Garment worker	0	0	2	3	2	2
Government official	0	0	1	2	1	1
Casual labour	1	4	2	3	3	4
Housewife	1	4	4	7	5	6
Other	0	0	5	8	5	6
Monthly income (riel)						
100, 000 – 1,000,000 riel	12	48	41	68	53	62
1,000,100 – 2,000,000 riel	8	32	14	23	22	26
2,000,100 – 4,000,000 riel	4	16	3	5	7	8
4,000,100 – 6,000,000 riel	1	4	2	3	3	4

without home gardens, respectively, had a moderate living standard. Sixty-two percent of households had an income ranging from 100,000 to 1,000,000 riel (25 to 250 USD), while the average income was 1,491,600 riel (372.89 USD). Farming was the major source of income.

Characteristics of home gardens

In terms of a home garden, 19 households have been involved in the project for three to four years. Seed, water tank, and fertilisers were provided. The size of the garden ranged from 20 to 200 square meters and some were >1000 square meters. Vegetables were grown about three to four times per year. Green mustard, luffa, cucumber, water spinach, chilli and round brinjal were the main types of vegetables grown. Vegetable gardening aims to increase vegetable consumption and provide additional income. A household could earn around 40,000 to 1, 00,000 riel (10 to 250 USD) from vegetable gardening.

Dietary diversity score of the children

With regards to dietary diversity score, 55 children had a medium score. Children from households without home gardens had higher low dietary diversity score than children from households with home gardens. Consumption of fruits, vegetables, meat and milk were higher among children residing in households with home gardens than in children residing in households without home gardens. Consumption of pulses/legumes or nut was not observed (Table 2).

Table 2. Dietary diversity score and nutritional status of children in households with and without a home garden

Variable	*With a home garden*		*Without a home garden*	
	n	%	*n*	%
Dietary Diversity Score				
High (6 and above)	3	12	2	3
Medium (4 to 5)	18	72	37	62
Low (below than 3)	4	16	21	35
Nutritional Status				
Weight-for-age				
Normal (±2*SD*)	17	68	39	65
Underweight (<-2*SD*)	8	32	17	28
Severely underweight (<-3*SD*)	0	0	4	7
Weight-for-height				
Normal (±2*SD*)	21	84	55	92
Wasted (<-2*SD*)	2	8	4	7
Severely wasted (<-3*SD*)	2	8	1	2
Height-for-age				
Normal (±2*SD*)	17	68	30	50
Stunted (<-2*SD*)	5	20	20	33
Severely stunted (<-3*SD*)	2	8	10	17
Tall (>2*SD*)	1	4	0	0

Nutritional status of the children

Underweight: Eight and 17 children from households with and without home gardens were underweight, respectively, while seven children residing in a household without home garden were severely underweight.

Wasting: Eight and seven percent of children from households with and without home gardens, respectively, were analysed to be wasted. Eight and two percent were severely wasted.

Stunting: Children in households with and without home gardens had 20% and 33% of stunting, respectively. Eight and 17 percent of the children were severely stunted.

Relationship of home garden ownership with dietary diversity score and nutritional status of children

Results of the study (Table 3) revealed that there were no significant differences in dietary diversity score, weight-for-age, weight-for-height, and height-for-age of children from households with and without a home garden. This outcome illustrated that the presence or absence of a home garden did not influence the dietary diversity and nutritional status of the children at the research sites. The similarities in living standard, household size, mother's educational attainment, and monthly income between households with and without home garden (Table 1), as well as the traditional home garden practice that have existed in these households without the project might have led them to have similar variety of food intakes and child feeding practices.

Relationship between socio-demographic and economic factors with dietary diversity score and nutritional status of children

Dietary diversity score

There was a negative, moderate significant association between dietary diversity score and age of the children from households with a home garden (p=0.01, r=-0.49). This implied that the increase in respondent or caregiver's age tend to lower the dietary diversity score of children in the family. This may be supported by the results of Oduor's study in 2018 which explained that the age of the caregiver influences his/her nutritional knowledge and attitudes towards food preparation (Oduor, 2018). Young people tend to understand more about food choices, cooking methods, and the ability to get food for the family. However, there were no significant relationships between dietary diversity score and sex, child's caregiver, educational attainment, respondents' occupation, family size, income, and vegetable consumption of families without a home garden.

Nutritional status

There were no significant relationships between socio-demographic characteristics and economic factors with weight-

Table 3. Correlations between households with and without a home garden with dietary diversity score and nutritional status of children

Variable	*Coefficient*	*P-value*
Dietary Diversity Score	0.235ns	0.01
Weight-for-age (underweight)	0.144ns	0.41
Weight-for-height (wasting)	0.160ns	0.33
Height-for-age (stunting)	0.253ns	0.14

for-age and height-for-age of the children. However, family size (p=0.01, r=0.5) and vegetable consumption of the family (p=0.01, r=0.53) were found to have a positive and moderate significant association with weight-for-height (wasting) of the children with home gardens. This implied that the consumption of more vegetables may reduce the prevalence of wasting. Sex (p=0.001, r=0.53) had a positive, strong association, while family size (p=0.02, r=-0.29) had a negative, very weak relationship with weight-for-height (wasting) of the children from households without a home garden (Table 4).

A large family size may result in more mouths to feed, thus decreasing the amount of available foods to feed the children. Food intake decreases per capita with an increase in family size (Vil, 1975). Studies have shown that the foods available per head to larger families were often lower than that available to smaller families, and this unequal food distribution reflected growth rate. Moreover, the nutrient intakes of the children were observed to be lower than the recommended dietary intake for children aged 2 to 5 years old (FNRI-DOST, 2015). Energy, protein and fat intakes of the children were significantly associated with the nutritional status of the children. Low food and nutrient intakes influence the growth of children. Protein intake in particular is important for growth because protein provides essential amino acids required for protein synthesis. Hence, deficiency of protein will limit a child's growth significantly (Braun, 2016).

DISCUSSION

The results of the nutritional status of children at the research sites were similar to a survey conducted by the Cambodia Demographic and Health Survey in 2014 in Siem Reap. About 35.9% of the children were reportedly stunted, while 11.3% were severely stunted. The majority were underweight (26.2%) and 6.4% consisted of those who were severely underweight; 9.5% existed in a wasted situation, and 2.3% were severely wasted (National Institute of Statistics, 2014). The probable causes of underweight were identified as follows: inadequate food consumption, an underlying illness, stress, obsessive exercise, lack of interest in eating, or a sudden growth spurt. Low mean energy intake of 653kcal (with home garden) and 640kcal (without a home garden) among the children respondents were observed. These were much lower in comparison to the recommended energy intake for males aged 1 to 2 years and 3 to 5 years old at 1000kcal and 1350kcal, respectively, as well as the recommended

Table 4. Relationship of socio-demographic and economic factors with dietary diversity score of children

Variable	*With*		*Without*	
	Coefficient	*P-value*	*Coefficient*	*P-value*
Age	-0.49*	0.01	0.08	0.56
Respondent's education level	0.18	0.38	0.08	0.52
Family size	-0.01	0.94	0.11	0.39
Monthly income	-0.27	0.20	0.07	0.58
Sex	0.18	0.66	0.92	0.77
Child's caregiver	0.44	0.15	0.23	0.64

*Correlation is significant at p<0.05

energy intake for females aged 1 to 2 years and 3 to 5 years at 930kcal and 1260 kcal, respectively (FNRI-DOST, 2015). In addition, the nutrients intake of the children from this study also showed that majority had low nutrients intake per day. Wasting, or low weight-for-height, is characterised as the failure to receive adequate nutrition in the period immediately preceding a survey, and a result of inadequate food intake or a recent episode of illness that has caused loss of weight and the onset of malnutrition (WHO, 2018). Research in Tanzania (Mgongo *et al.*, 2017) and Western Kenya (Bloss *et al.*, 2004) have found that children who had illnesses were more likely to have low weight-for-height (wasting). Wasting is a strong predictor of mortality among children under five years of age. Stunting, on the other hand, can begin before birth as a result of a mother's poor nutritional status and continues after birth if a mother cannot provide high quality breast milk. These undesirable conditions can make an undernourished child more likely to become sick.

According to the data of a district situation in 2015 for management and development, it was stated that farming is the main occupation of the people in the study area (86.36%). Women are major contributors, with other family members, in earning income for family support. Home gardening is a part of food consumption and family income. Low-income families were prioritised by the USAID (CESIAN) home gardening project to serve food security and consequently acquire income and health. Interestingly, majority of the households (73%) at the research sites had their own gardens even though they were not in the gardening project. Their gardens had an average size land of 160 meters square. They simply grew white Chinese cabbage, water spinach, herbs, green mustard, luffa gourd and so on.

Table 5. Relationship between socio-demographic and economic factors with nutritional status of children

Variable	*Weight-for-age*				*Weight-for-height*				*Height-for-age*			
	With a home garden		*Without a home garden*		*With a home garden*		*Without a home garden*		*With a home garden*		*Without a home garden*	
	Coeffi.	*P-value*	*Coeffi.*	*P-value*	*Coeffi.*	*P-value*	*Coeffi.*	*P-value*	*Coeffi.*	*P-value*	*Coeffi.*	*P-value*
Age	0.10	0.65	0.01	0.95	-0.21	0.31	-0.09	0.50	0.17	0.42	-0.07	0.57
Respondent's education level	0.22	0.30	-0.08	0.53	0.13	0.55	-0.17	0.20	0.04	0.85	0.08	0.53
Family size	0.17	0.42	-0.02	0.86	0.50*	0.01	-0.29*	0.02	-0.25	0.22	0.08	0.57
Monthly income	-0.02	0.91	-0.07	0.59	-0.13	0.53	-0.09	0.50	-0.05	0.82	-0.05	0.71
Sex	0.06	0.74	0.19	0.31	0.29	0.34	0.53*	0.00	0.163	0.88	0.13	0.58
Child's caregiver	0.35	0.38	0.29	0.28	0.39	0.25	0.08	0.99	0.35	0.43	0.22	0.69
Respondent's occupation	0.43	0.20	0.27	0.86	0.26	0.75	0.39	0.18	0.43	0.13	0.26	0.89

*Correlation is significant at $p<0.05$

Thus, there were many similarities in socio-demographic and economic characteristics of households with and without home gardens (educational attainment, occupation, income, etc.). Table 4 implies that there were no significant associations of some socio-demographic factors with dietary diversity score and nutritional status of the children. However, there were slightly higher prevalences of underweight, wasting, and severe wasting in children from households with a home garden in comparison to children from households without a home garden. Nevertheless, there is a possibility that these children may have had higher wasting and underweight rates than at present, before their families got involved in the project.

CONCLUSION

There were no associations between dietary diversity scores and nutritional status of children with and without home gardens. Among the socio-demographic and economic factors, age, sex, family size, and vegetable consumption were significantly associated with dietary diversity score and nutritional status of the children.

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Authors' contributions

LS, led the data collection, conducted the study, data analysis and interpretation, prepared the draft of the manuscript and reviewed the manuscript; ASB, advised on study methodology, provided advice and reviewed the manuscript; CBJ, JTD and WAH, provided advice and reviewed the manuscript.

Conflict of interest

There is no conflict of interest to declare.

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Environmental enteric dysfunction, soil transmitted helminthiasis and stunting among 36- to 59-month-old children in Quezon Province, Philippines

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ABSTRACT

Introduction: Environmental enteric dysfunction (EED) is relevant in public health as it is a potential cause of child stunting. In the Philippines, stunting affects 33.4% of children in 2015. As of date, no local studies on EED exist. This study primarily aimed to determine the prevalence of EED, soil transmitted helminthiasis (STH) and stunting; and their relationships. **Methods:** A cross-sectional study was conducted among 120 children aged 36-59 months old in Quezon Province, Philippines. EED was assessed via glucose hydrogen breath test with a cut-off of ≥20 ppm over baseline by 90 minutes suggestive of small intestinal bacterial overgrowth (SIBO), a biomarker of EED. Kato Katz Technique was used to determine STH. Stunting was determined using the 2006 World Health Organization Child Growth Standards. Descriptive and correlation analyses of data were done at 10% level of significance. The study received approval from the National Ethics Committee of PCHRD-DOST, Philippines. **Results:** Data on expired hydrogen level of <20ppm were documented. The prevalence of STH among children were 38.1% for *Ascaris lumbricodes,* 15.3% for *Trichuris trichiura,* and 1.7% for hookworm infections. A high rate of stunting at 40.0% was observed. There was a significant positive association between stunting and the presence of *Ascaris* (p=0.01). **Conclusion:** There was no case of EED detected in the study. The severity of stunting was high at 40.0%. Among the STH, the prevalence of 38.1% children having *Ascaris lumbricodes* was alarming in terms of morbidity control. Nutrition interventions including WASH practices and the use of anthelminthic drugs need to be intensified to address stunting and STH.

Keywords: environmental enteric dysfunction, stunting, small intestinal bacterial overgrowth, soil transmitted helminthiasis

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INTRODUCTION

Stunting among children younger than 5 years is a major global health priority that has gained international attention because of its significant consequences on health and development. In the Philippines, stunting has remained unchanged over the years, affecting 33.4% children in 2015 (FNRI-DOST, 2016). Recognising that the primary drivers of undernutrition are inadequate dietary intake and recurrent infection, strategies to prevent and/or to address undernutrition are focused on dietary solutions, as well as water, sanitation and hygiene (WASH) interventions. However, both interventions have small to moderate impacts on linear growth. Dietary interventions impact linear growth from only 0.12 to 0.28 in terms of length-for-age Z-score (Dewey & Adu-Afarwuah, 2008), while WASH interventions implemented with 99% coverage would reduce stunting prevalence by only 2.4% (Bhutta *et al.*, 2008). This indicates that the cause of stunting transcends beyond dietary inadequacy and infections. The pathogenesis of stunting is poorly understood (Owino *et al.*, 2016) and implicates that a subclinical disorder of the small intestine known as environmental enteropathy (EE) or environmental enteric dysfunction (EED) may have caused poor linear growth (Humphrey, 2009; Owino *et al.*, 2016).

EED is a condition when there are abnormalities in the intestinal mucosa and is characterised by the presence of bacterial overgrowth in the small intestine. Small intestinal bacterial overgrowth (SIBO) contributes to the pathogenesis of EED as overgrowth has been associated with EED domains such as intestinal inflammation and micronutrient malabsorption (Harper *et al.*, 2018; Donowitz *et al.*, 2016; Vonaesch *et al.*, 2018). EED is found at a high prevalence among stunted children living under poor sanitary conditions and is pandemic in developing countries with limited resources (Owino *et al.*, 2016).

The impact of helminth colonisation on EED has not been well studied, but considering that the prevalence of intestinal soil transmitted helminthiasis (STH) infections is high, this study attempted to establish an association between STH and EED. STH infections remain a public health problem in many areas in the Philippines where challenges related to water, sanitation and hygiene continue to be experienced. Specifically, the prevalence of STH in the Philippines was noted at 27.7% for *A. lumbricoides,* 31.3% for *T. trichiura,* and 13.3% for hookworm (Soares Magalhães, 2015). The estimated prevalence of STH in the Philippines in school age children and preschool age children have not satisfied the targets of <20% cumulative prevalence and zero heavy intensity STH recommended by the World Health Organization (WHO) in order to achieve morbidity control (Belizario *et al.*, 2015).

As of date, no local studies are available that looked into the occurrence of EED in the Philippines. Hence, this study aimed to determine the prevalence of EED, STH and stunting among 36- to 59-month-old children; and their relationships. It also aimed to identify determinants of stunting among the study children.

MATERIALS AND METHODS

A stratified multi-stage sampling was employed in this cross-sectional study among 36- to 59-month old children in the province of Quezon, Philippines, where stunting prevalence among children <5 years old was highest in the CALABARZON region at 33.9% (FNRI-DOST, 2016). This group of children was

selected because of their cognitive, social and motor skills that enabled them to comply with research protocols. All apparently healthy children who had no feeding problem and inborn/congenital/ severe illness; who were not suffering from disease at the time of recruitment; who were not taking antibiotics for the last four weeks; residents in the barangay or municipality for the past six months; and with signed informed consent were included in the study.

To select the study areas, all municipalities in the Province were grouped into two strata, namely: low- and high-income municipalities. Per stratum, one municipality was randomly selected to represent the low (5th class) and high (1st class) income municipalities. Using the list of children 0-71 months old obtained from the Municipal Nutrition Action Officers, children aged 36-59 months from all qualified barangays were constructed as the sampling frame. The required sample size was estimated based on the prevalence of stunting (33.9%) among children in Quezon and the total number (124,411) of children aged 36-59 months in Quezon with a sampling error of 8%, level of confidence of 90%, and a design effect of 1.3. A total of 120 children was computed and were randomly drawn from the master list obtained from the Municipal Nutrition Action Officers. A randomly selected replacement was considered in case of disapproval from mothers to participate, decided to terminate participation in any stage of the study, or failed to conform with the preconditions for hydrogen breath testing.

The mothers or caregivers of the selected children were invited for a meeting to explain the nature and purpose of the study, as well as the study protocols. Assistance from the municipal and barangay officials, health and nutrition workers, and day-care teachers who were oriented on the study were tapped to encourage participation of the respondents.

Glucose was used as the test substance for the diagnosis of SIBO using the Hydrogen Breath Test Sleuth by Breathe EZ Systems, Inc. In this study, EED was defined with SIBO as the biomarker for the pathophysiological changes of malabsorption and intestinal inflammation characterising EED. Study children were examined by a paediatrician before enrolment to ensure that they were physically/ medically fit for the Glucose Hydrogen Breath Test. Demonstration on how to breath using disposable mouthpiece and how to release expired air to the breath analyser was also conducted by the municipal nurse or the researcher. Mothers/caregivers were advised not to give their children any slowly digested foods, any fibre supplements and laxatives for 24 hours prior to the test, and to fast for 8-12 hours before the procedure. On the day of assessment, mouth rinse was first administered to children in order to avoid the possibility of an early peak in hydrogen associated with the action of bacteria in the mouth on the carbohydrates used in the test. Children were instructed not to swallow the mouthwash. The test began with the collection of a basal expired air (fasting sample). Baseline breath hydrogen was measured by letting children take a deep breath of air and expired into the mouthpiece until all the air was out of the lungs. The presence of bacterial overgrowth was ruled out before performing the glucose breath test. Children who were eligible but with a baseline expiratory breath of >10 ppm, which is indicative of bacterial overgrowth, were excluded from the study. Glucose was then administered orally. A glucose load of 1.75g/kg body weight up to a maximum of 75g (Harriet Lane Handbook, 2017) dissolved in 10ml/kg body weight up to a maximum

of 250 ml (Eisenmann *et al.*, 2008) was appropriate for children. Using this as reference, the glucose drink (Medic orange, glucose test beverage) formulated by the Medic Diagnostic Laboratory in Pasig City, Philippines was used as the test substance. Samples of expired air were measured at 30-, 60- and 90 minutes after glucose load. The 2017 North American Consensus was adopted such that the length of glucose testing was limited to two hours; and the diagnostic criteria used was a rise in expired hydrogen of ≥20 ppm from baseline by 90 minutes as the ideal criterion for a positive test to suggest the presence of SIBO (Rezaie *et al.*, 2017).

Height measurements were carried out following the standard procedures and recommended equipment as prescribed in the National Health and Nutrition Examination Survey (NHANES) Anthropometry Procedures Manual (CDC, 2013). Microtoise was used to measure the height of children in centimeters. Two measurements of height were taken and recorded to the nearest 0.1cm. A third reading was required when the two measurements were beyond the acceptable limits (0.5cm for height). Age in months of each child was also collected to enable the determination of nutritional status. The corresponding Z-scores were computed using the 2006 WHO Child Growth Standards (CGS) software to determine the nutritional status of the children. Children with Z-scores <-2 standard deviation (*SD*) for height-for-age were classified as stunted.

The collection, packaging, storage and transport of faecal samples followed the procedures prescribed by the Department of Parasitology, University of the Philippines, Manila, where parasitological assessment was done. Each mother/caregiver received an improvised paper triangle lined with wax paper instead of a plastic cup, wooden stick and re-sealable plastic bag for one stool specimen of each child. A demonstration of stool collection was done in such a way that thumb-size stool was to be placed in the paper triangle using the wooden stick, then folding the paper to secure the specimen and putting it in a re-sealable plastic bag. The respondents were informed of the schedule of collection either at a specified venue or via house visits. Immediately after submission, sealed and labelled specimens were placed in an insulated box (styrofoam box) packed with ice and transported to the laboratory within 24 hours after collection. Parasitological assessment utilised the Kato-Katz technique following the WHO's Bench Aids for the Diagnosis of Intestinal Parasites (WHO, 1994). Microscopic reading of the slides was done by the microscopist to determine the prevalence of individual STH species, namely: *Ascaris lumbricodes, Trichuris trichiura* and hookworm.

Data entry was done in Microsoft Excel and analysed using the Statistical Package for Social Sciences (SPSS) software version 23. Data cleaning for inconsistencies, errors, double entry were performed before data analysis.

The profile and distributional characteristics of the subjects were described using appropriate descriptive statistics and by constructing percent distributions. Correlation analysis was done to determine the significant associations between variables. In particular, point biserial correlation coefficient was used for correlating dichotomous variables with quantitative data, while Cramer's *V* coefficient (based on chi-square) was used for nominal variables. Significant factors were determined at 10% level of significance.

This research received ethical approval from the National Ethics Committee of the Philippine Council for Health Research and Development of the

Table 1. Distribution of nutritional status in children 36-59 months in Quezon Province, Philippines based on height-for-age Z-score (HAZ)

Nutritional status based on Height-for-age Z-score (HAZ) (n=120)	*Magnitude (%)*
Severely stunted (<-3*SD*)	7.5
Moderately stunted (<-2*SD* to -3*SD*)	32.5
Normal (-2*SD* to +2*SD*)	60.0

Department of Science and Technology (PCHRD-DOST), Philippines.

RESULTS

Overall, the prevalence of stunting in the study area was 40.0%, with 7.5% children classified as severely stunted and 32.5% moderately stunted (Table 1). With reference to the WHO cut-off points (WHO, 1995), the severity of stunting in this study was very high at 40.0% magnitude. This prevalence was higher compared with the 2015 national prevalence of 33.4% for stunting, but consistent with Quezon Province at 40.0% based on the FNRI National Nutrition Survey in 2015 (DOST-FNRI, 2016).

Results of the parasitological assessment revealed that *Ascaris lumbricodes* infections were seen in 38.1% of the study children; *Trichuris trichiura* infections were seen in 15.3% of the study children; and for hookworm infections, a prevalence of 1.7% was observed.

Figure 1 shows the expired hydrogen of 120 children from baseline and by 30-, 60-, and 90-minutes after ingestion of glucose. By definition, a positive test to suggest the presence of EED is a rise of ≥20ppm from baseline in breath

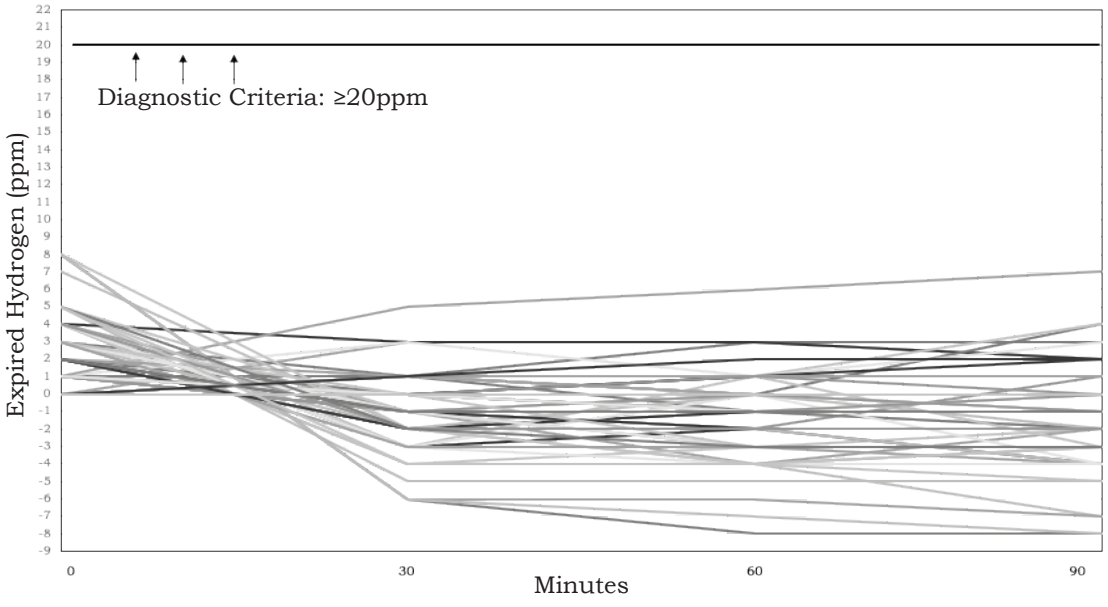

Figure 1. Expired hydrogen of 120 children aged 36-59 months at baseline, 30-, 60-, and 90-minutes after ingestion of glucose

Table 2. Measures of the associations between stunting and socio-demographic-economic variables, food intake, feeding and health practices, WASH variables, and STH in children 36-59 months in Quezon Province, Philippines

Variables	*Stunting*	
	Correlation coefficient	*p-value*
Socio-demographic-economic variables		
Household size	0.114[a]	0.22
Household income	0.118[a]	0.20
Nutrient intake		
Energy	-0.088[a]	0.34
Protein	-0.164[a]	0.07*
Calcium	-0.176[a]	0.05*
Phosphorus	-0.279[a]	<0.001***
Iron	-0.093[a]	0.31
Vitamin A	-0.198[a]	0.03**
Riboflavin	-0.273[a]	<0.001***
Niacin	-0.025[a]	0.77
Feeding and health practices		
Breastfeeding after birth	0.006[b]	0.95
End of exclusive breastfeeding	0.325[b]	<0.001***
Full immunisation	0.185[b]	0.04**
WASH variables		
Type of drinking water	0.185[b]	0.04**
Drinking water service level	0.292[b]	0.01**
Toilet classification	0.170[b]	0.06*
Toilet service level	0.181[b]	0.14
Hand washing service level	0.022[b]	0.81
Appropriate hand washing techniques	0.150[b]	0.10
Soil transmitted helminthiasis		
Ascaris presence	0.252[b]	0.01**
Trichuris presence	0.136[b]	0.14
Hookworm presence	0.027[b]	0.77

[a]correlation coefficient using Point Biserial
[b]correlation coefficient using Cramer's V
*significant at *p*-value<0.10
**significant at *p*-value<0.05
***significant at *p*-value<0.001

hydrogen by 90 minutes (Rezaie *et al.*, 2017). In this study, the mean expired hydrogen concentration at 90 minutes from baseline was 0.73ppm (Range: 0,8). Results showed that children demonstrated no increase in breath hydrogen greater than the diagnostic criteria of 20ppm. Hence, there was no case of EED detected in this study.

Correlation analysis is presented in Table 2. Results showed that stunting was significantly and negatively associated with nutrient intakes of protein (p=0.07), calcium (p=0.05), phosphorous (p<0.001), vitamin A (p=0.03), and riboflavin (p<0.001). Moreover, stunting was found to be significantly associated with the age of

Table 3. Summary of significantly associated factors with stunted or not stunted children aged 36-59 months in Quezon Province, Philippines.

Variables	*Stunting*	
	Not Stunted	*Stunted*
Nutrients† (Mean Percent EAR)		
Protein	220.6	197.0
Calcium	122.0	94.7
Phosphorus	150.1	119.4
Vitamin A	154.5	118.7
Riboflavin	228.2	163.5
End of exclusive breastfeeding‡ (%)		
< 6 months	65.1	34.9
≥ 6 months	46.9	53.1
Immunisation‡ (%)		
Fully immunised	62.7	37.3
Incomplete immunisation	30.0	70.0
Classification of drinking water‡ (%)		
Improved	68.2	31.8
Unimproved	50.0	50.0
Drinking water service level‡ (%)		
Basic	70.7	29.3
Unimproved	33.3	66.7
Toilet classification‡ (%)		
Improved	61.7	20.0
Unimproved	38.3	80.0
Soil transmitted helminthiasis‡ (%)		
Positive for Ascaris	44.4	55.6
Negative for Ascaris	69.9	30.1

†Comparison using *t*-test for independent samples
‡Comparison using chi-square test

child when exclusive breastfeeding was stopped ($p<0.001$), full immunisation ($p=0.04$), type of drinking water ($p=0.04$), drinking water service level ($p=0.01$), and toilet classification ($p=0.06$). With STH, there was a significant positive association between stunting and the presence of *Ascaris* ($p=0.01$).

The summary of significantly associated variables with stunting or no stunting is shown in Table 3. The mean estimated average requirement (EAR) for nutrients was higher in children who were not stunted compared to those who were stunted. The proportion of children who were not stunted were higher for those who were exclusively breastfed just under six months (65.1%) and fully immunised (62.7%). Higher proportion of children who were not stunted used improved drinking water (68.2%), basic water service level (70.7%), improved toilet facilities (61.7%), and practised appropriate hand washing techniques (67.9%). For those who used unimproved toilet facilities, more children were

stunted (80.0%) than those who were not (38.3%). The percentage of children found positive for *Ascaris* and stunted was higher (55.6%) than those who were not stunted (44.4%).

DISCUSSION

EED is becoming relevant in public health as it is a potential cause of child stunting. This cross-sectional study was conducted among 120 children aged 36-59 months old in Quezon Province, Philippines. It aimed to determine the prevalence of stunting, STH and EED; and their relationships. EED was assessed via glucose hydrogen breath test with a cut-off of ≥20ppm over baseline by 90 minutes suggestive of SIBO, a biomarker of EED.

Stunting remained a public health problem in the study area with a prevalence of 40%. A higher proportion of stunted children were using unimproved drinking water service level (66.7%) and unimproved toilet (80.0%). Similar patterns were observed in the study of Danaei *et al.* (2016) which highlighted that environmental factors, particularly unimproved sanitation, is attributable to 7.2 million cases of stunting worldwide. It also presented that environmental risk factors such as poor water quality, poor sanitary conditions, among others have the second largest impact on stunting globally. A cross-sectional study in Indonesia also highlighted that the chances of stunting was three times higher among children in households with untreated water and unimproved latrine (Torlesse *et al.*, 2016).

For soil transmitted helminthiasis, results showed that the prevalence of children having *Ascaris lumbricodes* was 38.1%, which is considered alarming in terms of morbidity control based on the WHO's recommended cut-off of <20% cumulative prevalence. In addition, *Trichuris trichiura* and hookworm infections were observed in 15.3% and 1.7% children, respectively. A cross-sectional study in Argentina among school age children showed a strong association between unimproved sanitation and unimproved drinking water with the presence of STH infection (Echasu, 2017). A meta-analysis of more than 40,000 studies (Strunz, 2015) further established the link between WASH factors and the presence of STH. It was estimated that an increase of 10,000 epg egg count of *A. Lumbricoides* will increase the odds of having stunting by 47% among children and adolescents (Jardim-Botelho *et al.*, 2008). Pathogen colonisation may promote chronic inflammation and induce changes to intestinal microbiota, contributing to stunting and EED (Humphrey, 2009; Owino *et al.*, 2016; Watanabe & Petri, 2016; Gilmartin & Petri, 2016; Harper *et al.*, 2018). Among the pathophysiological changes underlying EED, the study of Harper *et al.* in 2018 found strong evidence supporting the pathway between intestinal inflammation and stunting. Hence, further analysis of pathogen-induced changes to the intestinal microbiota as a pathway leading to stunting needs to be investigated (Owino *et al.*, 2016).

In this study, no case of EED was detected. Previous studies revealed that EED characterised by elevated expired hydrogen frequently occurs in developing areas with poor sanitation and hygiene (Korpe & Petri, 2012; Prendergast & Kelly, 2016), as well as limited public health resources, in association with microbial and parasitic contamination of food and water (Owino *et al.*, 2016). Pereira *et al.* (1991) investigated SIBO in a cohort of 340 children under the age of 5 years in rural Burma (Myanmar) and detected SIBO in 27.2% children. In the study of Dos Reis *et al.* (2007) among 5 to 11 years old in Brazil, bacterial

overgrowth was diagnosed in 18 (37.5%) of the 48 slum dwelling children and in only one (2.1%) of the 48 children in the control group. The homes of these slum dwelling children did not have running water or a sewerage system compared with the control group. Lin *et al.* (2013) supported these results in assessing the relationship of faecal environmental contamination and environmental enteropathy among children living in different levels of household environmental cleanliness across rural Bangladesh.

The absence of EED in this study was probably due to a better environmental condition of the study households compared with the above mentioned literatures. Specifically, majority of the households in the study areas had access to: (1) improved drinking water (55%); (2) improved sanitation (60.8%); and (3) availability of water (99.2%) and soap (70%) for hand washing. Only 11.7% of the households had earth floor material and only 39.2% raised animals.

Several limitations can be identified in the present study. The cross-sectional design of this study did not allow any inference with regards to causal relationships. Similar to the findings of Donowitz *et al.* (2016), non-detection of EED in this study might be due to the limitation of cross-sectional analysis which only detects children with SIBO at the time of testing and may have missed children who have had recent or prolonged SIBO in their lifetime. Furthermore, the study is only a representative of one Province and the results therefore cannot be generalised for the whole population of the Philippines. Regardless of these limitations, to our knowledge, this was the first study on EED among children in the country. In addition, helminth colonisation on EED has not been well studied, thus the study was an attempt to establish an association between STH and EED.

CONCLUSION

The study revealed that there was no prevalence of EED detected among 36- to 59-month-old children in Quezon Province. The prevalence of STH among children were 38.1% for *Ascaris lumbricodes,* 15.3% for *Trichuris trichiura,* and 1.7% for hookworm infections. Stunting prevalence was recorded at 40%. Nutrition interventions together with the use of anthelminthic drugs, provision of clean water, environmental sanitation, and promotion of hygiene practices must be intensified to address problems on stunting and STH. This study revealed trends that warrants further epidemiological studies. The Hydrogen Breath Test employed can be adapted for a case control study with stunted subjects in areas with poor environmental conditions.

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Authors' contributions

ARB, principal investigator, conceptualised and designed the study, conducted data collection and analysis, prepared the manuscript; CVCB, VBM and MMP reviewed and provided technical inputs on the study design, data analysis and manuscript; NAT, provided technical inputs on the study and sampling design, 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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Comparison of anthropometric profile, bone-related parameters, biochemical parameters, functional capacity, and vitamin D status – A study on postmenopausal Malaysian women

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ABSTRACT

Introduction: The optimal level of total 25(OH)D in Malaysian postmenopausal women that is necessary to ameliorate the combined effects of decreased bone mineral and muscle mass, and increase in adiposity, as seen in osteosarcopenic obesity, is unknown. Given that these are common pathologies existing in bone, muscle and excess fat-related disorders, this study investigated the association between body composition, functional capacity and total 25(OH)D levels. **Methods:** This was a cross-sectional study involving 117 postmenopausal Malaysian women aged 50-88 years. Total 25(OH)D was analysed by chemiluminescent microparticle immunoassay CMIA on Siemens® platforms. Bone mineral was measured by broadband ultrasound attenuation. Functional performance was assessed using modified components of short physical performance battery test. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Version 25.0). **Results:** Overall, 47% women were vitamin D deficient; 3% had none, 22% had one, 65% had two, and 10% had all three adverse body composition indicators (osteosarcopenic obesity). Individuals without any adverse body composition indicator had higher total 25(OH) D (74.7±7.0nmol/L, p=0.004) compared to all the other groups [1 (53.0±3.8nmol/L); 2 (48.7±1.9nmol/L) and 3 (58.3±5.4nmol/L)]. The single inflection point (ROC curve) for total 25(OH)D in predicting osteosarcopenic obesity was 58.9nmol/L. **Conclusion:** Higher vitamin D levels were associated with lower body fat, enhanced bone quality, and improved functional capacity. Maintaining vitamin D levels above >58.9nmol/L may protect Malaysian postmenopausal women against the combined pathologies of bone, muscle and excess fat.

Keywords: vitamin D, osteosarcopenic obesity, postmenopausal, Malaysian women

INTRODUCTION

Malaysia is expected to reach the status of an ageing nation by 2030, wherein 15% of the population would be aged 60 years and above (United Nations Library, 2017). Ageing is associated with a decrease in bone mineral (Riggs *et al.,* 1981) and muscle mass (Novak, 1972), and an increase in adiposity (Fukagawa, Bandini & Young, 2017). A decline

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in physical capacity associated with normal ageing can be attributed to the decline in muscle mass and a reduction in muscle strength (Larsson, Grimby & Karlsson, 2017). The loss of muscle mass with ageing, termed sarcopenia (Evans, 2011), along with its associated loss in muscle strength, increases the risk of fracture at various skeletal sites. The consequence is reduced mobility and a poor quality of life in advanced age.

Furthermore, a decline in bone mineral may be aggravated in individuals with obesity and those with obesity and sarcopenia combined, compared to individuals suffering from osteopenia/osteoporosis alone (Ormsbee *et al.,* 2014). A combination of adverse interrelationships in body composition is hypothesised to manifest in osteosarcopenic obesity (OSO), a term coined to depict the concurrent occurrence of these three phenotypes – osteopenia/osteoporosis, sarcopenia, and obesity (Ormsbee *et al.,* 2014).

The mechanical interactions between bone and muscle are well established, and a growing body of research suggests a common pathology in bone and muscle-related disorders (Gunton *et al.,* 2015). Increase in adiposity also exacerbates the loss of bone and muscle mass (Ormsbee *et al.,* 2014). OSO may lead to functional impairment and increases the risk of falls, fractures, and morbidity (Ilich *et al.,* 2014).

OSO is defined as T-score ≤-1.0, appendicular skeletal muscle mass index (ASMMI) ≤5.41kg/m^2 (20th percentile of ASMMI of the study population), and percent body fat ≥32% (Ilich, Kelly & Inglis, 2016). Interestingly, serum levels of 25-hydroxyvitamin D (25 (OH)D) have been shown to have a significant association with adiposity, bone and muscle-related disorders, which manifest as osteopenia/osteoporosis, sarcopenia, and obesity (Han *et al.,* 2014; Plawecki & Chapman-Novakofski, 2010). It is well documented that severe vitamin D deficiency is associated with proximal muscle weakness, which is thought to be associated with secondary hyperparathyroidism and induced hypophosphatemia (Larsson *et al.,* 2017). It is widely accepted that total serum 25(OH)D (D_2 plus D_3) concentration of <50nmol/L represents vitamin D deficiency (IOM, 2011, Bischoff-Ferrari *et al.*, 2006). There is a dearth of information on the relationship between vitamin D status of individuals with OSO.

The aim of this study was to investigate the association between OSO, body composition, functional capacity, and total 25(OH)D concentrations in postmenopausal Malaysian women. To the best of our knowledge, this is the first study that investigated the relationship between the indices of vitamin D status and OSO in a group of postmenopausal Malaysian women living in and around the town of Semenyih, Malaysia.

SUBJECTS AND METHODS

Study population

A total of 117 apparently healthy postmenopausal Malaysian women aged 50 – 88 years were recruited at random through advertisements and flyers that were distributed at schools, community centres, senior citizen clubs, residential areas, and religious centres around the town of Semenyih from April 2017 – March 2019. Postmenopausal was defined as having no menstrual period, bleeding, or spotting during the 12 months prior to enrolment. Interested participants completed a validated and structured health and lifestyle questionnaire, which included questions on ethnic background, past diseases, family history of past diseases, physical activity level, and substance abuse (Aguilar-Farias, 2016; The North American Menopause Society, 2005).

Consumption of calcium and vitamin D supplements, as well as daily servings of milk were recorded. Use of sunscreen was also recorded.

Apparently healthy and interested participants were screened for eligibility with the following inclusion criteria: (i) a woman, (ii) citizen of Malaysia (of Malay, Indian or Chinese ethnicity), (iii) postmenopausal (no menstrual period, bleeding, or spotting in the 12 consecutive months prior to enrolment). Exclusion criteria included: (i) inability to stand for height, weight, and gait speed assessments, (ii) presence of artificial limbs and/or metal implants, (iii) severe cardiac, pulmonary, or musculoskeletal disorders, (iv) severe cognitive impairment or any disability that makes communication impossible, (v) presence of terminal illness, (vi) on hormone replacement therapy, vii) on medications for anti-convulsant, cholesterolaemia, hypoglycaemia, and psychiatric disorders.

Ethics approval and consent to participate

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were reviewed and approved by the Science and Engineering Research Ethics Committee, University of Nottingham Malaysia (SEREC-NZA051016). Written informed consent was requested and obtained from all willing participants.

Measurement of anthropometric parameters

Height of individual was measured barefoot with a standard height rod (SECA 217, SECA GmbH & Co., Hamburg, Germany). Body composition including weight, fat mass (kg), skeletal muscle mass (kg), fat-free mass (kg), and body fat percent (%) of individuals were measured using a body composition analyser DSM-BIA (InBody 230, Seoul, Korea). Body mass index (BMI) was calculated by using the formula, weight in kilograms divided by the square of height in meters (kg/m^2). Waist circumference (WC) was measured at the midpoint between the lower margin of palpable rib and the top of iliac crest, while hip circumference was measured at the widest portion of the buttocks (WHO, 2008). Waist–hip ratio was calculated using the ratio of the circumference of the waist to the hip.

Appendicular skeletal muscle mass index (ASMMI)

ASMMI was computed from the sum of muscle mass of the four limbs, adjusted for height ($kg/height^2$) (InBody 230, Seoul, Korea). A threshold of 5.41kg/m^2 was used to define OSO (Ilich *et al.,* 2016).

Muscle strength

Muscle strength was determined by handgrip strength and was measured on both hands using a handgrip dynamometer (JAMAR Hydraulic Hand Dynamometer® Model PC-5030 J1, USA). Subjects were seated with shoulders adducted and neutrally rotated, elbow flexed at 90°, forearm in neutral, and wrist between 0 and 30° of dorsiflexion. Grip strength was measured twice at each hand, and the higher of the two values was used in the analysis (Fess & Moran, 1981).

Calcaneal broadband ultrasound measurements

Broadband ultrasound attenuation (BUA) measurement of the calcaneus was determined by contact ultrasound sonometry using SAHARA clinical bone sonometer (Hologic Inc, Waltham, MA, USA). All data were collected by the same operator. Participants were measured from April 2017 – March 2019 and as close as practically possible to the time

of blood sampling for markers of vitamin D status. The foot of the participant was placed in the foot well with ultrasound gel applied on either side of the heel. The foot was then strapped to the footrest. A button was switched on for the emission and receiver probes to close in on the heel. The ultrasound waves emitted from the emission probes through the bone was sensed by the receiver probe. Calcaneal ultrasound measurements were recorded by the data controller. The ultrasound sonometer was calibrated at the start of each session using a 'phantom'. Precision of the instrument was monitored on the heel of the same individual and the CV was calculated to be 4.1 %. Results were recorded as BUA (dB/MHz), speed of sound (SOS) (m/s), bone mineral density (BMD) (g/cm^2), T-Scores [the number of standard deviation (*SD*) units above or below the average of young adults], and Z-scores (the number of SD units above or below the average of age-matched individuals). Both T- and Z-scores were based on the Hong Kong Chinese Female reference trace of the manufacturer, the closest reference group to the study population.

Functional performance

Short physical performance battery (SPPB) test

Functional performance was assessed using modified components of SPPB test (Ilich *et al.,* 2016). The tests are described below:

One-leg stance

Participant was asked to stand on one leg while lifting the contralateral limb, for up to 30 seconds. This was performed on both the right and left legs. The test was stopped when the participant touched any surface or lowered the contralateral limb to the ground or, ultimately, at the end of 30 seconds. The mean value of the right and left legs was documented as the final score (Shin *et al.,* 2014).

Gait speed

Gait speed was measured by timing a 6-meter normal walk. The 6-meter course was marked by two cones or pieces of tape. Participants were allowed to use a cane or any other walking device they normally use when walking.

Sit-to-stand chair test

Participants were asked to sit in an armless chair, cross their arms over their chest, with back straight and feet flat on the floor. Participants were then asked to rise from the chair and sit down again. This cycle was repeated for 30 seconds. The number of consecutive, chair sit-to-stand cycles completed was recorded, with the last time the participant sat down in the chair being the final count.

Blood collection and biochemical analysis (total 25(OH)D, iPTH, calcium, albumin)

Fasting blood samples were collected from 8am to 10am by a trained phlebotomist, processed and stored at -80°C until analysis. Blood serum was collected using yellow-topped plasma tubes (SST) and plasma was collected using lavender-topped plasma tubes (EDTA as additive). The total 25(OH)D concentration was analysed by chemiluminescent microparticle immunoassay CMIA on Siemens® platforms, Agilent, USA. Serum albumin level was analysed by using BCG Dye bonding on ADVIA® 2400 Clinical Chemistry System, Siemens Healthcare GmbH, Germany. Serum intact parathyroid hormone (iPTH) level was analysed using 2-site sandwich microparticle immunoassay from Siemens® ADVIA® Centaur XP immunoassay system, Siemens Healthcare GmbH, Germany. Serum calcium level was analysed using Arsenazo III Method on ADVIA® 2400 Clinical Chemistry System Siemens Healthcare GmbH, Germany. Vitamin D deficiency was defined as

total 25(OH)D <50nmol/L (IOM 2011, Bischoff-Ferrari *et al.*, 2006).

Power and sample size calculation

Sample size was computed using the formula $n= [\sigma^2(z_{1-\beta} + z_{1-\alpha/2})^2] / (\mu_0 - \mu_1)^2$ (Rosner & Glynn, 2011), in which $z_{1-\alpha/2}$= 1.96 (5% of type 1 error); $z_{1-\beta}$ = 0.84 (a power of 80%); μ_0 = population mean; μ_1 = expected mean of study population; n = sample size of study population; σ = standard deviation (*SD*) of reference population.

To estimate the average total 25(OH) D levels in postmenopausal Malaysian women, with a mean value (SD) of 44.4(10.6) nmol/L (Rahman *et al.,* 2004), and a threshold of <50nmol/L for total 25(OH)D levels indicating vitamin D deficiency; 28 participants were required for this study to reach a significance level of 5% ($z_{1-\alpha/2}$ = 1.96) and power of 80% ($z_{1-\beta}$ = 0.84). Assuming an attrition rate of 20%, 34 participants were estimated to be enrolled in this study.

Data analysis

Data were expressed as mean ± standard error (SE) or frequency (percentage). The normality of distribution was checked for all study parameters. The number of adverse body composition indicators was categorised as 0 (normal; without low bone mass, low muscle mass, or high percent body fat), 1 (having any one of the components), 2 (having any two of the components), and 3 (OSO; having all three components).

Chi-square test was performed to assess the differences in categorical variables between the groups. One-way analysis of covariance (ANCOVA) was performed to assess the relationship effects between the number of adverse body composition indicators and anthropometric parameters, bone-related parameters, blood biomarkers, and functional capacity tests, after adjusting for covariates. Adjustments for covariates like age, height, BMI, ethnicity, physical activity level, daily servings of milk, use of sunscreen, consumption of calcium and vitamin D supplements, were applied where appropriate.

Receiver operating characteristic (ROC) curve was constructed to determine the total 25(OH)D level (single inflection point based on the point closest to 0,1 corner of the ROC plane) to predict OSO (Perkins & Schisterman, 2006). A statistical probability level of p<0.05 (two-sided) was considered significant. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS Version 25.0).

RESULTS

A total of 117 participants participated in this study. Regarding the number of adverse body composition indicators, 3% had none, 22% had one, 65% had two, and 10% had all three criteria (categorised as OSO). The prevalence of vitamin D deficiency (total 25(OH) D <50nmol/L) was 47% in our study population (Table 3).

Table 1 presents the relationship between the number of adverse body composition indicators and general characteristics. Physical activity level, milk intake, use of sunscreen, and consumption of calcium and vitamin D supplements are presented. Significant associations were found between the number of adverse body composition indicators with ethnic groups (p=0.022) and vitamin D supplementation (p=0.039). These variables were adjusted as covariates in the following analysis.

The relationship between the number of adverse body composition indicators and anthropometric parameters, bone-related parameters, blood biomarkers, and functional capacity tests are presented in Table 2. Adjustments for covariates like age, height, BMI, ethnicity, physical activity level, daily

Table 1. Comparison between the number of adverse body composition indicators and general characteristics

Variables	*Overall mean (n=117)*	*0 (n=4)*	*1 (n=26)*	*2 (n=76)*	*3 (n=11)*	*p-value*
Ethnicity, *n* (%)						
Chinese	47 (40.2)	1 (25.0)	6 (23.1)	36 (47.4)	4 (36.4)	0.022*
Malays	34 (29.0)	3 (75.0)	13 (50.0)	14 (18.4)	4 (36.4)	
Indians	36 (30.8)	0 (0.0)	7 (26.9)	26 (34.2)	3 (27.3)	
Physical activity level, *n* (%)						
Sedentary/inactive	57 (48.7)	1 (25.0)	11 (42.3)	39 (51.3)	6 (54.5)	0.543
At least 10 minutes/week of moderate activity	25 (21.4)	2 (50.0)	4 (15.4)	15 (19.7)	4 (36.4)	
20-60 minutes/week of moderate activity	16 (13.7)	0 (0.0)	5 (19.2)	10 (13.2)	1 (9.1)	
60-180 minutes/week of moderate activity	11 (9.4)	0 (0.0)	3 (11.5)	8 (10.5)	0 (0.0)	
> 180 minutes/week of moderate activity	8 (6.8)	1 (25.0)	3 (11.5)	4 (5.3)	0 (0.0)	
Daily serving of milk, *n* (%)						
None	86 (73.5)	3 (75.0)	18 (69.2)	56 (73.7)	9 (81.8)	0.302
1 serving/day	25 (21.4)	0 (0.0)	6 (23.2)	18 (23.7)	1 (9.1)	
2 servings/day	5 (4.3)	1 (25.0)	1 (3.8)	2 (2.6)	9 (9.1)	
3 servings/day	1 (0.9)	0 (0.0)	1 (3.8)	0 (0.0)	0 (0.0)	
Use of sunscreen, *n* (%)						
Yes	39 (3.3)	2 (50.0)	8 (30.8)	25 (32.9)	4 (36.4)	0.890
No	78 (66.7)	2 (50.0)	18 (69.2)	51 (67.1)	7 (63.6)	
Consumption of calcium supplementation, *n* (%)						
Yes	27 (23.1)	0 (0.0)	9 (34.6)	15 (19.7)	3 (27.3)	0.291
No	90 (76.9)	4 (100)	17 (65.4)	61 (80.3)	8 (72.7)	
Consumption of vitamin D supplementation, *n* (%)						
Yes	14 (12.0)	0 (0.0)	1 (3.8)	9 (11.8)	4 (36.4)	0.039*
No	103 (88.0)	4 (100)	25 (96.2)	67 (88.2)	7 (63.6)	

*$p<0.05$ was considered significant, based on chi-square test.
0, had no; 1, had one; 2, had two; 3, had all three adverse body composition indicators (low bone mass, low muscle mass, or high percent body fat).

servings of milk, use of sunscreen, and consumption of calcium and vitamin D supplements were applied where appropriate. Our results revealed that individuals with three adverse body composition indicators had significantly lower fat-free mass (p=0.007), muscle mass (p=0.004), and ASMMI (p<0.001) compared to those with zero, one and two adverse body composition indicators. They also had significantly lower body weight (p<0.001) and BMI (p<0.001) compared to those with two adverse body composition indicators; and significantly lower height (p=0.002) compared to those with no and one adverse body composition indicator. Individuals with one, two and three

Table 2. Comparison of anthropometric profile, bone-related parameters, biochemical parameters, and functional capacity between number of adverse body composition indicators.

Variables	*Overall mean (n=117)*	*0 (n=4)*	*1 (n=26)*	*2 (n=76)*	*3 (n=11)*	*p-value*
Anthropometric parameters, mean±*SD*						
Age (years)	60.7±0.7	59.0±3.2	60.4±1.4	60.5±0.8	63.0±2.8	0.703
Height (cm)	152.7±0.6	159.0±2.0[b]	154.9±1.1[b]	152.3±0.7	148.0±1.8[a]	0.002*
[1]Weight (kg)	63.5±1.2	52.5±0.9	61.6±2.9	66.5±1.3[a]	50.8±1.5[b]	<0.001*
[1]BMI (kg/m^2)	27.3±0.5	20.8±0.8	25.7±1.2	28.7±0.6[b]	23.2±0.5[a]	<0.001*
[1]WC (cm)	84.6±1.2	72.2±2.5	79.3±2.6[b]	87.8±1.4[a]	79.5±2.6	0.007*
[1]Body fat mass (kg)	26.7±0.9	13.8±1.4[b]	23.8±2.2	29.5±1.0[a]	19.6±1.0[b]	<0.001*
[1]Percent body fat (%)	40.9±0.7	26.2±2.6[b]	36.8±1.8[a]	43.5±0.8[a]	38.3±0.9[a]	<0.001*
[1]Fat-free mass (kg)	36.7±0.4	38.7±1.3[b]	37.9±1.0[b]	37.1±0.5[b]	31.3±0.7[a]	0.007*
[1]Muscle mass (kg)	19.6±0.4	20.9±0.7[b]	20.4±0.6[b]	19.1±0.3[b]	16.2±0.4[a]	0.004*
[1]Appendicular skeletal muscle mass index (kg/m^2)	6.1±0.1	6.1±0.2[b]	6.3±0.1[b]	6.3±0.1[b]	5.1±0.1[a]	<0.001*
Bone-related parameters, mean±*SD*						
[2]BMD (g/cm^2)	0.4±0.0	0.6±0.04[b]	0.6±0.03[b]	0.4±0.0[a]	0.4±0.0[a]	<0.001*
[2]BUA (dB/MHz)	69.3±1.7	91.1±5.9[b]	84.2±3.7[b]	64.2±1.6[a]	61.2±3.0[a]	<0.001*
[2]SOS (m/s)	1521.9±3.2	1564.3±10.4[b]	1551.0±7.2[b]	1511.3±3.0[a]	1510.6±7.1[a]	<0.001*
[2]T-score	-1.9±0.1	-0.4±0.4[b]	-0.8±0.3b	-2.3±0.1[a]	-2.4±0.2[a]	<0.001*
[2]Z-score	0.02±0.1	1.2±0.3[b]	0.9±0.3b	-0.3±0.1[a]	-0.4±0.2[a]	<0.001*
Biochemical parameters, mean±*SD*						
[2]Total 25(OH)D (nmol/L)	51.7±1.7	74.7±7.0[a]	53.0±3.8[b]	48.7±1.9[b]	58.3±5.4[b]	0.004*
[2]Calcium (mmol/L)	2.4±0.01	2.4±0.1	2.4±0.0	2.4±0.0	2.4±0.0	0.662
[2]Albumin (g/L)	44.6±0.3	45.8±2.0	46.0±0.5	44.0±0.7	45.2±0.7	0.436
[2]Intact parathyroid hormone (pmol/L)	5.9±0.3	5.1±0.8	4.9±0.5	6.4±0.3	4.4±0.5	0.111
Functional capacity tests, mean±*SD*						
[2]Right hand grip strength (kg)	19.5±0.5	22.8±2.5	20.4±1.1	19.2±0.7	18.0±1.5	0.946
[2]Left hand grip strength (kg)	18.0±0.5	22.3±2.9	18.6±1.1	17.6±0.6	17.7±1.6	0.460
[2]Sit to stand test in 30 sec (times)	11.8±0.4	11.3±1.3	13.4±0.7	11.4±0.5	11.0±0.5	0.195
[2]Gait speed (m/sec)	0.9±0.0	1.0±0.1	1.0±0.1	0.9±0.1	0.9±0.1	0.666
[2]One-leg stance (s)	20.7±1.1	26.4±3.6	20.7±2.3	20.8±1.4	17.3±1.1	0.405

One-way ANCOVA with Bonferroni test was performed to determine the differences in the mean values of anthropometric parameters, bone-related parameters, blood biomarkers, and functional capacity tests between groups, after adjusting for covariates in different models [1]age, height, ethnicity and physical activity level; [2]model [1]+BMI, daily serving of milk, use of sunscreen, and consumption of calcium and vitamin D supplements

0, had no; 1, had one; 2, had two; 3, had all three adverse body composition indicators (low bone mass, low muscle mass, or high percent body fat)

BMI= body mass index; WC= waist circumference; BMD= bone mineral density; BUA= broadband ultrasound attenuation; SOS=speed of sound

*p<0.05 was considered significant.

[a,b] Figures not sharing a common letter are significantly different at p<0.05

Table 3: Differences in mean values (±SE) of anthropometric parameters, bone-related parameters, blood biomarkers, and functional capacity tests between vitamin D deficient and non-deficient groups

Variables	*Optimal cut off*	*Vitamin D deficient n=55*	*Vitamin D non-deficient n=62*	*p-value*
Anthropometric parameters				
Age (years)		60.9±1.0	60.5±0.9	0.820
Height (cm)		151.3±0.9	154.0±0.7	0.019*
[1]Weight (kg)		65.6±1.9	61.6±1.4	0.077
[1]BMI (kg/m^2)		28.7±0.8	26.0±0.6	0.086
[1]WC (cm)		85.2±1.8	84.1±1.6	0.921
[1]Body fat mass (kg)		29.2±1.4	24.7±1.1	0.047*
[1]Percent body fat (%)		43.2±1.0	38.8±1.0	0.052
[1]Fat-free mass (kg)		36.5±0.7	37.0±0.6	0.424
[1]Muscle mass (kg)		19.5±0.4	19.8±0.3	0.470
[1]Appendicular skeletal muscle mass index (kg/m^2)		6.2±0.1	6.1±0.1	0.397
Bone-related parameters				
[2]BMD (g/cm^2)		0.4±0.02	0.5±0.02	0.900
[2]BUA (dB/MHz)		67.4±2.7	70.6±2.1	0.813
[2]SOS (m/s)		1517.0±5.3	1525.5±3.9	0.949
[2]T-score		-2.1±0.2	-1.9±0.2	0.782
[2]Z-score		-0.1±0.2	0.1±0.1	0.798
Biochemical parameters				
[2]Total 25(OH)D(nmol/L)	50[a]	36.8±1.2[†]	64.9±1.7[†]	<0.001*
[2]Calcium (mmol/L)		2.4±0.02	2.4±0.01	0.636
[2]Albumin(g/L)		44.7±0.3	44.6±0.4	0.449
[2]Intact Parathyroid hormone (pmol/L)	4.2[b]	7.2±0.4[†]	3.8±0.3	<0.001*
Functional capacity tests				
[2]Right hand grip strength (kg)	18[c]	18.5±0.8	20.4±0.7[†]	0.077
[2]Left hand grip strength (kg)	18[c]	16.9±0.7	19.0±0.6	0.037*
[2]Sit to stand test in 30 sec (times)		11.4±0.5	12.1±0.5	0.665
[2]Gait speed (m/sec)	0.8[c]	0.9±0.04	1.0±0.04[†]	0.116
[2]One-leg stance (s)		19.2±1.8	21.7±1.3	0.492

Vitamin D deficiency was defined as total 25OHD Vitamin D <50nmol/L (Bischoff-Ferrari *et al.,* 2006).

One-way ANCOVA was performed to determine the differences in the mean values of anthropometric parameters, bone-related parameters, blood biomarkers, and functional capacity tests between different vitamin D deficient and non-deficient groups, after adjusting for covariates in different models [1]age, height, ethnicity and physical activity level; [2]model 1+BMI, fat mass, muscle mass, percent body fat, fat-free mass, daily serving of milk, use of sunscreen, and consumption of calcium and vitamin D supplements

BMI=body mass index; WC= waist circumference; BMD= bone mineral density; BUA= broadband ultrasound attenuation; SOS=speed of sound

[a] Bischoff-Ferrari *et al.* (2006)

[b] Holick *et al.* (2012)

[c] AWGS (Chen *et al.,* 2014)

*p<0.05 was considered significant

[†]p<0.05 was considered significantly different compared to the respective cut-off values, based on one-sample *t*-test

adverse body composition indicators had significantly higher percent body fat compared to those with no adverse body composition indicator.

With respect to bone-related parameters, individuals with two and three adverse body composition indicators had significantly lower BMD (p<0.001), BUA (p<0.001), SOS (p<0.001), T-score (p<0.001), and Z-score (p<0.001) compared to individuals with no and one adverse body composition indicator. Moreover, individuals without any adverse body composition indicator had significantly higher total 25(OH)D (74.7±7.0nmol/L, p=0.004) compared to all the other groups of one (50.0±3.8nmol/L), two (48.7±1.9nmol/L), and all three (58.3±5.4nmol/L) adverse body composition indicators.

Participants were categorised into vitamin D deficient and non-deficient groups (vitamin D deficiency defined as total 25(OH)D <50nmol/L) (Bischoff-Ferrari *et al.*, 2006). The differences in the mean values of anthropometric parameters, bone-related parameters, blood biomarkers, and functional capacity tests between the vitamin D deficient and non-deficient groups are reported in Table 3. Forty-seven percent (47%, n=55) of the participants were vitamin D deficient. The analysis revealed that the vitamin D deficient group had significantly lower height (p=0.019), left hand grip strength (p=0.037), and total 25(OH)D (p<0.001), but significantly higher fat mass (p=0.047) compared to the non-deficient group, after adjusting for covariates. Moreover, non-vitamin D deficient group had significantly higher right hand grip strength and gait speed compared to the respective cut-off values for diagnosing sarcopenia (Chen *et al.*, 2014) (<18kg and <0.8m/s, respectively) (p<0.05). However, such differences were not found in the vitamin D deficient group (p>0.05).

Figure 1 illustrates that the area under the curve (AUC) was 0.683 (95% CI = 0.500–0.867, p=0.04). The single inflection point (optimal level of total 25 (OH)D in predicting OSO based on the point closest to the (0,1) corner of the ROC plane) with a maximum sensitivity of 0.727 and specificity of 0.723 was 58.9nmol/L.

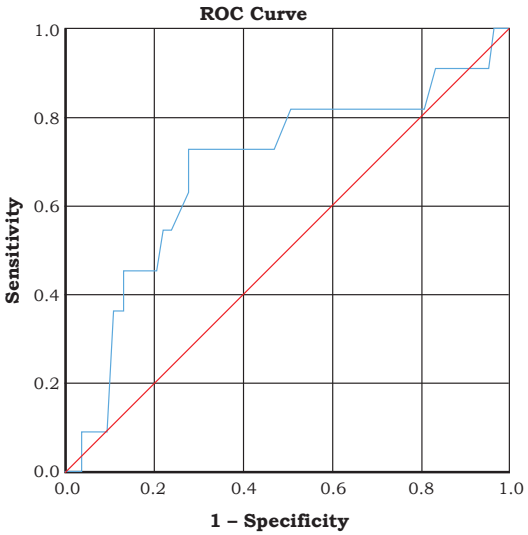

Figure 1. ROC curve for identifying optimal total 25(OH)D cut-off point in predicting osteosarcopenia obesity (OSO)
'Osteosarcopenia obesity' was defined as T-score ≤-1.0, Appendicular skeletal muscle mass index ≤5.41kg/m^2 (20th percentile of ASMMI of the study population), body fat percentage ≥32% (Ilich *et al.*, 2016). Number of participants with OSO = 11 (10%).

DISCUSSION

Vitamin D and bone

The results from the present study revealed a high prevalence of vitamin D deficiency and secondary hyperparathyroidism in the study population. With 50nmols/L as the threshold value, 47% of the current

population was found to be vitamin D deficient. The mean iPTH levels of these individuals was significantly higher than the non-deficient group, which was also significantly higher than the threshold for defining secondary hyperparathyroidism based on intact PTH >40 pg/ml (4.2 pmol/L) (Holick *et al.,* 2012)) (Table 3). Declining levels of 25(OH)D are associated with a rise in iPTH levels, which may have adverse effects on the skeleton. However, the level of 25(OH)D at which this occurs may differ in population groups. The overall mean value of BUA of the study participants was 69.3±1.7 dB/MHz (Table 2). This BUA value is comparable and in fact, even higher than the average BUA of age-matched and ethnically similar healthy reference females at 62.34 dB/MHz (Lim, Chan & Lam, 2005). Interestingly, BUA of participants identified as vitamin D deficient was close to the reference value at 67.4±2.7 dB/MHz (Lim *et al.,* 2005). Lowe *et al.* (2010) reported that the South Asian postmenopausal women in their study had significantly higher serum iPTH and lower 25(OH)D concentrations than their Caucasian counterparts, however, this was not associated with significantly higher markers of bone resorption or reduced bone quality (Lowe *et al.,* 2010).

African Americans, who have lower levels of vitamin D compared to other American groups, have adaptive responses to low 25(OH)D, that may reduce the harmful effects of low 25(OH) D on bone (Harris, 2006). There is evidence that blacks may have intestinal resistance to the actions of 1,25(OH) D (Dawson-Hughes *et al.,* 1995). This is relevant for bone health as 1,25(OH) D may have favourable effects on bone formation that are independent of its effect on increasing calcium absorption. Another adaptive response may be skeletal resistance to iPTH stimulated bone resorption (Cosman *et al.,* 1997). Therefore, in terms of bone health, the level of vitamin D, which poses a threat to bone health, may vary in different ethnic groups. The current study participants were overweight, with an average BMI of 27.3±0.5 kg/m^2 and percent body fat 40.9±0.7. It is suggested that adverse skeletal effects of low levels of 25(OH)D may have been counteracted by positive skeletal effects of increased oestrogen synthesis from adipocyte aromatase or adipocyte leptin secretion (Walsh *et al.,* 2016).

Functional capacity of study participants

It is to be noted that left hand grip strength was significantly higher in the non-vitamin D deficient group (19.0±0.6 vs. 16.9±0.7, p=0.037). Although not statistically significant, all other functional capacity scores were also higher in the non-vitamin D deficient group compared to the deficient group. A greater muscle gain was found in patients with a higher baseline of serum 25-(OH) vitamin D in the PROVIDE study (Verlaan *et al.,* 2018). The authors suggested that the mechanism of muscle gain via 25(OH) D may be explained by synergistic action of vitamin D with leucine and insulin to stimulate muscle protein synthesis. Furthermore, a higher dose of vitamin D or longer supplementation to achieve serum 25(OH)D concentrations of 20 to 40 micrograms/L (50-100nmol/L) has been suggested to increase muscle mass (Verlaan *et al.,* 2018). The average serum 25(OH)D concentrations of the vitamin D deficient group in the current study was 36.8±1.2 nmol/L. The current study has arrived at the threshold level of 58.9nmol/L, below which the study participants are at risk of suffering from OSO. Data presented in Table 3 clearly indicated that the vitamin D deficient group, with average 25(OH)D levels of 36.8±1.2nmol/L (significantly lower than 58.9nmol/L) were compromised

with respect to their functional capacity performance. This study therefore highlights that timely intervention to upgrade vitamin D status in the study population is necessary. With age, loss of lower limb power and limb strength may inevitably increase the risk of falls, injury and disability (Nevitt *et al.,* 1989).

Muscle and fat

The results from the current study showed that 10% of the study participants were with OSO. This is of concern as this condition is generally without pain yet progressively degenerative. This calls for strategies to be in place for screening and timely intervention. Authors of earlier studies have proposed possible mechanisms that lead to progressive losses of bone and muscle mass with increase in adipose tissue. As reported earlier in the text, increase in adiposity is correlated with loss of skeletal muscle function and size (sarcopenia). The consequence of sarcopenia is the loss of skeletal contractile power, particularly in the lower limb, which is essential for rising from a chair or climbing up stairs. In the current population, non-vitamin D deficient group had significantly higher right handgrip strength and gait speed compared to the respective cut-off values for diagnosing sarcopenia (<18kg and <0.8m/s, respectively) (Chen *et al.,* 2014). Loss of lower limb power and strength is the greatest risk factor for falls, injury and disability (Moreland *et al.,* 2004; Nevitt *et al.,* 1989). About 8% of muscle mass is lost per decade between the ages of 40 and 70, and this progresses to about 15% per decade after 70 years of age. Moreover, with age, there is an increase in lipid content and direct deposition of lipids within the muscle fibres (Visser *et al.,* 2005).

This study has identified that at least in the current population, a minimum level of 58.9nmol/L of 25(OH) D is necessary to prevent the risk for the occurrence of osteosarcopenia in the participants. The Malaysian population will reach the status of an 'ageing' nation by 2030. Prevention of debilitating disorders of motor functions, such as OSO, is no doubt the best way to counter this social and economic burden. Simple diagnostics, as reported in the current study in asymptomatic women could reduce the risk of adverse health outcomes at a later age and pave the way for better mobility among free-living individuals of any community.

Strengths

This was the first study that investigated OSO in a group of postmenopausal Malaysian women. This study reports muscle mass, as well as muscle performance via grip strength measurements and SPPB test. Furthermore, this study has arrived at a threshold level of 58.9nmol/L for 25(OH)D, below which participants will be considered at risk of OSO. This study also classified participants on the phenotypic debilities of bone, muscle and physical performance based on established and published thresholds.

Limitations

The sample size of the study was comparatively small. This may have limited the outcome of the ROC curve analysis. Bone health was assessed by ultrasound and body composition by BIA. Although dual-energy x-ray absorptiometry (DXA) is considered the "gold standard" test for BMD determination, it is not widely available in primary care, thus using it as a screening tool at primary settings is not feasible due to constraints of resources. There is an increasing body of evidence demonstrating that low ultrasound readings at the calcaneus are associated with increased fracture risk and correlate well with DXA in postmenopausal women (Cook *et al.,*

2005). Portable ultrasound devices, such as the one used in this study, are relatively inexpensive and easy to operate within the primary care setting and can be used to screen patients with low bone density for further investigation (Evans *et al.,* 2005; Lee *et al.,* 2006). There are reports that sunscreen use may increase the risk of vitamin D deficiency. Therefore, we collected information on the use of sunscreen in our participants to assess, whether there were any significant differences between the groups with adverse body composition indicators (Table 1). No significant difference was found on the latter criterion. Furthermore, for the same reason, 'use of sunscreen' was included as a covariate and adjusted for in data analysis. Another limitation of this study is that no information was collected on sunshine exposure and skin type.

CONCLUSION

The study participants had an average BUA higher than the average BUA of age-matched and ethnically similar healthy reference females. Increased serum vitamin D levels were associated with lower body fat, enhanced bone quality, and improved functional capacity of the postmenopausal women in this study. Approximately half of the participants were vitamin D deficient. The average serum 25(OH)D concentration of the vitamin D deficient group in the current study was 36.8±1.2 nmol/L, which is considerably lower than the threshold of 50 nmol/L and the threshold of 58.9 nmol/L for predicting OSO. Therefore, maintaining serum vitamin D levels above >58.9 nmol/L (at least in postmenopausal Malaysian women) may protect them against the combined abnormalities of bone, muscle and body composition in mid- and later life. This study identified 10% of the study participants with OSO. Screening for OSO with simple diagnostics (as reported in the current study) in asymptomatic obese women could reduce the risk of adverse health outcomes at a later age and pave the way for better mobility among ageing individuals. Population-specific thresholds for total 25(OH)D should be arrived at in order to screen for specific diseases where vitamin D deficiency is incriminated.

List of abbreviations

ANCOVA, analysis of covariance; ASMMI, appendicular skeletal muscle mass index; BIA, bioelectrical impedance analysis; BMD, bone mineral density; BMI, body mass index, BUA, broadband ultrasound attenuation; DEXA, dual-energy x-ray absorptiometry; VDBP, vitamin D binding protein; 25(OH)vitamin D, 25-hydroxyvitamin D; OSO, osteosarcopenic obesity; iPTH, intact parathyroid hormone; ROC, receiver operating characteristic curve; SE, standard error of mean; SOS, speed of sound; SPPB, short physical performance battery; WC, waist circumference; WHR, waist-hip-ratio.

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Authors' contributions

MSR, designed the study, was responsible for recruitment of the participants, analysed the data and wrote the paper; ANZ, designed the study, was responsible for recruitment of the participants, collected and analysed the data.

Conflict of interest

The authors do not have any conflict of interest to report. The lead author affirms that this manuscript is an honest, accurate, and transparent account of the study being reported. The reporting of this work is in compliance with the STROBE guidelines.

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Association between dietary intake, physical activity and stress level with constipation among undergraduate students

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Abstract

Introduction: Constipation is one of the most common health problems among university students. This study aims to determine the association between socio-demographic characteristics, dietary intake, physical activity, and stress level with constipation among undergraduate students. **Methods:** The study was a cross-sectional study and the participants comprised of 140 undergraduate students (27.9% males and 72.1% females). A 3-day dietary record was obtained to determine energy, fibre and fluid intakes, while physical activity was determined using the International Physical Activity Questionnaire. Stress level and constipation were assessed using the Cohen's Perceived Stress Scale and Agachan's Constipation Score System Questionnaire, respectively. Data were analysed using IBM SPSS Statistics version 23. Multivariate linear regression analysis was done to determine factors that were associated with constipation. **Results:** Mean age of the participants was 20.9±1.5 years old and majority were third year students (32.1%). Mean intakes of energy, dietary fibre, and fluids per day were 1567±438kcal, 5.6±3.5g, and 2301±946ml, respectively. The median score for physical activity was 1135.5 MET-minutes/week. Most of the participants (77.1%) had a high or a very high stress level and 64.3% had slight constipation. Age, dietary fibre, fluid, energy, and perceived stress scale explained a significant amount of the variance in the occurrence of constipation [$F(6.133)=16.373$, $p<0.001$, $R^2=0.425$, $R^2_{Adjusted}=0.399$]. **Conclusion:** Age, energy, fluid and fibre intakes, as well as perceived stress were factors that were associated with constipation among undergraduate students. Therefore, undergraduate students should be encouraged to practise a healthy lifestyle to modify these identified risk factors.

Keywords: constipation, energy intake, dietary fibre, fluid intake

INTRODUCTION

Constipation is one of the most common gastrointestinal (GI) disorders encountered in clinical practice and is defined as having three or fewer times of bowel movements per week. Patients with constipation may experience prolonged, incomplete, difficult and/or painful defecation, which can greatly affect their quality of life. Furthermore, this straining during bowel movement may also lead to the development of haemorrhoids, which can cause further pain, itchiness or even bleeding

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during bowel movement (American Gastroenterological Association, 2013). In Asia, about 10% to 98.4% of adults have experienced symptoms of constipation. The core symptoms of constipation can be further divided into 'straining' that are experienced by 82.8% of adults, 'lumpy and hard stool' (74.2%), and 'incomplete evacuation' in another 68.1% of adults (Patimah *et al.*, 2017).

Utilising the Rome II criteria, Karakaya *et al.* (2015) reported that the prevalence of constipation among university students was 20.6%. Meanwhile, Lim *et al.* (2016) documented that the prevalence of functional constipation among undergraduate students was 16.2% based on the Rome III criteria and it was recognised as one of the most common health problems among undergraduate students. Less intakes of energy, dietary fibre and water, and physical inactivity, as well as having stress were found as risk factors for constipation in another study (Leung *et al.,* 2011). Consumption of lower calories for long term may also decrease the stimulation of colonic motility as limited amount of food passes from the stomach into the upper part of the small intestine (Malone & Thavamani, 2020).

Furthermore, undergraduate students are known for practising undesirable health-related behavioural habits such as increased consumption of unhealthy foods since they are living away from home. When they stay away from their family and indulge in eating out, they often feel relieved when all their food desires can be easily satisfied. These habits are mostly attributed to a higher consumption of high caloric snacks and fast foods, as well as a lower intake of fruits and vegetables (Sogari *et al.*, 2018). Studies found that only 11% of the undergraduate students fulfilled the recommendation of taking 20 - 30 g of fibre per day (Koo *et al.*, 2019; NCCFN, 2017). The latest findings from the National Health and Morbidity Survey (NHMS) in 2019 showed that 95% of Malaysian adults do not consume enough fruits, vegetables and plain water (IPH, 2020). Inadequacy of dietary fibre and plain water intakes can worsen the symptoms of constipation. Besides insufficient dietary fibre intake, it was also found that inadequate intake of fluid is strongly associated with aggravated constipation symptoms (Mazlyn *et al.,* 2013).

A study found that majority of youths have a moderate level of active lifestyle (Salamudin & Harun, 2013). NHMS 2019 also reported that one in four adults are not physically active (IPH, 2020). Youths aged 21 years old and below are more active than other age categories. However, above this age category, youths have a reduced physical activity level. This may be due to time constraint which makes them spend less time on exercising and more time revising their studies (Salamudin & Harun, 2013). It was identified that undergraduate students pursuing the medical programme had a lower physical activity level (26%) as compared to other academic programmes. It is very important to be more physically active as it will reduce the risk of chronic constipation (Chang *et al.,* 2015).

A study conducted by Gan *et al.* (2011) found that 21.6% of university students experience moderate to high levels of stress. Stress can lead to serious health issues in many ways. It was found that unhealthy dietary pattern often occurs in female students who are stressed. They tend to consume more energy dense foods such as high sugary food because they believe it will reduce their stress levels (Mikolajczyk, Ansari & Maxwell, 2009). The brain and gut work very closely together, thus any disturbances in mental capability due to stress will invariably disrupt the gastrointestinal tract function, which

may then lead to constipation (Lingu *et al.,* 2012).

There are several local studies looking at the factors associated with constipation (Patimah *et al.*, 2017). However, there are limited number of studies that focus on constipation among university students. Therefore, the purpose of this study was to determine the association between socio-demographic characteristics, dietary intake, physical activity, and stress level with constipation among undergraduate students. The study offered an opportunity to those with constipation to rectify this chronic health problem that may have arisen as they grow older. Through this study, more knowledge on the factors that can contribute to constipation can be acquired in hope that constipation can be prevented at an early stage.

MATERIALS AND METHODS

A cross-sectional study was conducted among undergraduate students to determine the association between socio-demographic characteristics, dietary intake, physical activity, and stress level with constipation. A total of 140 participants were recruited by using the cluster sampling method. There are sixteen faculties in UPM and two out of sixteen faculties were randomly selected. The two participating faculties were the Faculty of Medicine and Health Sciences and the Faculty of Computer Science and Information Technology. In each participating faculty, two academic programmes were further randomly selected. All of the undergraduate students in the selected academic programmes were invited to participate in this study. Lastly, the participants were recruited by using inclusion and exclusion criteria. The inclusion criteria for this study were undergraduate students of both genders and all ethnic groups. International students, post-graduate students, those with physical disabilities, and were pregnant during the time of enrolment were excluded. The total participants from the Faculty of Medicine and Health Sciences were 92 participants, meanwhile from the Faculty of Computer Science and Information Technology were 48 participants. Data collection was conducted from December 2016 to February 2017. The sample size of the study was computed using Epi Info version 7 by considering the following assumptions: the prevalence of constipation among university students was 20.6% (Karakaya *et al.*, 2015), a 95% level of significance, and a power of 80%. Finally, the sample size of 127 was obtained with an addition of a 10% non-response rate.

Socio-demographic characteristics of the participants such as age, sex, ethnicity, year of study, and monthly food expenditure (RM) were captured. Dietary intake was assessed by using a three-day dietary record (two weekdays and one weekend). The three-day dietary record was prepared in the form of a booklet and was given during the interview session. During the interview session, the researcher guided the participants on how to estimate the portion size and quantity of foods consumed (Suzana *et al.*, 2009). After three days, the researcher contacted the participants to collect and verify the records. Participants were told to record all foods and beverages intake including cooking methods and brand names of processed foods. The Nutritionist Pro software and information from the Malaysian Food Composition Table (Tee *et al.,* 1997) and the Food Composition Guide Singapore (Health Promotion Board Singapore, 2003) were used in the analysis. Data on total energy, fibre and fluid intakes were retrieved from the analysis.

Short form version of the International Physical Activity Questionnaire (IPAQ) was used to assess the participants' physical activity level for the past seven days. IPAQ short form version comprised of seven questions, which included physical activities such as walking, moderate-intensity and vigorous-intensity physical activities. The physical activity levels were categorised into three levels, which were low, moderate and high. The formula used was: MET level × minutes of activity × frequency per week (International Physical Activity Questionnaire Group, 2002).

Stress level was assessed by using the Cohen's Perceived Stress Scale (PSS), which consisted of ten items. The participants were asked about their feelings and thoughts during the past one month and they were asked to answer these questions quickly without thinking about the actual number of times they have experienced it. A five-point Likert scale was used to rate the responses, ranging from never (0), almost never (1), sometimes (2), fairly often (3) to very often (4). Positively worded items at numbers 4, 5, 7 and 8 were scored inversely (0=4, 1=3, 2=2, 3=1, 4=0). Higher scores were associated with higher levels of perceived stress. The perceived stress level was grouped into "very low" (0-7 marks), "low" (8-11marks), "average" (12-15 marks), "high" (16-20 marks), and "very high" (≥21 marks).

The Agachan's Constipation Score System Questionnaire contains eight items (frequency of bowel movements; difficulty in evacuation; completeness of evacuation; abdominal pain; time per attempt; type of assistance; number of unsuccessful attempts for evacuation per 24 hours; and duration of constipation). A five-point Likert scale was used to rate the seven items in a range of 0 (none of the time) to 4 (all of the time), and one item in the range of 0 to 2. The total score ranged from 0 to 30, which was then grouped into "none" (<5 marks), "slight" (5-9 marks), "moderate" (10-14 marks), and "severe" (≥15 marks). The severity of constipation increases with a higher total score (Ng *et al.*, 2016).

Data collected were analysed by using the IBM SPSS Statistics version 23 with the significance level set at $p<0.05$. Univariate analysis was used to analyse all descriptive data. The results for categorical variables were presented as frequencies and percentages, while the results for continuous variables were presented as mean and standard deviation. Multivariate linear regression analysis using stepwise method was done to identify the factors associated with constipation.

Ethics statement

Prior to the commencement of the study, ethical approval was obtained from the Ethics Committee for Research Involving Human Subjects, Universiti Putra Malaysia [reference number: UPM/TNCPI/RMC/1.4.18.2 (JKEUPM)]. Permission for data collection was also obtained from the Faculty of Computer Science and Information Technology, UPM. Prior to data collection, participant information sheets were distributed to the participants and informed consent was obtained.

RESULTS

Participant's socio-demographic characteristics are presented in Table 1. Mean age of the participants was 20.9±1.5 years old and majority were females (72.1%). Majority (63.6%) of them were Malays and third year students (32.1%). More than half of the participants (70.0%) spent about RM100 to RM300 on food, while the remaining participants (30.0%) were living with a higher monthly food expenditure of between RM301 to RM600. The mean intakes of energy, dietary fibre, and fluids

per day were 1567±438 kcal, 5.6±3.5 g, and 2301±946 ml, respectively. The median score and interquartile range for physical activity among the participants were 1135.5 and 1796 MET-minutes/ week, respectively. Majority of the participants (60.7%) were considered as having a moderate physical activity level. It was found that most of the participants (77.1%) had a high or a very high stress level and 64.3% had slight constipation.

Table 2 shows the mean energy, fibre and fluid intakes of the participants.

Table 1. Socio-demographic characteristics, dietary intake, physical activity, stress level, and constipation of participants

Characteristics	*n (%)*	*Mean±SD*	*Range*
Socio-demographic characteristics			
Age (years)		20.9±1.5	
Sex			
Male	39 (27.9)		
Female	101 (72.1)		
Ethnicity			
Malay	89 (63.6)		
Chinese	41 (29.3)		
Indian	10 (7.1)		
Year of study			
First year	40 (28.6)		
Second year	38 (27.1)		
Third year	45 (32.1)		
Fourth year	17 (12.1)		
Monthly food expenditure		287.8±108.6	100.00 – 600.00
RM 100 – 300 ($ 24.57 – 73.62)	98 (70.0)	70.7±26.7	
RM 301 – 600 ($ 73.62 – 147.43)	42 (30.0)		
Dietary intake			
Energy (kcal/day)		1567±438	
Dietary fibre intake (g/day)		5.6±3.5	
Fluid intake (ml/day)		2301±946	
Physical activity			
Total score (MET-minutes/week)		1135.5†	
Low (<600)	32 (22.9)		
Moderate (600-2999)	85 (60.7)		
High (≥3000)	23 (16.4)		
Stress level		19.9±5.4	
Lowest (0-7)	0 (0)		
Low (0-11)	7 (5.0)		
Average (12-15)	25 (17.9)		
High (16-20)	49 (35.0)		
Very high (≥21)	59 (42.1)		
Constipation			
None (<5)	24 (17.1)		
Slight (5-9)	90 (64.3)		
Moderate (10-14)	24 (17.1)		
Severe (≥15)	2 (1.4)		

†median score

Table 2. Dietary intake among participants

Dietary Intake	*Recommendation*†	*n (%)*	*Mean±SD*
Total energy intake (kcal/day)			1567±438
Male	2240 kcal/day		1890±402
<2240		35 (89.7)	
≥2240		4 (10.3)	
Female	1840 kcal/day		1443±386
<1840		92 (91.1)	
≥1840		9 (8.9)	
Dietary fibre intake (g/day)	20-30 g/day		5.6±3.5
<20		100 (100)	
20-30		0 (0)	
>30		0 (0)	
Total fluid intake (ml/day)	2000 ml/day		2301±946
<2000		60 (42.9)	
≥2000		80 (57.1)	

†Recommendation is based on RNI for Malaysians (NCCFN, 2017) for energy (at PAL 1.6) and fluid intakes; MDG (NCCFN, 2010) for dietary fibre intake.

Only 10.3% of males and 8.9% of females achieved the recommended nutrient intake (RNI) for energy intake. None of the participants met the minimum amount of dietary fibre intake, which is 20 g per day. Table 3 shows the association between age, dietary intake, and perceived stress with constipation among the participants. Factors associated with constipation were age (p=0.007), dietary fibre (p=0.044), fluid (p=0.050), energy (p=0.040), and perceived stress (p<0.001). All these factors explained a significant amount of the variance in the occurrence of constipation [F(6.133)=16.373, p<0.001, R^2=0.425, $R^2_{Adjusted}$ = 0.399].

DISCUSSION

The present study found that more than half of the participants had slight constipation, followed by a small percentage of participants with no or moderate constipation, as well as severe constipation. This study demonstrated that socio-demographic characteristics, dietary intake, physical activity, and perceived stress were associated with constipation among the participants.

Table 3. Factors related to constipation among university students

Model	*Unstandardised Coefficients*		*Standardised Coefficients*	*t*	*p*	*95% CI*
	Beta	*Std. Error*	*Beta*			
Age	0.354	0.129	0.191	2.744	0.007	(0.990, 0.196)
Dietary fibre (mg)	-0.114	0.056	-0.145	-2.035	0.044	(-0.225, -0.003)
Fluid (ml)	0.000	0.000	-0.142	-1.976	0.050	(-0.001, 0.000)
Energy (kcal)	0.001	0.000	0.149	2.074	0.040	(0.000, 0.002)
Perceived stress scale	0.291	0.036	0.561	7.999	<0.001	(0.219, 0.362)

R^2=0.425, *Adj.* R^2=0.399; F=16.373, p<0.001

Further analysis showed that the factors associated with constipation were age, dietary fibre, fluid, energy, and perceived stress.

According to a systematic review conducted by Mugie *et al.* (2011), the prevalence of constipation worldwide among the general population was very variable, with a range of 2.5% to 79.0% among adults. A study revealed that the prevalence of constipation was higher among hospitalised patients, their attendants, and medical students in the age group of 18 to 30 years old (Khatri *et al.*, 2011). In comparison to the present study, most of the participants had slight constipation and a small percentage had severe constipation. A previous study by Lim *et al.* (2016) reported that the prevalence of constipation was higher than this present study, although it was conducted at the same university. This inconsistent finding may be due to the different target population and age range of participants, in which the previous study was conducted not only among undergraduate students, but involved participants between the age range of 18 to 30 years (Lim *et al.*, 2016).

In the process of ageing, a lot of health changes can be observed and one of the changes is increased risk towards constipation. According to the American Gastroenterological Association (2013), the median overall prevalence of constipation was 16% in adults, with a rate of 33.5% in adults aged 60 to 101 years old in North America. In the present study, age was significantly associated with constipation scores among the participants. Gras-Miralles & Cremonini (2013) addressed that increasing age was associated with a progressive increase in the risk of constipation. Furthermore, this finding was also supported by a study from Talley *et al.* (2003). They found a strong association between increasing age and the risk of chronic constipation among participants aged 11 to above 75 years old. Pelvic Floor Dysfunction (PFD) and slow colonic transit are two major aetiologies of constipation, of which those without any identified cause are classified as having normal transit constipation. Elasticity, compliance, altered macroscopic structural changes (example diverticulosis), and altered control of the pelvic floor are affected by age-related cellular dysfunctions. These molecular and physiologic changes are possible explanations for the association between age and constipation.

Only 10.3% of males and 8.9% of females achieved the RNI for energy intake. This finding was consistent with another cross-sectional study performed by Gan *et al.* (2011) where more than half of the Malaysian undergraduate students did not meet the RNI for total energy intake. Similarly, Mazlyn *et al.* (2013) reported that only 12.2% of Malaysian adults aged between 18 to 60 years old met the RNI for total energy intake. The present study revealed that total energy intake had a statistically significant correlation with constipation among undergraduate students. This finding was in contrast with another study by Bouchoucha *et al.* (2006), which reported that constipation is contributed by a low total calorie intake due to diminished gastrocolic reflex in response to eating. The gastrocolic reflex is a physiological reflex that controls the motility of the lower gastrointestinal tract following a meal. The colon will have increased motility in response to the stretch of the stomach on ingestion of food. The gastrocolic reflex allows room for consumption of more food via control over peristalsis and movement of ingested food distally towards the rectum. Thus, with limited amount of food intake, colon motility will decrease (Malone & Thavamani, 2020).

However, in the present study, most of the participants did not achieve

the energy requirement based on the RNI for Malaysia (NCFFN, 2017) and majority of them fell in the category of slightly constipated. Therefore, these two variables were positively associated. Another previous study showed that refined grains was a major food that causes constipation, as they have a low dietary fibre level that can lead to constipation (Mazlyn *et al.,* 2013). Perhaps the participants in this present study may have consumed foods that are high in refined grains, which have caused a higher energy intake and increased risk of constipation. However, in this study, Food Frequency Questionnaire (FFQ) or Semi-food Frequency Questionnaire (SFQ) were not used, and therefore, there was a limitation to further investigate the link between refined grains intake and risk of constipation.

In the present study, the mean dietary fibre intake of the participants was low and all of them had a daily dietary fibre intake of <20 g. The finding was consistent with previous studies examining the status of fibre intake in Malaysians. An earlier study found that dietary fibre intake among Malaysian adults aged between 22 to 60 years was less than the recommended dietary fibre intake, which was between 10.0 g to 15.6 g (Ng *et al.,* 2016). In the present study, lower dietary fibre intake also contributed to constipation. A study conducted by Khatri *et al.* (2011) among Malaysian adolescents, adults, and the elderly showed similar results in which there was a negative correlation between dietary fibre intake with constipation for all subjects combined. Alternatively, the higher the dietary fibre intake, the lower the risk of getting constipation. Increased dietary fibre can reduce the risk of getting constipation by increasing faecal bulk, defecation frequency and stool consistency, as well as reducing transit time of the faecal material through the large intestines.

The mean total fluid intake reported among the participants was 2301±946 ml per day, and more than half of the participants in the present study (57.1%) achieved the RNI for recommended total fluid intake of 2000 ml per day. There was an inverse correlation between total fluid intake and constipation. This result was consistent with a previous study by Khatri *et. al.* (2011), which found that total fluid intake was negatively correlated with constipation. In other words, as fluid consumption increases, the risk of constipation decreases. This is because adequate fluid intake can facilitate dietary fibre to increase the weight of stool and produce soft stools.

In the present study, majority of the participants had stress levels ranging from moderate to very high. These findings were not in agreement with a previous study conducted among undergraduate students (Mikolajczyk *et al.,* 2009), which indicated that only 21.6% of undergraduate students experienced moderate to high levels of stress. Apart from academic overload, it was found that stress levels among university students increased due to other factors as well such as personal problems, financial problems, college activities and others (Rahim *et al.,* 2016). Based on a study conducted by Bataineh (2013), it was suggested that a high level of stress among undergraduate students was due to several reasons such as a heavy academic workload, low motivation, and competition with other students, meeting with failures, high family expectations, and lack of purchasing power.

A positive correlation was found between stress scores and constipation scores among the participants. This finding from the current study was in line with an earlier study which reported depression and stress as being statistically, significantly correlated with abnormal anorectal function,

indicating that a higher stress level may contribute to functional constipation (Leung *et al.,* 2011). Similar finding was suggested in another study, where there was a statistically significant association between perceived stress level and constipation symptoms among 715 nursing students in Korea (Lee *et al.*, 2011). Disturbances of the gastrointestinal tract function may occur due to stress, thus increasing the risk of getting constipation (Suzana *et al.*, 2009).

It was proven that the risk of chronic constipation was decreased significantly by doing regular physical activity (Chang *et al.*, 2015). A systematic review by Peters, De Vries, Vanberge-Henegouwen & Akkermans (2001) found that gastrointestinal diseases can be prevented by doing regular physical activity at a relatively low intensity. However, in the present study, physical activity did not significantly contribute to constipation. The level of physical activity would be expected to increase the level of fluids required by the body, and dehydration may explain why higher levels of physical activity could be associated with more severe constipation since fluid intake in the body appear to be inadequate (Mazlyn *et al.*, 2013). In the present study, more than half of the participants achieved the recommended fluid intake even though they were not physically active. Therefore, physical activity in the present study was not one of the contributing factors as most of them had low to moderate levels of physical activity and consumed enough fluids.

There were several limitations in the present study that should be taken into consideration. The cross-sectional design of the present study has limited its findings from showing the causal relationship between the dependent and independent variables, although multivariate analysis was used to determine these factors. All data were self-reported by the participants via self-administered questionnaires, which was highly dependent on the honesty and memory of the participants, especially on questions relating to physical activity and stress levels. As a result, this can lead to reporting bias. Another important limitation was the unavailability of anthropometry data to strengthen the results, such as to capture the issue of under-reporting (UR). This is because the present study did not measure anthropometric metrics, which led to the inability to calculate basal metabolic rate that is vital to address UR.

Another important limitation that needs to be addressed is the limitation associated with the Agachan's Constipation Score System Questionnaire, which was used to assess the prevalence and severity of constipation. It had several categories to classify the severity of constipation, but this questionnaire required a score of more than 15 to meet the definition of "constipation". According to this classification, only two participants were identified as having constipation. Therefore, Multiple Logistic Regression (MLR) was not performed.

It was noted that in the present and previous studies, there are several risk factors of getting constipation, which include low total energy intake, low dietary fibre intake, low water intake, physical inactivity, and stress (Leung *et al.*, 2011). But, in real life, people consume mixed foods that contain various nutrients instead of certain foods or single nutrients. Dietary pattern is a dynamic state that takes into account the interactions and inter-correlations between nutrients and foods, as well as their cumulative effects on health. Therefore, it is recommended that future studies focus on dietary pattern in relation to constipation among university students. Besides

that, the present study only recruited undergraduate students from UPM, which may limit the generalisability of the study. Hence, future studies are recommended to involve students from different universities in Malaysia in order for the study findings to be generalised to all university students in the country.

CONCLUSION

Age, energy, fluid and fibre intakes, as well as perceived stress were factors that were associated with constipation among undergraduate students. Therefore, undergraduate students should be encouraged to consume adequate energy, dietary fibre, and fluids in their daily life. On top of that, they also require attention from various parties, especially counsellors in universities, as well as health care professionals to assist them to reducing their stress levels.

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Authors' contributions

NBMY, principal investigator, conceptualised and designed the study, led the data collection, prepared the draft of the manuscript and reviewed the manuscript; QAMY, conducted data analysis, assisted in drafting of the manuscript and reviewed the manuscript.

Conflicts of interest

The authors report no conflicts of interest.

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Fulfilment of minimum acceptable diet (MAD), short birth length and family income level are associated with stunting in children aged 6-23 months in Central Jakarta

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ABSTRACT

Introduction: Stunting is a condition characterised by retardation in the growth and development of children as a result of chronic inadequate nutritional intake and/or recurrent infectious diseases. This research aims to determine the factors related to stunting occurrence among children aged 6-23 months. **Methods:** This cross-sectional study was carried out in 2019. The sample was 231 children selected by multistage random sampling technique from 13 Posyandu (integrated healthcare centres) in six administrative villages located across three sub-districts of the Central Jakarta region. **Results:** The results showed that the prevalence of stunting was 26.0% and minimum acceptable diet (MAD) was only met by 31.6% of the children studied. Chi-square analysis revealed that short birth length (*OR*=2.176; 95% CI: 1.155–4.098) and family income level (*OR*=0.388; 95% CI: 0.201–0.749) were significantly associated with stunting. Logistic regression showed that fulfilment of MAD, short birth length (*OR*=0.471; 95% CI: 0.244–0.909), and family income (*OR*=0.387; 95% CI: 0.197–0.759) were significant factors for stunting among children 6-23 months in Central Jakarta in 2019, with fulfilment of MAD as a dominant factor (*OR*=3.29; 95% CI: 1.171–9.241). **Conclusion:** More efforts need to be done to achieve the recommended MAD for all children aged between 6-23 months and to prevent short birth length. Large scale studies to explore the role of MAD in reducing stunting and qualitative studies to identify the constraints and promoting factors to better infant and young child feeding practices are imperative for programme improvement.

Keywords: stunting, fulfilment of minimum acceptable diet, children aged 6-23 months.

INTRODUCTION

Stunting is defined as impaired growth and development that children experience from poor nutrition, repeated infection, and inadequate psychosocial stimulation, which in many cases, happen in the 1st 1000 days of life and is characterised by height-for-age

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z-score (HAZ) of <-2 standard deviations (*SD*) (WHO, 2010). Stunting is a high-risk health problem associated with obstructed development of body organs, decreased child performance at school, increased mortality risk in children (UNICEF, 2013; Tiwari, Ausman & Agho, 2014; Sarma *et al.*, 2017), increased risk of non-communicable diseases, short body posture as adults, and reduction in quality of life in the future (Dewey, 2016; Black, 2013; Rahab *et al.*, 2018). In 2007, 2010, 2013, and 2018 (Indonesian Ministry of Health, 2018), the prevalence of child stunting in Indonesia were 36.8%, 35.6%, 37.2%, and 30.8%, respectively. *Daerah Khusus Ibukota* (DKI) Jakarta Province has a stunting prevalence of 22.7% and Central Jakarta has the highest prevalence compared with other regions of Indonesia, at 29.2% (Indonesian Ministry of Health, 2016; Indonesian Ministry of Health, 2018), which is considered as a moderate public health problem (WHO, 1995).

The causes of stunting are multifactorial and include immediate causes (inadequate dietary intake and infection); underlying causes (household food insecurity, inadequate care and feeding practices, unhealthy household environment, and inadequate health services), and basic causes (household characteristics, sociocultural, economic and political context). Feeding practices play an important role in stunting and can be assessed in relation to the fulfilment of minimum acceptable diet (MAD), minimum dietary diversity (MDD), and minimum meal frequency (MMF), which are three out of the eight core indicators for assessing infant and young child feeding practices (UNICEF, 2012). Minimum dietary diversity or MDD for both breastfed and non-breastfed children aged 6-23 months is defined as receiving four or more food groups out of the seven food groups (grains, roots and tubers; legumes and nuts; dairy products; flesh foods; eggs; vitamin A-rich fruits and vegetables; and other fruits and vegetables) (UNICEF, 2012). In this case, non-breastfed children are favoured as infant formula is included under the dairy food group, while breast milk is not counted. However, because MDD is a proxy indicator for mean micronutrient density adequacy of a diet, the exclusion of breast milk from the seven food groups is considered fine (UNICEF, WHO, FANTA III & USAID, 2017). Minimum meal frequency is defined as the minimum frequency in provision of solid, semi-solid, or soft foods of two (for age 6-8 months) or three (for age 9-23 months) times for breastfed children, and four times (6-23 months) (including formula) for non-breastfed children (UNICEF, WHO, FANTA III & USAID, 2017). Thus, it captures the caloric sufficiency of a child's diet. Minimum acceptable diet (MAD) is a summary indicator of 6-23 months children meeting both MDD and MMF. This study aimed to determine the factors related to the occurrence of stunting among children aged 6-23 months in Central Jakarta in 2019.

MATERIALS AND METHODS

The study was a descriptive cross-sectional survey. This survey was conducted in three sub-districts which represented the urban areas in Central Jakarta, DKI Jakarta Province, Indonesia. It was carried out during April and May 2019. The samples of the population selected for this study were mothers and their children (6–23 months) residing in the study area. In its final analysis, this research used primary data from 231 children aged 6-23 months, based on sample inclusion criteria. Children with infections and allergies at the time of the study were excluded. The sample calculation in this study used a two-different-proportion

hypothesis test with 80% power, 95% CI, and a design effect of 1.5.

A multistage procedure was adopted in selecting respondents for this study. The sampling involved a three-stage process in the study area, which was composed of eight sub-districts, each containing 5-6 administrative villages. In the first stage, three sub-districts were selected by balloting from the eight sub-districts. Next, from each of the three chosen sub-districts, two administrative villages were selected by random sampling, giving a total of six administrative villages selected. Finally, from each of the administrative villages, two or three Posyandu were selected by random sampling to provide the sample population needed for the study (13 Posyandu = 231 children). From each household, only one mother-child pair was selected for the study. Ethical clearance was obtained from the Ethics Committee of the Public Health Faculty of Universitas Indonesia (ID: 128/UN2. F10/PPM.00.02/2019). Consent was obtained in writing from each participant after the purpose of the study was explained to them. All identities of the participants were kept confidential.

Information were collected from respondents using a content-validated, interviewer-administered questionnaire. The present questionnaire was adopted from three valid questionnaires - the Indonesia Demographic and Health Surveys (IDHS), National Basic Health Surveys (Riskesdas), and National Growth Monitoring Surveys (PSG), respectively. We did not test the validity and reliability of our questionnaire, with assumption that these questions were already pretested. Data collection was assisted by enumerators who had been trained and met the specified criteria. Data were collected by measuring children's length and through interviews with respondents conducted by trained data collectors**.** Secondary data used in this study were geographical data and the number of children in each Posyandu.

The height measurement of each child was obtained and converted into height-for-age (HFA) as an indicator of nutritional status. This was then expressed as z-score based on the standard reference of measurements using the World Health Organization (WHO) Anthro software. Children were classified as stunted (HFA <-2 z-score), severely stunted (HFA <-2 z-score) or not stunted (HFA ≥-2 z-score). Maternal knowledge regarding children's health and nutrition was assessed, and their overall knowledge was classified as 'met' or 'not met'. Children's dietary feeding practices were measured through 24-hour dietary recalls using the WHO Infants and Young Children Feeding (IYCF) guidelines. These guidelines were designed to measure the fulfilment of MDD when the child ate at least four or more varieties of foods from the seven food groups in a 24-hour time period; fulfilment of MMF when the child received complementary foods in the minimum recommended number of times in the past 24 hours; and MAD, which is a composite indicator of MDD and MMF. With that, meeting the WHO recommended MAD meant that currently breastfed children aged 6-23 months met both the MDD and the MMF based on what the child ate and drank in the past day and night (Khanal, Sauer & Zhao, 2013). However, meeting the MAD was slightly different for non-breastfed children. Dietary diversity was calculated by using six food groups (excluding dairy products) at least four times a day and combining milk-related products (formula milk, milk, or yoghurt) with at least two feedings a day.

The other independent variables used were initiation of breastfeeding, colostrum feeding, exclusive breastfeeding, history of infection,

Table 1. Distribution of height-for-age (HAZ) in children aged 6 – 23 months in Central Jakarta, *n*=231

Height-for-age (HAZ)	*%*
Severe stunting (<-3 *SD*)	8.7
Moderate stunting (-3 *SD* to <-2 *SD*)	17.3
Not stunting (≥-2 *SD*)	74.0

immunisation status, vitamin A supplementation, low birth weight, short birth length, family characteristics including mother's education level, family income level, family size, and birth order. These information were collected using questionnaires. Chi-squared test was used for bivariate analysis and logistic regression test was used for multivariate analysis, using the Statistical Package for Social Sciences (SPSS) software with a statistical significance of $p<0.05$. Normality test with Kolmogorov-Smirnov test showed that data in this study were normally distributed with a *p*-value of 0.200 ($p>0.05$).

RESULTS

Table 1 shows that the overall prevalence of stunting was 26.0% (8.7% severe stunting and 17.3% moderate stunting). Boys had a higher prevalence of stunting (26.6%) than girls (25.2%). In addition, older children (aged 12-23 months) had a higher prevalence of stunting (30.6%) than younger children (aged 6-11 months) (17.9%). Table 2 shows the specific fulfilment of each indicator for child feeding practices used in this study. The proportion of children who fulfilled MDD was >50%, while >70% fulfilled MMF. However, the proportion of children who fulfilled MAD was lower than both MDD and MMF (31.6%). Along with the increasing age of children, MDD achievement increased as evidenced by the highest MDD achievement in children aged 18-23 months. MMD achievement was then categorised into two based on the status of breastfeeding, namely children who were still given breast milk and who were not given breast milk. In all age groups, the percentage of those achieving MDD were higher than those who did not achieve MDD, except for children who were not given breast milk (age group 6-11 months), whereby the percentage of those achieving and not achieving MDD were the same, that was 50%, respectively. In this study, it was also shown that MDD achievement in children who were still given breast milk (48.7%) was lower compared to children who were not given breast milk (72.2%).

Table 3 shows the percentage of each dietary diversity score (number of types of food groups consumed by children). Among children who were still given breast milk, the highest value for dietary diversity score in the 6-11 months and 12-17 months age groups was 3 (34.4% and 37.7%), while those in the 18-23 months age group was 4 (42.9%). The values of dietary diversity score in children who were not given breast milk were 3 (45%), 4 and 5 (34.8%), and 4 (41.7%) in children aged 6-11 months, 12-17 months, and 18-23 months, respectively. Table 3 also illustrated that the order of the most consumed food groups were foods from group 1 (grains, roots, and tubers) at 97.4%, foods from group 6 (fruits and vegetables rich in vitamin A), foods from group 4 (meat), foods from group 3 (dairy products), foods from group 7 (fruits and other vegetables), foods from group 5 (eggs), and foods from group 2 (nuts and processed products). From Tables 3 and 4, it was observed that 2 children (0.4%)

Table 2. Distribution of the fulfilment of MDD, MMF and MAD in children aged 6 – 23 months in Central Jakarta

	Age (months)						*Total*	
	6–11		*12–17*		*18–23*		*n*	*%*
	n	*%*	*n*	*%*	*n*	*%*		
Fulfilment of MDD (breastfed and non-breastfed)	84		76		71		231	
Not met		57.1		40.8		29.6		43.3
Met		42.9		59.2		70.4		56.7
Fulfilment of MDD in breastfed children	64		53		35		152	
Not met		59.4		50.9		37.1		51.3
Met		40.6		49.1		62.9		48.7
Fulfilment of MDD in non breastfed children	20		23		36		79	
Not met		50.0		17.4		22.2		27.8
Met		50.0		82.6		77.8		72.2
Fulfilment of MMF	64		53		35		152	
Not met		25.0		31.6		28.2		28.1
Met		75.0		68.4		71.8		71.9
Fulfilment of MAD	20		23		36		79	
Not met		76.2		65.8		62.0		68.4
Met		23.8		34.2		38.0		31.6

only consumed breast milk without complementary foods, meanwhile there were 150 children (64.9%) who consumed breast milk accompanied by complementary foods, and 79 children (34.3%) who did not consume breast milk, but consumed complementary foods that were mostly accompanied by consumption of formula milk. Less than 50% of children reportedly received initial breastfeeding, but at most received colostrum (88.7%), while only one-third (29.4%) were exclusively breastfed during the period of 0–6 months. Two-third of children had infection history (69.3%), while 7.8% had low birth weight (LBW), and 26.4% had short birth length (SBL). More than 70% of children had been fully immunised for their age, and >80% had received vitamin A supplementation. More than half of the children's mothers had a relatively high education level (59.7%). The proportion of children with a family size of ≤4 members was 62.3%, while 64.1% were in the first or second birth order in their families. In addition, the proportion of mothers with correct knowledge on exclusive

Table 3. Distribution of Dietary Diversity Score (DDS) and food groups consumed by children aged 6 – 23 months in Central Jakarta

	Age (months)						*Total*	
	6–11		*12–17*		*18–23*			
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*
Number of food groups consumed (DDS)								
In breastfed children	64		53		35		152	
0		3.1		1.9		0.0		2.0
1		7.8		3.8		0.0		4.6
2		14.1		7.5		14.3		11.8
3		34.4		37.7		22.9		32.9
4		25		34		42.9		32.2
5		12.5		7.5		17.1		11.8
6		3.1		7.5		2.9		4.6
In non-breastfed children	20		23		36		79	
1		0.0		0.0		2.8		1.3
2		5.0		0.0		5.6		3.8
3		45.0		17.4		13.9		22.8
4		30.0		34.8		41.7		36.7
5		20.0		34.8		30.6		29.1
6		0.0		8.7		5.6		5.1
7		0.0		4.3		0.0		1.3
Food groups consumed							231	
Grains, roots, and tubers								97.4
Nuts and their processed products								17.7
Dairy products (milk, yoghurt, and cheese)								54.1
Meat (various meats, fish, poultry and offal)								63.6
Eggs								24.2
Fruits and vegetables rich in vitamin A								69.7
Other fruits and vegetables								38.5

Table 4. Factors associated with stunting (severe and moderate) in children aged 6 – 23 months in Central Jakarta

Characteristics	*Nutritional status (HAZ)*				*Total*		*OR (95% CI)*	*p-value*
	Stunting		*No stunting*					
	n	*%*	*n*	*%*	*n*	*%*		
Fulfilment of MDD								
Not met	28	28.0	72	72.0	100	100	0.831	0.644
Met	32	24.4	99	75.6	131	100	(0.460-1.501)	
Fulfilment of MMF								
Not met	19	29.2	46	70.8	65	100	0.794	0.590
Met	41	24.7	125	75.3	166	100	(0.419-1.507)	
Fulfilment of MAD								
Not met	38	24.1	120	75.9	158	100	1.362	0.413
Met	22	30.1	51	69.9	73	100	(0.734-2.530)	
Breastfeeding initiation								
No	30	24.8	91	75.2	121	100	1.450	0.419
Yes	30	27.3	80	72.7	110	100	(0.691-3.042)	
Colostrum feeding								
No	5	19.2	21	80.8	26	100	1.540	0.552
Yes	55	26.8	150	73.2	205	100	(0.554-4.284)	
Exclusive breastfeeding								
No	40	24.5	123	75.5	163	100	1.281	0.545
Yes	20	29.4	48	70.6	68	100	(0.681-2.410)	
History of infection								
Yes	16	22.5	55	77.5	71	100	1.304	0.528
No	44	27.5	116	72.5	160	100	(0.677-2.513)	
Birth weight								
Low birth weight	7	38.9	11	61.1	18	100	1.921	0.260
Normal birth weight	53	24.9	160	75.1	213	100	(0.709-5.208)	
Birth length								
Short birth length	23	37.7	38	62.3	61	100	2.176	0.023*
Normal birth length	37	21.8	133	78.2	170	100	(1.155-4.098)	
Immunisation status								
No	16	28.6	40	71.4	56	100	0.840	0.738
Yes	44	25.1	131	74.9	175	100	(0.428-1.646)	
Vitamin A supplementation								
No	10	26.3	28	73.7	38	100	0.979	1.000
Yes	50	25.9	143	74.1	193	100	(0.444–2.158)	
Mother's education level								
Low	28	30.1	65	69.9	93	100	0.701	0.306
High	32	23.2	106	76.8	138	100	(0.387–1.269)	
Family income level								
Low (< Rp3,940,973.96)	45	32.8	92	67.2	137	100	0.388	0.006*
High (> Rp3,940,973.96)	15	16.0	79	84.0	94	100	(0.201–0.749)	
Family size								
Large (> 4 members)	22	25.3	65	74.7	87	100	1.059	0.976
Small (≤ 4 members)	38	26.4	106	73.6	144	100	(0.576–1.948)	
Birth order								
Third or above	22	26.5	61	73.5	83	100	0.958	1.000
First or second	38	25.7	110	74.3	148	100	(0.520–1.765)	

*p-value<0.05 indicates statistical significance

Table 5. Multivariate analysis on the factors associated with stunting in children aged 6 – 23 months in Central Jakarta

Independent variables	*p-value*	*OR*	*95% CI*
Fulfilment of MDD	0.062	0.435	0.181–1.044
Fulfilment of MMF	0.119	0.529	0.238–1.178
Fulfilment of MAD	0.024*	3.290	1.171–9.241
Short birth length	0.025*	0.471	0.244–0.909
Family income level	0.006**	0.387	0.197–0.759

*p-value<0.05 indicates statistical significance
**p-value<0.01 indicates statistical significance

breastfeeding was 88.3% from the total population. Two-third of mothers had correct knowledge on the right timing for introducing complementary foods (65.8%) and most of them introduced complementary foods based on the consistency of food – semi-solid and soft (40.7%).

Bivariate analysis is presented in Table 4 and indicated that birth length and family income level were significantly associated with stunting in children aged 6–23 months, while other variables were not significantly associated with stunting. From this study, there were no significant association between birth length and family income level according to fulfilment of MDD, MMF and MAD. The logistic multivariate analysis was conducted through several stages: bivariate analysis to select candidate variables, multivariate modelling, and interpretation of the modelling results. The results of the bivariate selection showed that only SBL and family income level fulfilled as candidates for multivariate analysis; however, we also included the fulfilment of MAD, MDD, and MMF because these variables were important and substantial in this study. Multivariate analysis in Table 5 indicated that fulfilment of MAD (*OR*=3.29; 95% CI: 1.171–9.241), SBL (*OR*=0.471; 95% CI: 0.244–0.909), and family income (*OR*=0.387; 95% CI: 0.197–0.759) were variables significantly associated with stunting, after controlling for other selected variables (fulfilment of MDD, fulfilment of MMF, SBL, and family income level). Based on this research, fulfilment of MAD was a dominant factor in stunting in children aged 6–23 months in Central Jakarta.

DISCUSSION

Stunting in children is caused by many factors. Non-optimal feeding practices is one of the highest risk that leads to retardation in the growth and development of children. MAD is a useful indicator to see the progress of quantity and quality of children's food intake (WHO, 2010). Low dietary diversity and meal frequency practices are determinants for health and growth in children <2 years of age. They increase the risk of under-nutrition, illness, and mortality in infants and young children (Beyene, Worku & Wassie, 2015). Even with optimum breastfeeding, children will become stunted if they do not receive sufficient dietary diversity and frequency over 6 months of age (Issaka *et al.*, 2014). The study conducted by Jemide *et al.* (2016) found that there is a relationship between fulfilment of MAD and stunting. Another study from Kakati and Baruah (2015) found that the risk of stunting is greater in children who did not meet the fulfilment of MAD compared to those who met the fulfilment of MAD.

The prevalence of stunting in this population was high, with 26% of children aged 6-23 months affected, and this level of stunting in Central Jakarta is categorised as a moderate public health problem by WHO (1995). The prevalence of stunting was higher in children aged 12–23 months than in children aged 6–11 months, and this finding is similar to results from Rwanda (Nsereko, 2018). As children age, their growth curve deviates from the normal curve in line with the increase in their nutritional needs (Terati & Susanto, 2018). Stunting in male children was more prevalent than in females, reflecting the lower likelihood for females than males to become stunted during infancy and childhood, as well as the higher survival rate of infant females than infant males (Boylan, 2017). Based on children's feeding practices, this study found that the fulfilment of MAD indicator was lower than for other indicators (MDD and MMF), because the fulfilment of MAD is a combination of the fulfilment of the other indicators (that is, in order to meet MAD, children should meet both MDD and MMF). In this study, we found that the fulfilment of MDD was lower than MMF, and so this result influenced the lower proportion of fulfilment of MAD.

This study also showed that MDD achievement in children who were still given breast milk was lower compared to children who were not given breast milk. This can happen because children who were still given breast milk considered breast milk to be the main source of their nutrition and breast milk was not included in the food group for the achievement of MDD; while children who were not given breast milk consumed more complementary foods than children who were still given breast milk to meet their energy and nutrient needs (Marriot, 2011). Therefore, MDD achievement in children who were still given breast milk versus those who were not given breast milk cannot be compared because breast milk was not counted in this indicator when used to determine the quality of complementary feeding practices. Besides that, it is known that children who are not given breast milk would achieve better MDD because they are routinely given formula milk or other milk products that are included as one of the food groups in MDD (WHO, 2010). Therefore, MDD achievement indicators are more suitable for use in children who are not given breast milk because children would then only rely on complementary foods to meet their nutritional needs (Arimond & Ruel, 2004).

Short birth length (SBL) was found to be a significant variable associated with stunting, with 2.176 *OR* value (95% CI: 1.155–4.098). This proves that in our sample, children with SBL had 2.176 times higher risk of stunting than those who did not. This finding is similar to other research that showed the importance of maternal pregnancy nutrition for children's nutritional status (Dewi, Dewi & Murti, 2019). After conducting further analysis, it was found that 23% of children who had a history of SBL also had a history of LBW. In addition, it was proven from this study that there were 37.7% of children who had a history of SBL. Family income level is a protective factor against stunting. Given the 0.388 *OR* value found in this study, children with higher family income level will consume more nutritionally rich foods than those with lower family incomes (Beal *et al.*, 2018), whose intake varies less in terms of quantity of various important nutrients for children's growth such as protein, minerals, and vitamins (Lestari *et al.,* 2018). This is related to the high purchasing power of children with high family income (Nshimyiryo *et al.,* 2019).

Other variables had no significant relationships with stunting in this study in terms of fulfilment of MDD, MMF, and MAD because the 24-hour recall method used did not include specific portions of foods and this may have led to biased results. Breastfeeding initiation, colostrum feeding, and exclusive breastfeeding were found to have no significant relationships, probably because mothers thought that these could provide children's nutritional needs without knowing that other variables also played an important role in meeting children's nutritional needs. History of infection had no significant relationship with stunting, probably because we only asked about infection in the preceding month, which was not representative of a child's entire history of infection. Low birth weight, immunisation status, vitamin A supplementation, and family characteristics also had no significant relationships in this study, because there were other significant factors that affected stunting, such as children's feeding practices and children's history of infection (UNICEF, 2013).

Multivariate analysis results showed that fulfilment of MAD, SBL, and family income were significant factors for stunting among children 6-23 months, with fulfilment of MAD as a dominant factor. MAD is a summary indicator of 6-23 months of children meeting both MDD and MMF. Due to the multidimensional feeding of adequate complementary foods to children aged 6-23 months, it is very important to use a combination of indicators that can identify the extent to which adequate feeding practices are being met (Guirindola, 2018). The results of this study showed that children who did not meet MAD were 3.29 times more likely to be stunted. The fulfilment of MAD is therefore very important because it represents the effect of both food quantity and quality on children's linear growth (Nsereko, 2018). A cohort study conducted in Bangladesh found that the fulfilment of MAD was a predictor of linear growth in children aged 6-24 months (Owais *et al.,* 2016). The limitation of this study was that if the feeding practices conveyed by respondents to the researcher were different from the actual feeding practices in children, then this would have affected the accuracy of the research data. MAD was obtained from the calculation results of more than one variable, of which its accuracy is based on the feeding practices reported by respondents.

CONCLUSION

Given that the fulfilment of MAD is the strongest factor affecting stunting, we therefore recommend that programmes aimed at improving mothers' knowledge and behaviour related to child feeding practices need to be strengthened through education to pregnant and lactating women, especially those from low-income families. The fact that the fulfilment is worse among younger children indicates that the transition to complementary feeding needs special and early efforts. Short birth length indicates problems of growth and development in the womb, and therefore we recommend that mothers should be in good health and nutritional status both before and during pregnancy. Large scale studies to explore the role of MAD in reducing stunting and qualitative studies to identify the constraints and promoting factors to better IYCF practices are imperative for programme improvement.

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Authors' contributions

M, principal investigator, conceptualised and designed the study, led the data collection in Central Jakarta, conducted the study, prepared the draft of the manuscript, and reviewed the manuscript; EA, conducted the study, advised on data analysis and interpretation, and reviewed the manuscript; EAL, conceptualised and designed the study, advised on data analysis and interpretation, assisted in drafting of the manuscript, reviewed the manuscript, and provided the final approval for publication.

Conflict of interest

None declared.

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Stability of lactoferrin and lysozyme in human milk at various temperatures and duration of storage

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ABSTRACT

Introduction: Exclusive breastfeeding, especially in the first six months, is essential for infants as it provides nutrition and protection against various diseases. Colostrum, which is found in the first breast milk produced, contains various protective factors, such as lactoferrin and lysozyme. Human milk can be stored at room temperature, refrigerated, or kept frozen. Several factors affect the stability of the bioactive content in human milk, such as temperature and storage time. The aim of this study was to measure the stability of lactoferrin and lysozyme levels in human milk during the first six hours (h) at different temperatures and compare it with that of frozen human milk. **Methods:** Human milk samples were obtained from 11 breastfeeding mothers using certain criteria. The human milk was stored at room temperature and 4°C for 1, 3, and 6 h and classified as never frozen, while frozen human milk was stored at -20°C for 1, 3, and 6 days. Measurement of the lactoferrin and lysozyme levels was performed using enzyme-linked immunosorbent assay. **Results:** The results showed that storage at room temperature significantly reduced lactoferrin and lysozyme levels. Lactoferrin levels in frozen human milk did not significantly decrease during the first six days. Meanwhile, the lysozyme levels in frozen human milk decreased significantly. **Conclusion:** The levels of lactoferrin and lysozyme in frozen human milk stored for the first six days were more stable than those stored at room temperature and 4°C in the first 6 h.

Keywords: human milk, lactoferrin, lysozyme, storage duration, temperature

INTRODUCTION

Breast milk is an important source of nutrition for newborns and infants. According to the World Health Organization (WHO), exclusive breastfeeding for the first six months is essential for infants and is recommended until the age of 2 years (WHO, 2009). The neonatal period is a critical period for an infant due to the susceptibility to various types of antigens, including bacteria and viruses, or others (Lawrence & Lawrence, 2016). Exclusive breastfeeding protects infants from various diseases, such as otitis media, respiratory tract infections, gastroenteritis, sepsis, childhood cancer, asthma, and meningitis (El-Agamy, 2011; Palmeira *et al.*, 2016; Basir *et al.*, 2019). Human milk contains numerous components of the immune system which are essential and vital for infants (Abdullah & Saleh, 2019). Colostrum

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in human milk, which is first produced before birth up to approximately four days postpartum, contains various protective factors, such as enzymes (lactoferrin, lysozyme, and others), immunoglobulin (Ig), especially IgA, cytokines, leukocytes, fats, hormones, and others that contribute to the maturation of the infant's immune system (Kasiati *et al.*, 2013; Palmeira *et al.*, 2016; Basir *et al.*, 2019).

Lactoferrin (Lf) is a glycoprotein from the transferrin protein group with a mass of approximately 78-80 kDa (Lawrence & Lawrence, 2011), and was first discovered in cow's milk as a red protein in whey (Czosnykowska-Łukacka *et al.*, 2019). Lf has antibacterial (Bruni *et al.*, 2016), antibiotic, and antiviral properties (Berlutti *et al.*, 2011). The geographic location, nutritional status, socioeconomic status, race, genetic polymorphism, birth process, and infant condition determine the Lf levels in human milk (Czosnykowska-Łukacka *et al.*, 2019). Lysozyme is a 14 kDa cationic protein and lytic enzyme. Lysozyme was first discovered by Alexander Fleming in 1921, when droplets from Fleming's nose fell on a petri dish filled with bacteria. Lysozyme is secreted by the mucous glands, neutrophils, and macrophages (Ganz, 2006). Lysozyme is known to have antibacterial activity against both gram-positive and gram-negative bacteria (Masschalck & Michiels, 2003), antiviral activity (Małaczewska *et al.*, 2019), and anti-inflammatory properties (Tagashira *et al.*, 2018).

Human milk is typically given fresh to the infant; however, there are certain situations wherein the human milk cannot be consumed directly by the infant, such as suckling issues of the infant, a wound on the mother's nipple, or mothers returning to work. Therefore, mothers of infants use breast pumps to help collect their milk and store it. Human milk can be stored at room temperature, refrigerated, or frozen. The factors of temperature and storage time of human milk have been determined to affect the bioactive content in human milk, including the levels of lactoferrin and lysozyme. Human milk stored at -20°C for 3 months and six months showed significantly decreased lactoferrin levels up to 37% and 46%, respectively, compared to fresh human milk (Rollo *et al.*, 2014). Chang *et al.* (2013) also reported that lysozyme levels significantly decreased to 39.8% in frozen human milk stored at -20°C for four weeks compared to fresh human milk (Chang *et al.*, 2013). However, there is limited research related to the levels of Lf and lysozyme in fresh human milk at various temperatures and storage times.

The Academy of Breastfeeding Medicine's clinical protocol states that freshly expressed human milk with very low bacterial count can be stored at room temperature for a maximum of 6-8 h (Eglash *et al.*, 2017). The aim of this study is therefore to provide information about the stability of lactoferrin and lysozyme levels in fresh human milk at different storage times, specifically for the first 6 h, and storage temperatures, as well as compared with frozen breast milk. With clearer information, guidance can be given on the storage condition and duration that can best preserve the bioactive content in human milk to ensure that the infant is provided with optimum nutrition.

METHODS

Collection of human milk

Human milk samples were obtained from breastfeeding mothers registered in the *Pondok Kesehatan Desa, Sumber Porong*, Malang, Indonesia. Eleven breastfeeding mothers were selected by consecutive sampling based on three criteria, namely (i) days 4 to 30 postpartum, (ii) underwent normal

delivery process, and (iii) in good health. Data related to general characteristics, including age, weight, height, medical records, therapeutic treatment, and pregnancy history were carefully recorded. Data regarding infant's weight and height were recorded. Data consent from breastfeeding mothers had been obtained before samples of human milk were collected. Human milk was collected between 7:00 and 10:00 a.m. using an electric breast pump. All samples were collected in 10ml sterile bottles. Frozen breast milk was stored at -20°C (freezer) for one, three, and six days. On the 6th day, fresh human milk was collected again and stored for 1, 3, and 6 h at different temperatures, i.e. room temperature and 4°C (Figure 1). This research was approved by the ethics committee of Poltekkes Kemenkes Malang (number 925/KEPK-POLKESMA/2020).

Measurement of lactoferrin and lysozyme levels

The measurement of Lf and lysozyme levels in human milk was performed using enzyme-linked immunosorbent assay (ELISA). Liquid human milk was centrifuged at 3000 rpm for 20 minutes at 4°C. The supernatant was collected for ELISA. The ELISA kits for Lf (Fine Test Biotech, Cat. no. EH0396, China)

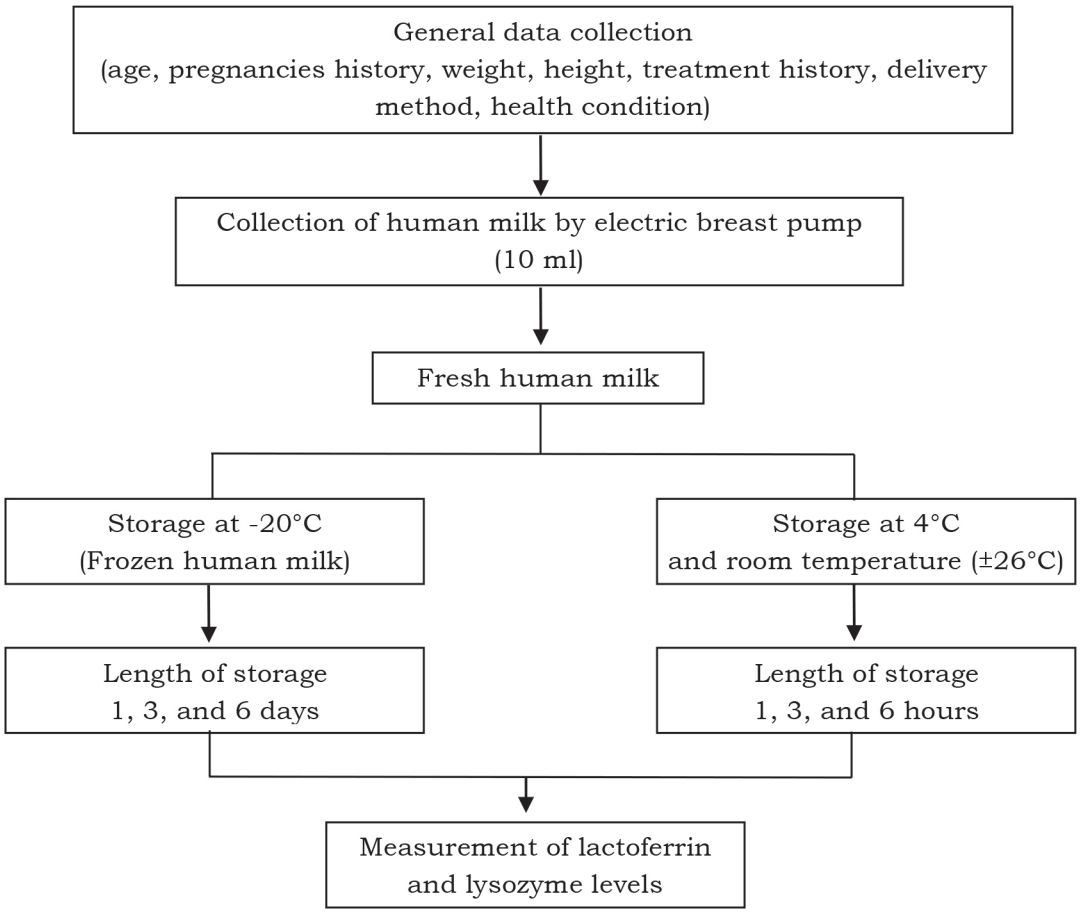

Figure 1. Data collection flow

and lysozyme (Fine Test Biotech, Cat No. EH3314, China) were used to carry out tests according to the manufacturer›s instructions. The Lf and lysozyme levels were measured at a wavelength of 450 nm. A standard curve was used to determine the lactoferrin and lysozyme levels.

Statistical analysis

Data on Lf and lysozyme levels in human milk were analysed using a two-way analysis of variance (ANOVA) (SPSS 23.0, SPSS Inc., Chicago, IL, USA). The significant differences between groups were analysed by Duncan's multiple range test as a post-hoc test. A *p*-value <0.05 was considered as statistically significant.

RESULTS

The results showed that the levels of Lf in human milk stored at room temperature in the first 6 h decreased significantly ($p<0.05$) compared to those stored at 4°C and frozen (Figure 2). The lowest level of lactoferrin was observed upon 6 h of storage at room temperature. Storage at 4°C for 3 and 6 h decreased the level of lactoferrin compared with frozen human milk. Based on these results, the lactoferrin levels were more stable at cold temperatures and were significantly decreased after the first 3 h of storage at room temperature.

The results showed that the lysozyme levels at room temperature were significantly reduced compared to those in frozen human milk ($p<0.05$) (Figure 3). Frozen human milk stored for the first six days was observed to have a significantly decreased lysozyme level after storage for three and six days ($p<0.05$). Frozen human milk stored for one day had the highest levels of lysozyme.

DISCUSSION

Human milk provides the essential nutritional standards for newborns and infants. Lf and lysozyme are the enzymes present in human milk, which play a role in the immune system of infants. Lactoferrin and lysozyme levels in breast milk can decrease due to changes in the environment. The protein aggregation in lactoferrin and lysozyme is induced by several conditions, such as changes in pH that are too acidic or alkaline, a dramatic increase or decrease in the

Table 1. General information of mothers and infants

Donors	*Age (years)*	*Previous pregnancies*	*Weight (kg)/ Height (cm)*	*Body Mass Index (BMI) (kg/m²)*	*BMI category*	*Newborns' weight (kg) / length (cm)*
A	31	1	55.0/150	24.44	normal	3.4/49
B	25	-	65.0/158	26.04	overweight	3.2/51
C	31	3	53.0/151	23.24	normal	2.7/50
D	38	1	60.5/160	23.63	normal	3.0/48
E	26	2	49.5/149	22.30	normal	3.0/48
F	38	1	61.0/163	22.96	normal	3.5/51
G	33	1	56.0/160	21.88	normal	3.7/53
H	33	2	60.0/150	26.67	overweight	2.9/49
I	34	2	68.0/158	27.24	overweight	3.3/54
J	28	2	58.0/152	25.10	overweight	3.9/48
K	35	2	60.0/154	25.30	overweight	3.1/49

temperature, and high concentrations of organic/inorganic salts (Venkataramani *et al.*, 2013; Wang *et al.*, 2019). The results showed that storage of human milk at room temperature caused a significant reduction in the lactoferrin levels in the first 3 h of storage. Storage at 4°C for 1 h and frozen breast milk maintained the levels of Lf. However, the lactoferrin levels began to decrease after storage for 3 h at 4°C. Meanwhile, the level of lysozyme in human milk stored at room temperature was significantly lower than that in frozen breast milk. The lowest lysozyme level in human milk was found in samples stored at room temperature. Howland *et al.* (2020) reported cleavage in the peptide structure of the amino acid lysine, arginine, and glutamate due to an increased thrombin activity and other protease enzymes in human milk stored at room temperature. Lysozyme or muramidase is a part of group of glycosidic hydrolases with 14.7 kDa of atomic mass in human lysozyme. Furthermore, aspartate-52 and glutamate-35 amino acid play a role in enzymatic activity of human lysozyme (Gálvez-Iriqui *et al,* 2020). The cleavage mechanism in those amino acid might be associated with lysozyme reduction in human milk.

The lactoferrin levels in the first month of lactation are known to decrease, but remain stable subsequently for up to eight months (Affolter *et al.*, 2016; Yang *et al.*, 2018). The lactoferrin levels in colostrum can reach up to 5.5g/L, while in mature breast milk lactoferrin levels are only 1.5-3.0g/L (Czosnykowska-Lukacka *et al.*, 2019). The lactoferrin in cow's milk is 1.5-2 times lower than the lactoferrin in human milk (Soboleva *et al.*, 2019), that is up to 1.5 mg/L in colostrum and 0.5 mg/L in mature cow's milk (Yang *et al.*, 2018). The lysozyme

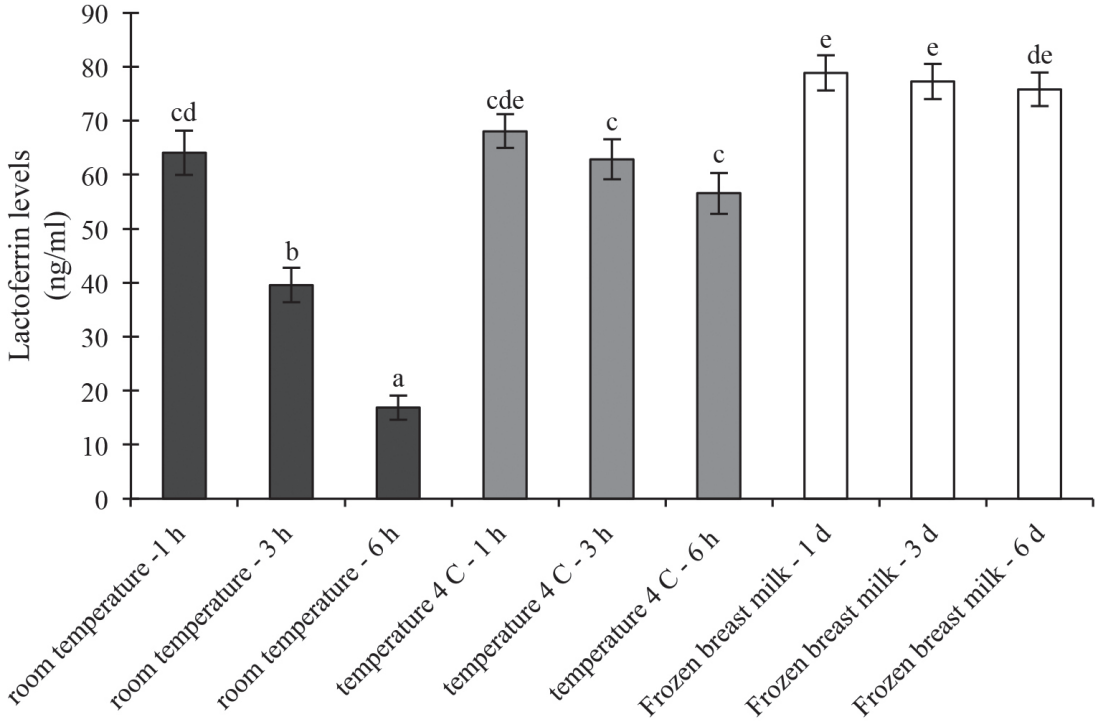

Figure 2. The levels of lactoferrin in human milk at different temperatures and duration of storage times. Different letters showed significant difference compared to other groups based on the DMRT post-hoc test.

levels in human milk are commonly about 60-400 µg/ml (Yang *et al.*, 2011; Chang *et al.*, 2013) and higher than in cow's milk, which is found to be only 0.05-0.22 µg/ml (Yang *et al.*, 2011). Chang *et al.* (2013) reported that frozen and pasteurised human milk produced lower lysozyme levels than fresh human milk while the level of freshly expressed human milk is up to 60 µg/mL while frozen human milk only up to 40 µg/mL. The proteins in lysozyme can undergo denaturation at either high or low temperatures, depending on the length of storage.

The Australian Breastfeeding Association (ABA) recommends storing fresh human milk at room temperature (≤26°C) for 6-8 h (Australian Breastfeeding Association, 2011). Eglash *et al.* (2017) stated that human milk can only be stored at room temperature (10-29°C) for 6-8 h, depending on the conditions and hygiene of the storage area. This has an impact on the bacterial growth development in human milk during storage. Rollo *et al.* (2014) reported that the Lf levels in human milk were more stable during storage at -20°C during a five-day period. However, storage at -20°C for a week causes a significant decrease in the macronutrients of human milk compared to fresh human milk (Kim *et al.*, 2019); and if continued until three months of storage, this increases the acidity of human milk (Vázquez-Román *et al.*, 2016). Based on this research, the recommended duration for storage of frozen human milk is not >6 days, while fresh human milk can only be stored at room temperature and 4°C for <3 h.

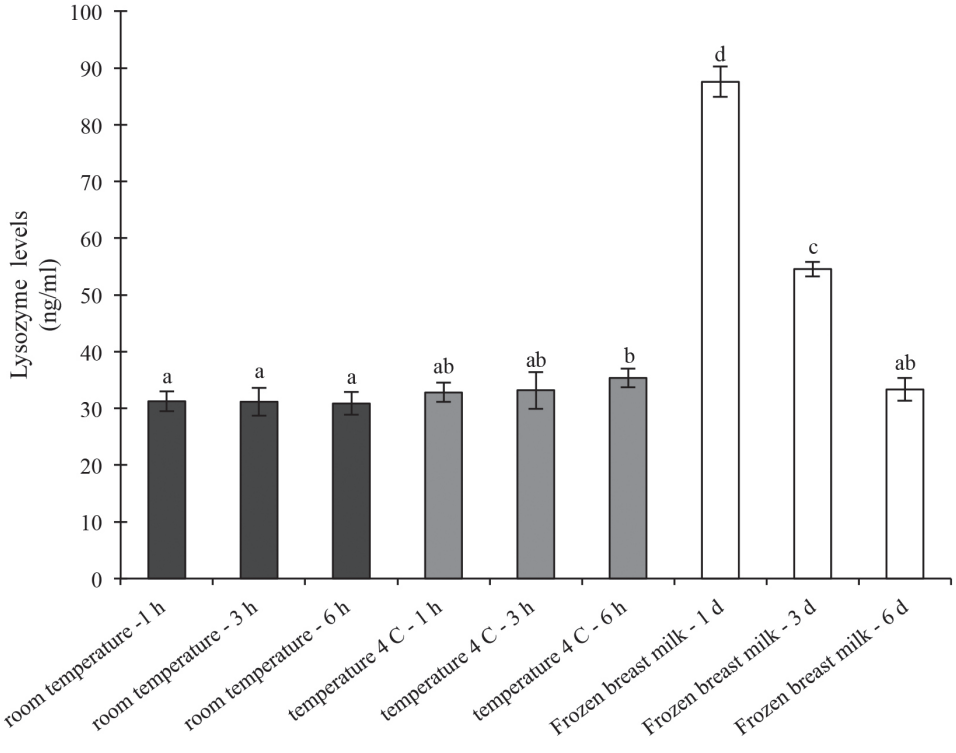

Figure 3. The levels of lysozyme in human milk at different temperatures and duration of storage time. Different letters showed significant difference compared to other groups based on DMRT post-hoc test.

CONCLUSION

The levels of lactoferrin and lysozyme in human milk are more stable and optimal at a storage temperature of -20°C (frozen human milk), which can be used to maintain their levels for the first six days. Further research is necessary to provide evidence of the antibacterial activities of lactoferrin and lysozyme in human milk at optimal storage time.

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Authors' contributions

K, principal investigator, conceptualised and designed the study, conducted the study, prepared the draft of the manuscript, reviewed the manuscript, and obtained funding; SBP was involved in data collection, prepared the draft of the manuscript, and reviewed the manuscript; ASU was involved in data collection and reviewed the manuscript; SNA assisted in data analysis and interpretation, assisted in drafting of the manuscript, and reviewed the manuscript.

Conflict of interest

Authors declare no conflict of interest.

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Association between nutrition knowledge and nutrition practice among Malaysian adolescent handball athletes

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ABSTRACT

Introduction: Previous literature suggests that good nutrition knowledge does not necessarily translate into desired eating behaviours among adolescent athletes, which may affect their sports performance. The purposes of the study were twofold, which were to examine nutrition knowledge and practice between the sexes, and to evaluate the magnitude of relationship between nutrition knowledge and practice among adolescent handball athletes in Malaysia. **Methods:** Three hundred and twelve male and female adolescent handball players competing in a national level competition voluntarily participated in this study. The participants were asked to complete a questionnaire which consisted of items on their demographic information and about their nutrition knowledge and eating practices. Independent t-test and Pearson's correlation were used to compare nutrition knowledge and practice between the sexes and to describe the relationship between variables, respectively. **Results:** The results showed that there were no significant differences (p>0.05) observed for nutrition knowledge and practice between the sexes. Besides, the results demonstrated that nutrition knowledge had a significant but weak positive correlation with eating habit (r=0.114, p=0.003) among the athletes. **Conclusion:** This study showed that nutrition knowledge and practice were moderately adequate, and that knowledge does not necessarily translate into good dietary practices. Therefore, the athletes would benefit from a comprehensive and innovative nutrition education, a strategy which warrants further investigation.

Keywords: nutrition knowledge, dietary practice, young athletes, handball

INTRODUCTION

Nutrition is one of the determinants for sports performance among athletes. Nutritional requirement changes as an individual move through different life stages. As such, adolescent athletes require unique nutritional consideration because they undergo altered hormonal, metabolism, and body composition changes following the onset of puberty. Young athletes need adequate energy to fulfil the requirements for both growth and development, as well as the demands for daily training and competition. Indeed, the Sports Dietitians Australia (SDA) has established a position statement about nutrition for adolescent athletes to address the aforementioned

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issue. SDA recommends that energy intake from carbohydrate, fat and protein should follow the guidelines set for adult athletes with some adjustments to match the energy demands (Desbrow *et al.*, 2019).

Muia *et al.* (2016) stated that it is common for adolescent athletes to experience inadequate energy due to heavy training. Nutrition adequacy depends on many factors such as nutrition knowledge, eating habit, training intensity, socioeconomic status, access to foods, to name a few. Much of the literature showed that athletes do not have adequate nutritional status for their respective sports because they do not follow the dietary guidelines (Heikura *et al.*, 2017). For instance, Jenner and colleagues (2019) concluded that professional and semi-professional team sport athletes did fulfil the recommendations for protein and fat intakes during training and competition, but not energy and carbohydrate recommendations. It is crucial that athletes consume sufficient energy and micronutrients to achieve optimal sports performance and to enhance recovery.

Likewise, nutritional inadequacy among adolescent athletes is caused by poor nutrition knowledge (Spronk *et al.*, 2015; Andrews *et al.*, 2016; Condo *et al.*, 2019). Lack of nutrition knowledge may be attributed to incorrect information received by the athletes. Generally, coaches are the person in closest contact with the athletes, thus athletes often rely on their coaches for nutrition information. However, a systematic review on athletes' and coaches' nutrition knowledge showed that coaches were not the best source of valid information for general and sports nutrition (Trakman *et al.*, 2019). Torres-McGehee *et al.* (2012) compared the nutrition knowledge among 579 student-athletes, team coaches, athletic trainers, and strength conditioning experts, and found that coaches and student-athletes had lower nutrition score than the other two groups.

Besides, disparities in knowledge level and practice between the sexes were observed in a few studies. Spronk *et al.* (2015) showed that Australian elite females were more knowledgeable in terms of nutrition knowledge and diet quality compared to their male counterparts. In contrast, Hull *et al.* (2017) reported that the National Collegiate Athletic Association (NCAA) male athletes had less favourable eating habits than female athletes, who have associated body image and dieting concerns. Therefore, in order to maintain an ideal weight, they tend to practise healthy diet, which requires a good knowledge in nutrition. However, another study by Saribay & Kirbas (2019) showed otherwise. They evaluated the knowledge between adolescent male and female athletes in Turkey and revealed that no significant differences existed in nutrition knowledge between the sexes. These inconsistencies in data warrant further studies to assess whether the levels of nutrition knowledge and practice may differ between the sexes.

Nutrition knowledge is often associated with healthy dietary practices. However, a comprehensive review on nutrition knowledge and dietary intake suggested that athletes have adequate nutrition knowledge, but it does not always translate into proper nutrition practices (Spronk *et al.*, 2015). According to the Health Belief Model, a person's willingness to change their health behaviour depends on their health perception. This portrays that knowledge should be a powerful tool to alter athletes' perception about nutrition and thus habit, but unfortunately, this was not the case as shown by previous literature. This situation is not only prevalent among athletes, but also seen in medical students in a few countries,

such as Australia (Perlstein *et al.*, 2017) and Saudi Arabia (Eman *et al.*, 2015), whom we assume are groups of people who would adhere to the knowledge they possess to serve as role models for their patients. It is prudent to suggest that all population, regardless of their socioeconomic status, require solid nutrition knowledge to help them make wise food selections for healthy living and to sustain physical activity performance. Thus, it warrants further studies to assess whether the level of nutrition knowledge may influence dietary habits.

In Malaysia, there is still a scarcity of data regarding this issue, particularly in young athletes under 18 years old. Much of the available data were derived from adult athletes and non-athlete population (Razalee & Tan, 2014; Siti Soraya *et al.*, 2018). Therefore, this study aimed to compare the levels of nutrition knowledge and practice between male and female adolescent handball athletes competing in a national tournament, as well as to examine whether correlation exists between these variables. We hypothesised that there will be no significant differences in terms of nutrition knowledge and practice between the sexes, and that no significant association will be observed between nutrition and dietary practice among the athletes.

MATERIALS AND METHODS

Study population

A cross-sectional study was conducted among the adolescent handball athletes aged between 13 to 18 years old (under 18 years old category) who participated in the national level Majlis Sukan Sekolah Malaysia (MSSM) competition for school athletes in 2019. MSSM is a prestigious tournament which comprises of the best athletes representing all 14 states of Malaysia. All athletes have undergone systematic training sessions with their own team coach before the competition. A total of 336 athletes were asked to participate in this study and the response rate was 92.8%. There were 155 male and 157 female athletes who agreed to volunteer for this study. The sample size of 312 was considered sufficient to detect a difference at a significance level of 0.05 and 95% confidence based on Krecjie & Morgan (1970). Parental and athletes' informed consents were obtained after being given verbal and written information about the study. The study protocol was reviewed and approved by the Universiti Pendidikan Sultan Idris Ethical Advisory Committee (Ethical approval serial no: 2019-0037-01).

Instrument

In this descriptive study, the participants were asked to complete a questionnaire adapted from Razalee & Tan (2014). The permission to use and modify the questionnaire was obtained from the original authors before commencing the study. Before administering it to the participants, the questionnaire was evaluated for content validity by three experts in nutrition. The content validation index (CVI) of the revised questionnaire scored 0.98 for relevance and ambiguity, and 0.99 for clarity and simplicity. The validated questionnaire was then piloted among 30 young athletes to assess its reliability. The *r*-value for the questionnaire was 0.89 as calculated using Cronbach's alpha, suggesting that the questionnaire had high internal reliability.

The participants answered their demographic information in Part A, while Parts B and C consisted of items about nutrition knowledge and practices, respectively. There were 11 statements about nutrition knowledge with True and False options. A correct response was coded as 1 and 0 for incorrect answer. The total score of 11

Table 1. Source of nutrition information of the participants (*n*=312)

Source of nutrition information	*Frequency (n)*	*Percentage (%)*
Internet		
Yes	170	54.4
No	142	45.5
Magazines, newspapers		
Yes	96	30.7
No	216	69.3
Social media		
Yes	7	2.2
No	305	97.8
Nutritionist		
Yes	31	9.9
No	281	90.1
Doctors		
Yes	90	28.8
No	222	71.2
Physical and health education class		
Yes	181	58.0
No	131	42.0
Team coach		
Yes	286	91.7
No	26	8.3

items were converted into percentage and characterised as poor nutrition knowledge (50% and below), moderate (51-79%), and good knowledge (80-100%), which was predetermined prior to the study.

To measure nutrition practice, 14 items were asked with a scale ranging from 1 (never; does not occur at all), 2 (sometimes; 1-2 day/week), 3 (often; 3-4 day/week), and 4 (always; 5-7 day/week). There were eight statements about positive nutrition habit and the score was given in descending order from 4 (always) to 1 (never). On the contrary, for the remaining six items of undesirable behaviours, the score was reversed ranging from 1 for inappropriate to 4 for very appropriate practice. The possible total scores for nutrition practice range from 14 to 56. The higher the total score, the better the dietary habits of the participants.

Procedure

Data of the athletes participating in the 2019 MSSM Handball Championship was obtained from the Education Department of Perak as event coordinator. The coaches of each state were informed about the study and were asked for their permission to allow their athletes to participate in the study on voluntary basis. During the seven days of the tournament period, the athletes were recruited and informed consent was obtained from the athletes and their parents.

Body weight and height were measured closest to 0.1kg and 0.1cm using calibrated digital weighing scale (Omron HN-288, Germany) and stadiometer (Charder HM-200P, USA), respectively. The participants were given approximately 20 minutes to answer the questionnaire in a quiet area to minimise interference from the crowd who were watching the games.

Statistical analysis

Data analysis was conducted using the Statistical Package for Social Sciences

Table 2. Frequency and percentages of correct answer on nutrition knowledge of the athletes (*n*=312)

Item		*Correct responses n (%)*
1.	Skipping breakfast can negatively affect athletic performance	282 (90.4)
2.	Nutrition affects sports performance	294 (94.2)
3.	According to the Food Pyramid Malaysia, one should consume 4-8 servings from the bread, cereal, rice and pasta group	191 (61.2)
4.	According to the Food Pyramid Malaysia, one should consume 2 servings of fruits and 3 servings of vegetables	261 (83.7)
5.	According to the Food Pyramid Malaysia, one should consume 2-3 servings from the dairy group	218 (69.9)
6.	According to the Food Pyramid Malaysia, one should consume 1/2-2 servings from the meat group	226 (72.4)
7.	Eating breakfast can improve concentration	297 (95.2)
8.	If you are not thirsty, then you must not be dehydrated	70 (22.4)
9.	Athletes should not consume drinks during training	125 (40.1)
10.	Coaches should not allow the athletes to consume drinks during training	140 (44.8)
11.	Coaches should not allow the athletes to consume drinks during competition	147 (47.0)

(SPSS) version 23.0 (IBM Corp, Chicago, USA). All data sets were found to have a normal distribution using the Shapiro-Wilk test. Parametric data were reported as mean±standard deviation (*SD*). Descriptive data were presented as frequency and percentage. Independent t-test was used to compare the nutrition knowledge and practice between the sexes, while correlations between variables were determined by Pearson's correlation test. A significant level was set at $p<0.05$.

RESULTS

One hundred fifty-five males and 157 female adolescent athletes participated in this study. They had a mean age of 16±1 years, height and body weight of 165.9±0.9cm and 62.2±12.9 kg, respectively. Table 1 shows the source of nutrition information of the participants. The participants listed team coaches (91.7%) as the most frequent source, followed by parents (68.3%), physical and health education class (58.0%), internet (54.5%), doctors (28.8%), magazines and newspapers (30.7%), nutritionist (9.9%), and social media (2.2%).

The frequency and percentages of correct answers for nutrition knowledge items are listed in Table 2. More than 60% of the participants had correct answers on the importance of breakfast and the Malaysian Food Pyramid. However, less than 50% of them responded correctly on hydration and fluid intake for training and competition. In terms of nutrition knowledge level, 55.3% of 312 participants scored well in nutrition knowledge, meanwhile 43.1% and 1.6% of them had moderate and poor nutrition knowledge, respectively.

Table 3 shows the frequency and percentages of nutrition practice among the participants. Majority of the participants reported good dietary habit on items such as consumed breakfast regularly (74.4%), did not skip three meals a day (70.6%), ate enough fruits (78.8%), consumed a lot of plain water (95.8%), and took sufficient carbohydrate (81.1%)

Table 3. Frequency and percentages on nutrition practice of the athletes (*n*=312)

Item	*Never* *n (%)*	*Sometimes* *n (%)*	*Often* *n (%)*	*Always* *n (%)*
1. How often do you eat breakfast in the morning?	9 (2.9)	71 (22.8)	76 (24.4)	156 (50.0)
2. Based on three meals per day, how often do you skip at least one meal per day?	61 (19.6)	159 (51.0)	73 (23.4)	19 (6.1)
3. How often do you take vitamin supplements?	135 (43.3)	132 (42.3)	29 (9.3)	16 (5.1)
4. How often do you take mineral supplements?	150 (48.1)	114 (36.5)	36 (11.5)	12 (3.8)
5. How often do you eat fish, meat, or seafood?	6 (1.9)	54 (17.3)	115 (36.9)	137 (43.9)
6. How often do you drink plain water?	0 (0.0)	13 (4.2)	48 (15.4)	251 (80.4)
7. How often do you drink carbonated beverages?	26 (8.3)	64 (20.5)	197 (63.1)	25 (8.0)
8. How often do you eat breads, cereals, pasta, potatoes, or rice?	3 (1.0)	56 (17.9)	117 (37.5)	136 (43.6)
9. How often do you eat fruits, such as apples, bananas, or oranges?	6 (1.9)	60 (19.2)	148 (47.4)	98 (31.4)
10. How often do you eat vegetables, such as broccoli, tomatoes, carrots, or salad?	27 (8.7)	06 (34.0)	89 (28.5)	90 (28.8)
11. How often do you eat dairy products such as milk, yoghurt, or cheese?	25 (8.0)	137 (43.9)	92 (29.5)	58 (18.6)
12. How often do you eat berry jams, cookies, candies, or other sweets?	8 (2.6)	160 (51.3)	106 (34.0)	38 (12.2)
13. How often do you eat fast food?	12 (3.8)	101 (32.4)	166 (53.2)	33 (10.6)
14. How often do you seek out nutrition information?	71 (22.8)	185 (59.3)	44 (14.1)	12 (3.8)

Table 4. Nutrition knowledge and practice between the sexes (*n*=312)

Variables	*Male athletes (n=155) Mean±SD*	*Female athletes (n=157) Mean±SD*	*95% CI*	*p-value*
Nutrition knowledge (number of correct answers)	8.5±1.4	8.7±1.3	(-1.894, 0.049)	0.179
Nutrition knowledge (% of correct answers)	77.3±12.1	78.6±13.1	(-0.093, 0.494)	0.109
Nutrition practice score	36.7±5.0	37.6±3.6	(-0.842, 4.489)	0.076

and protein sources (80.8%). However, 71.1% of the participants reportedly consumed carbonated beverages quite regularly, 42.7% seldom ate vegetables, 46.2% took sweets, 63.5% ate fast foods, and only 17.9% of them looked for information about nutrition.

Table 4 presents the mean differences in nutrition knowledge and practice between the sexes. Both groups had no significant differences in mean score of nutrition knowledge in terms of numbers and percentages of correct answers. On average, they correctly answered eight out of 11 questions about the food pyramid, the importance of breakfast, as well as dietary intake for sports performance. The mean score for percentages of correct answer for male and female athletes were 77.3±12.1% and 78.6±13.1%, respectively, indicated that both groups of athletes had

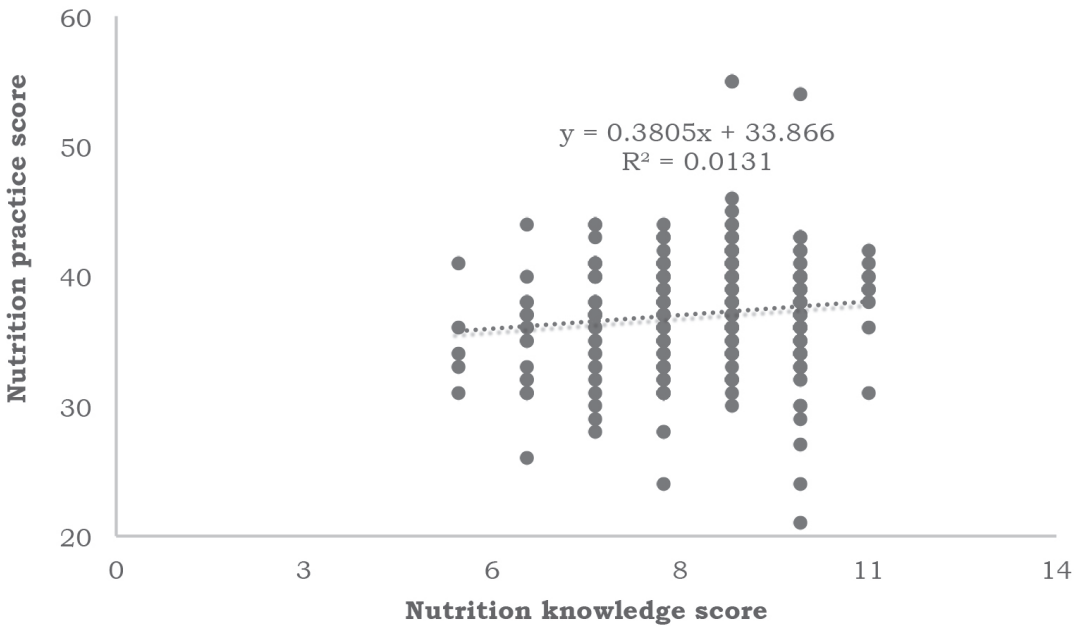

Figure 1. Relationship between nutrition knowledge and practice among adolescent handball athletes (*n*=312)

moderate level of nutrition knowledge. Furthermore, the findings on nutrition practice showed that female athletes scored higher than their male counterparts, but the difference was not significant. Both groups only scored a little higher than 50% of the total mark, which inferred that they did not practise proper eating habits.

A correlation between nutrition knowledge and practice of the athletes is shown in Figure 1. Pearson's correlation test reported a significant but weak positive relationship between these variables (r=0.114, p=0.003). This suggests that nutrition knowledge had a small, yet significant influence on the dietary habits of athletes.

DISCUSSION

Overall, this study revealed that both male and female adolescent handball athletes had moderate level of nutrition knowledge and practice, which were not significantly different between groups. Although they had moderate nutrition knowledge, it only contributed slightly to their dietary habits.

Most participants gave a correct answer to an average of eight out of 11 questions tested about general nutrition and sports nutrition, giving a mean knowledge score within the range of 77-78%, which is at a moderate level. Many of the participants in our study knew that skipping breakfast has a negative effect on cognitive and sports performance, and were aware about meal suggestions based on the food pyramid. However, they were lacking in terms of the importance of fluid intake to prevent dehydration during training (Table 2). The American College of Sports Medicine (ACSM) has established in a position stand on fluid replacement that athletes should drink fluid sufficiently before and during training or competition to prevent at least 2% body mass loss in order to sustain sports performance (Sawka *et al.*, 2007).

Our results were consistent with previous literature. For instance, Walsh *et al.* (2011) who evaluated the nutrition knowledge among 203 senior school male rugby athletes in Ireland concluded that the athletes had poor knowledge of the foods required for refuelling, appropriate use of sports drinks, and the role of protein in muscle formation, with an overall knowledge score of 59.6%. Similarly, Heikkila *et al.* (2018) revealed that the nutrition knowledge among young Finnish endurance athletes was relatively low (73%), particularly in the area of nutrition recommendations for endurance athletes and dietary supplements. It is very important for athletes to have correct information on the nutrition recommendations for their sports to ensure they get sufficient energy to perform optimally on-field. Indeed, ACSM and the Academy of Nutrition and Dietetics highly recommend that athletes should be referred to registered dietitians and nutritionists for a personalised nutrition plan because well-chosen nutrition strategies are crucial to athletes' performance and recovery (Thomas, Erdman & Burke, 2016).

Results on the sources of nutrition information showed that 91.7% of adolescent handball athletes get their nutrition information from their coaches. Some studies have pointed out that team coaches may not be the best person to consult regarding nutrition as they may not have the proper knowledge necessary to give accurate advice to the athletes (Torres-McGehee *et al.*, 2012; Botsis & Holden, 2015). Even though our study did not assess the nutrition knowledge of coaches, this issue remains a concern since coaches are the nearest person and are an important resource for the athletes as shown in our

study. The National Coaching Academy of National Sports Institute (AKK-ISN) of Malaysia has been continually offering Sports Science courses (Level 1 to Level 3), which comprise all elements of sports sciences, including sports nutrition, to coaches. But, up to date, no data is available on how many coaches have participated in and benefited from these courses. Therefore, future investigation is necessary to examine the Malaysian coaches' nutritional knowledge and to determine whether or not it is sufficient to avoid misconception among athletes.

Besides coaches, online platform was also a popular source of nutrition information among half of our participants, which ranked third in the list (54.4%). During this digital technology era, it is obvious that most nutrition information is widely available online. However, less is known about the information provider, which may lead to misinformation among the readers (Kitchen, Harle & Li, 2014). Therefore, digital literacy should be introduced to young athletes to help them make better judgement towards the abundance of nutrition information available online. With the enthusiasm and engagement of the young population towards digital technology, it is timely for nutritionists and dietitians to make full use of the online platform in disseminating correct information about nutrition.

It has been shown that from our study and previous literature that adolescent athletes are struggling to acquire different aspects of nutrition knowledge. Despite that, a similar trend has been observed that most athletes have inadequate level of nutrition knowledge. Without good knowledge, it is impossible to change the attitude and dietary habit to a better one. An individual must possess health literacy in order to appreciate nutrition knowledge. According to Peerson & Saunders (2009), health literacy is one's ability to obtain, read, understand, and use health information and services to make appropriate everyday health care decisions. The National Health and Morbidity Survey (NHMS) 2019 reported that one in three individuals in Malaysia had low health literacy (IPH, 2020). One of the factors that affects health literacy is access to information. Therefore, with the progress in digital technology, innovative nutrition education strategies utilising online access are needed to improve the knowledge of athletes.

Moreover, the results of nutrition practice showed that our athletes had practised healthy dietary habits moderately with regards to breakfast consumption and fluid intake during training and competition. Many studies showed similar trends in eating habit among athletes. Condo *et al.* (2019) who studied the nutritional intake and sports nutrition knowledge among adult female Australian athletes concluded that their athletes did not practise proper nutritional habits as there were inadequate intakes of carbohydrate and calcium. Another study by Normah (2014) on the hydration knowledge, attitude and fluid consumption behaviour among student athletes in a sport school in Malaysia showed that the athletes had misconception about the use of salt tablets to prevent dehydration, inappropriate use of thirst as an indicator for dehydration, and the need to consume sports drinks within two hours after exercise or training. ACSM has established a statement that sports drink is highly recommended for individuals undergoing training or exercise of more than an hour because it offers glucose for recovery, sodium to retain body water, and of course, fluid to replace water loss (Sawka *et al.*, 2007).

The results of the current investigation found that there were no significant differences in nutrition

knowledge and practice between male and female athletes. To the best of our knowledge, this was the first study that looked into the nutrition knowledge and practice among competitive adolescent handball athletes in Malaysia, and the results obtained were quite the opposite to cited literatures from other countries. Hull *et al.* (2016) who conducted a survey among male and female NCAA Division 1 athletes concluded that women tend to have more desirable habits such as cooking meals for themselves and not skipping breakfast. In contrast, male athletes showed higher occurrence of undesirable eating behaviours such as consuming fast foods or restaurant meals more frequently and having a higher consumption of alcohol during competitive season. On the other hand, Fortes *et al.* (2014) reported that female athletes had higher frequency of high-calorie diet restriction and were more dissatisfied with their body image compared to male adolescent athletes, which may lead to eating disorders. In our study, no differences were observed in terms of nutrition knowledge and practice between the sexes. This is likely due to the types of sports they play, namely team sport. Thus, they would not have much concerns about weight management and physical appearance as long as they can perform well in the tournament, and thus have less risk of engaging in unhealthy eating habits. Evidence has suggested that most unhealthy eating behaviours occur in sports that emphasise leanness or weight category, particularly in female athletes (Fortes *et al.*, 2014). Therefore, this topic remains inconclusive and requires additional investigation, in particular the comparison of dietary practices among athletes from different sports categories.

Furthermore, findings of the current study concluded that there was a significantly weak positive relationship between nutrition knowledge and practice among the athletes. Athletes who have higher knowledge would presumably practise desirable eating habits, but the relationship did not hold true for the majority of our respondents. If we look at the results on the sources of nutrition information, 58% of our participants claimed that they obtained their nutrition information from attending physical and health education classes. In Malaysia, nutrition education is embedded in the school curriculum and taught in Health Education in both primary and secondary schools. The lengthy period of nutrition exposure should have become a good nutrition base for our athletes. However, it is not assured that they will gain and adhere to the knowledge despite having attended nutrition-related courses over a period of school years. This statement is supported by a study from Andrews *et al.* (2016), who examined sports nutrition knowledge among 123 Division 1 NCAA student-athletes. They concluded that previous exposure to nutrition course did not significantly improve nutrition knowledge among athletes, and that this was probably because they were less motivated to focus on the nutrition component of their own sport and performance.

In fact, some studies also showed similar findings as our study, that even though the subjects had adequate knowledge, it did not translate into favourable eating behaviours (Lohman, Carr & Condo, 2019; Walsh *et al.*, 2011). They reasoned that the misalignment of this concept was due to attitude towards food intake and eating behaviours. Walsh *et al.* (2011) measured nutrition knowledge, attitude and behaviour among adolescent rugby athletes and concluded that they demonstrated poor knowledge and nutrition practice

despite having positive attitude towards nutrition. Our study did not measure the attitude of the athletes towards eating behaviour, but based on previous research, we speculate that the absence of good attitude towards nutrition among our athletes may have also contributed to inadequate knowledge and improper dietary practices. This highlights the need for some educational intervention to increase awareness towards nutrition and desirable dietary practices among adolescent athletes. An example of educational strategy which could be considered by educators is game-based learning. Many literature in the field of information technology, computer science, game design and engineering have shown the effectiveness of gamification in motivating and engaging students compared to traditional lectures (Licorish *et al.*, 2019). Johnson and colleagues (2016) concluded from their systematic review that out of 19 papers presenting empirical evidence about gamification for health and well-being, 59% reported positive outcomes, while 41% had moderate to lower quality of evidence. Therefore, research on gamification in health and well-being is warranted to improve the knowledge and behaviours of learners.

Limitation of the study

Despite offering some new insights into the knowledge and practice of nutrition among competitive adolescent athletes, our study also had some drawbacks. The use of self-administered report has been argued as being less trustworthy because the participants may not respond honestly to the questionnaire (Torstveit, Rosenvinge & Sundgot-Borgen, 2008). However, our study used a large sample involving more than 300 respondents in a field-setting, thus, a questionnaire was the recommended instrument because it was easy, convenient and inexpensive. In fact, we had at our very best effort explained the questionnaire verbally and with written instruction to the participants, and made sure they answered in our presence in designated areas. Another limitation of the study was that we limited the data collection to one specific sport, which is handball. Therefore, our data only represent the nutrition knowledge and practice of adolescent team sport athletes and may not be true for athletes in the individual sport categories. Future studies should include a more comprehensive sample of competitive adolescent athletes from different sport categories.

CONCLUSION

This was the first study to assess the relationship between nutrition knowledge and practice among competitive adolescent handball athletes in Malaysia. This study demonstrated a significant but weak positive relationship between nutrition knowledge and practice in the athletes. The overall nutrition knowledge and practice were similar in both sexes, with only a moderate level of understanding of nutrition and practice of desirable behaviours, highlighting the need for nutrition education interventions. Future research should consider the role of health literacy in order to strengthen understanding of nutrition knowledge and enhance translation of knowledge into healthful practices that will benefit the athletes' well-being and sports performance.

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Authors' contributions

NJ, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript, and reviewed the manuscript; JLFL, led the data collection, advised on data analysis and interpretation, and reviewed the manuscript; RYT, assisted in data collection and reviewed the manuscript; SHA, assisted in data collection and reviewed the manuscript; AS, advised on data analysis and interpretation, and reviewed the manuscript.

Conflict of interest

The authors have no conflict of interest or relevant financial relationship in this study.

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Proximate, mineral and fatty acid compositions of healthy recipes used in Fit, Eat, Active, Training (F.E.A.T) programme

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ABSTRACT

Introduction: Many people are seeking knowledge and skills about preparing healthy meals at home. This study aimed to determine the content of nutrients in 60 healthy dishes prepared for the recipe book *Mudah, Sihat, Sedap* used in the F.E.A.T nutrition intervention programme. The recipe book was developed as an educational material to guide participants in preparing healthy meals at home. **Methods:** A needs assessment survey was done to identify food preferences for breakfast, lunch and dinner of obese and overweight respondents. Recipes for these foods were then modified to reduce the calorie, fat, and sugar contents, while adding in fibre sources. Proximate analysis was done based on standard methods of AOAC, while total carbohydrate and calorie contents were calculated using the differentiation and Atwater methods, respectively. Mineral content was determined using atomic absorption spectrophotometer, while fatty acids content was analysed by gas chromatography using the FAME method. **Results:** Proximate analysis results showed that *Kobis Goreng* had the most calories (305 kcal/100g) (p<0.05) in all categories. Mineral analysis showed that sodium was significantly highest (p<0.05) in *Asam Pedas Daging* (554 mg/100g). For fatty acid analysis, *Masak Lemak Telur Itik* had the highest amount of MUFA (45.1%), *Masak Lemak Sotong* had the highest amount for SFA (71.8%), and *Masak Lemak Cili Api Udang* had the highest amount of PUFA (33.7%). **Conclusion:** This study successfully modified original recipes of selected cuisines used in the recipe book, which contained lower calories compared to original recipes. The calorie and nutrient values for each recipe will be included as nutrition composition information in the recipe book.

Keywords: obesity, calories, proximate contents, mineral, fatty acid

INTRODUCTION

The World Health Organization (WHO, 2020) defines being overweight and obese as an excessive accumulation of fat which poses a health risk. The leading cause of obesity is an imbalance in caloric intake and calories expended, in addition to increased consumption of

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energy-dense foods which are high in fat and sugar. Based on the National Health and Morbidity Survey (NHMS) 2019, one in two adults in Malaysia are overweight or obese. In addition, findings from both NHMS 2015 (IPH, 2015) and NHMS 2019 (IPH, 2019) have indicated that the obesity rate among women is higher than among men. Since overweight and obesity are correlated with increased risk of developing a broad range of non-communicable diseases, such as diabetes, cardiovascular diseases, cancers, and chronic respiratory diseases, public health authorities have identified the prevention of obesity as a high priority initiative to improve public health (WHO, 2020; Fukuda *et al.* 2016).

Obesity prevention initiatives around the world have focused primarily on two approaches: (1) encouraging healthy eating, with a focus on reducing energy intake through reducing total calories consumed from fats and sugars, while increasing portion of fruits, vegetables, and whole grains; and (2) increasing physical activity, by engaging in regular physical activity for about 150 minutes per week for adults (WHO, 2020). Jordan *et al.* (2010) showed that nutrition education could help in the adoption of a healthy eating lifestyle. Healthy foods are those that provide nutrients to sustain the body's well-being and retain energy. Water, carbohydrates, fat, protein, vitamins, and minerals are the key nutrients that make up a healthy and balanced diet.

In this study, laboratory analyses which include moisture, ash, lipid, protein, carbohydrate, mineral, and fatty acid analyses measure the actual levels of nutrients in foods, thus providing a high level of accuracy of the analyses. Moisture or water content of food is an essential constituent in the food composition databases. The variability of water content in foods affects its composition (Nielsen, 2017). Ash is an important part of proximate analysis, which comprises of inorganic residue remaining after water and organic matters have been removed by the heating process to obtain nutritional information. Proteins are important constituents of foods for different reasons. They are a major source of energy, as well as containing essential amino acids, which are essential to human health, but which the body cannot synthesise. Proteins are also the major structural components of many natural foods, often determining their overall texture, e.g., tenderness of meat or fish products. Lipids, proteins, and carbohydrates constitute the main structural components of foods. An accurate and precise analysis of lipids in foods is important for accurate nutritional labelling, meeting manufacturing specifications, and determining whether a food meets the standard of identity (Nielsen, 2017). Mineral element determination in human diets is very important for human health, due to the presence of essential and toxic elements in foods or their incorporation in the manipulation of cooking process.

This study hypothesised that improving diet by modifying meal ingredients to contain less calories, fat, sodium, and sugar, while increasing fibre may be among the most effective strategies for weight management in obese and overweight individuals. Nutrition information provided in recipe books are useful for the public to get information on healthy nutrition and further prevent obesity. Therefore, this study was conducted to determine the calorie and nutrient compositions of modified recipes from selected cuisines listed in the healthy recipe book *Mudah, Sihat, Sedap* that was used in the Fit, Eat, Active, Training (F.E.A.T) programme.

MATERIALS AND METHODS

The study protocol was approved by the Research Ethics Committee of Universiti Kebangsaan Malaysia (UKM PPI/111/8/JEP-2016-392). All research participants gave their permission to be part of the study and signed the informed consent form to participate. The food preferences of participants for breakfast, lunch and dinner were collected in Malacca. Malacca was selected as the study location since it had the second highest prevalence of obesity in Malaysia prior to the implementation of the study (IPH, 2015). Although Wilayah Persekutuan Putrajaya showed the highest prevalence of obesity in Malaysia at that time (43%), it was however not selected as the research area of choice because of logistic consideration by the research team.

The interview questions comprised of the participants' food preferences and favourite foods. This was to identify eating habits, most frequently consumed foods, and foods usually prepared at home. The 23 overweight and obese respondents provided the names of 41 types of foods which were then modified into healthier cuisines (Table 1) using the Nutritionist Pro™ software (Axxya Systems, United States), and thereafter prepared by professional chefs. The preferred cuisines reported by the respondents was part of the needs assessment results (Table 1) prior to the development of recipe book and implementation of the F.E.A.T programme. Another 12

Table 1. List of food preferences by respondents

Meal time	*Top five foods preferred*	*n*	*Percentage of foods mentioned (%)*
Breakfast (*n*=46)	*Nasi Lemak*	8	17.4
	Fried Rice	7	15.2
	Biscuits	5	10.9
	Bread	5	10.9
	Fried Noodle	5	10.9
Lunch (*n*=52)	*Asam Pedas*	10	21.7
	Masak Sambal/Pedas	10	21.7
	Ikan/Ayam Masak Lemak	6	11.5
	Stir-fried Vegetables	5	9.6
	Curry	5	9.6
Afternoon tea (*n*=24)	Banana Fritters	9	37.5
	Cekodok	4	16.7
	Bread	4	16.7
	Biscuits	3	12.5
	Others	1	4.1
Dinner (*n*=34)	*Asam Pedas*	6	17.6
	Ikan/Ayam Masak Lemak	6	17.6
	Masak Sambal/Pedas	5	14.7
	Noodle-based cuisines	3	8.8
	Curry	2	5.9

low calorie juices were developed by nutritionists using sources of fruits and vegetables, which contained less sugar. Sensory analysis comprising of attributes like taste, smell and texture of the cuisines was conducted among all nutritionists, research team members and chefs following the preparation of these modified recipes.

This paper focuses on the proximate analysis of foods listed in the recipe book. The study design was laboratory analysis based on standard methods. This study only reported the food analysis of the modified recipes. The food intake of the respondents is reported in the effectiveness of the F.E.A.T programme and is published elsewhere.

Sample preparation

The recipe book consisted of recipes for 48 dishes and 12 types of beverages. All recipes were modified to be healthier, by using cooking methods, such as steaming, shallow frying, boiling, baking, or no usage of oil at all, instead of deep frying, substituting coconut milk with low fat milk or yoghurt, reducing use of sugar, increasing use of high fibre ingredients such as *atta* flour instead of wheat flour, and preparing juices using fruits, low fat milk, vegetables, oats and honey or raisins instead of sugar as shown in Table 1. The cuisines were prepared at two different times and had 4 replicates for proximate and mineral analyses; 14 recipes that included yoghurt and low-fat milk as ingredients were prepared in duplicates for fatty acids analysis. All samples were weighed per serving and recorded (Table 2). The samples were homogenised, stored in labelled containers and kept at -20°C prior to analysis.

Chemical analysis

Moisture content

The moisture content of the food and beverage samples was determined according to the oven drying method introduced by the Association of Official Analytical Chemists (AOAC) (2007). According to this method, moisture content was reported as the difference between the weight of a sample before and after drying. The edible portion of the food was separated and homogenised using a blender. Approximately 5 g of sample was weighed and dried overnight at 105˚C until constant weight was obtained.

Total ash content

The ash content of all the samples were determined according to a method provided by AOAC (2007). The ash content of the sample symbolised the inorganic residue after burning of the organic matter. A porcelain crucible with its lid was pre-ignited overnight at around 450-550°C in a muffle furnace, cooled in a desiccator, and weighed after it reached room temperature. Approximately 5 g of sample was weighed and placed on a hot plate until the sample has turned black and no smoke was produced. The crucibles were then placed in the furnace at 550˚C overnight.

Crude protein content

The crude protein content of all the samples were determined according to the Kjehdahl method by AOAC (2007) using a Kjeltec System 2200 Distilling Unit (Foss Tecator, Hoganas, Sweden). The Kjeldahl method involves the digestion of food with a strong acid so that nitrogen is released, which is then quantified using a titration technique. Protein quantity is then calculated from the nitrogen concentration of the food using a conversion factor (usually 6.25, which is equivalent to 0.16 g nitrogen per gram of protein). Approximately 2 g of sample was placed into the digestion tubes. Potassium sulphate and copper sulphate were added into each digestion tube as catalyst. About 12 ml of

concentrated sulphuric acid (H_2SO_4) was carefully added to the digestion tube, which was then gently shaken. The digestion procedure was performed using pre-heated (420˚C) digestion block for 60 minutes until a clear green solution was obtained. The digested sample was then cooled for 30 minutes. The distillation procedure was then performed using the distillation unit of Kjeltec 2200. Boric acid mixture was titrated with 0.2N hydrochloric acid (HCl) until the colour changed to that of the indicator. The volume of acid required in the titration was recorded.

Crude fat content

The fat content in all the samples were determined according to the Soxhlet method by AOAC (2007), using the 2055 Soxtec Avanti Manual System, Foss Tecator, Sweden. Soxhlet method is a traditional technique used for extracting lipids in foods, whereby the sample is grounded into small particles, and placed into a porous thimble. Mainly it has three compartments - flask, extraction chamber, and condenser. The sample is placed in a thimble and once the flask is heated, the solvent is evaporated and will move up to the condenser, where it is converted into a liquid and collected into the extraction chamber containing the sample. When the solvent passes through the sample, it extracts the fats and carries them into the flask. After completion of the extraction, the solvent is evaporated, and the mass of lipid remaining is measured and used for analysis (Nielsen, 2017). The instrument uses a solution of 40-60 % petroleum ether to extract the fat in the samples. Initially, samples of foods (3 g) and beverages (5 g) were weighed and placed in the thimbles. About 2-3 spatulas of celite were mixed with samples of beverages before being heated in a water bath for two (2) hours. The samples were then homogenised with sodium sulphate before being transferred to the thimbles for fat extraction. When the extraction process had completed, extraction cups were dried in the oven at 100°C for 15 minutes. The extraction cups were left to cool in the desiccator and they were reweighed until consistent weight has been reached. The fat content was calculated in percentage based on initial sample weight.

Carbohydrate content

Carbohydrate content was calculated using the formula [Carbohydrate=100 % - % (moisture + ash + crude protein + fat)] (Merrill & Watt, 1973).

Calorie content

The number of grams of carbohydrate, protein, and fat were multiplied by 4, 4, and 9, respectively and the values were added, based on the Atwater general factors and reported in the unit of kcal (Atwater & Bryant, 1900).

Mineral content

Mineral compositions (sodium, potassium, calcium, magnesium, iron, and zinc) were analysed using ash obtained during ash analysis (AOAC, 2007). These minerals were chosen due to their important roles and benefits to the human body (Gharibzahedi & Jafari, 2017). Approximately 5 ml of concentrated hydrochloric acid was poured into the crucibles containing ash to dissolve the samples. The crucibles were then placed in a hot water bath until the samples dried. About 2 ml of concentrated hydrochloric acid was then added to dissolve the leftover residue. Distilled water was added next into the crucibles and then the solution was poured into a volumetric flask for filtration process. Following that, the solution was cooled to room temperature, and deionised water was added until the solution reached 100 ml. Ash solutions were then analysed using Atomic

Absorption Spectrophotometer (Analyst 400, Perkin Elmer, United States) and a calibration curve was prepared for each analysed mineral using the standard solutions prepared.

Fatty acids composition

Fatty acids were extracted from the samples using the standard method of International Union of Pure and Applied Chemistry (IUPAC) 2.301 (1987) at an accredited food analysis laboratory. The profiling was done using gas chromatography equipped with a fluorescent ion detector (GC-FID). The FAMEs were identified by comparing their relative and absolute retention times. Fatty acids composition was reported as a relative percentage of the total peak area.

Statistical analysis

Data were analysed using the SPSS version 22.0 (SPSS Inc., Chicago, II, USA) software. Descriptive analysis, in the form of mean and standard deviations, was used to present the data. Analysis of variance (ANOVA) test was used to compare differences in the means of moisture content, ash content, crude protein content, carbohydrate content, fat content, and mineral content of food and beverage samples. After performing an ANOVA, a post-hoc test (Tukey's Honestly Significant Difference, Tukey's HSD) was conducted to determine the differences between the groups.

RESULTS AND DISCUSSION

Table 2 shows the comparison of calorie content between the original recipes based on estimation and laboratory analysis results of modified recipes for each cuisine. Based on the laboratory analysis, all 41 dishes showed lower calories after modifications compared to the original recipes, except for *Kobis Goreng* and *Sambal Ikan Bilis*. Another seven dishes created by nutritionists showed calorie values of within 47 to 295 kcal/100g; for juices, the calories were within 12 to 49 kcal/100g. Total calories for healthy juices analysed in this study were <50 kcal/100g and this result is consistent with the study conducted by Hegde, Adhikari & D'Souza (2013), which stated that fruits, such as lemon, orange, apple, and papaya, are low in calories. The energy value for *Kobis Goreng* after modification was found to be higher (305 kcal/100g) compared to the original recipe (205 kcal/100g). This might be due to the presence of fried soya bean curd (220 kcal/100g) and dried salted prawn (229 kcal/100g) in the modified recipe. For *Sambal Ikan Bilis*, the calorie content after modification (178 kcal/100g) was found to be nearly the same with estimation of the original recipe (170 kcal/100g), however the value was lower compared to that reported in the Malaysian Food Composition Database (MyFCD) (316 kcal/100g). Laboratory analysis results showed that for vegetable dishes, *Kobis Goreng* had significantly the highest (p<0.05) amount of calories (305 kcal/100g), followed by *Kerabu Telur* for meat and poultry cuisines (295 kcal/100g). In the fish and seafood category, *Asam Pedas Tenggiri* and *Sambal Udang* had the highest number of calories (221 kcal/100g and 129 kcal/100g, respectively) due to the high fat content in both recipes. The high fat content in *Cucur Ikan Bilis* was the major contributor to the total calories in that recipe (261 kcal/100g). This was due to the use of *atta* flour (358 kcal/100g) and anchovies (264 kcal/100g), which contributed a fair amount of calories to this recipe (MyFCD).

Moisture and total ash content

Table 3 showed that *Jus Labu Bersusu* had the highest (p<0.05) moisture

Table 2. List of cuisines and beverages from the recipe book [Cont'd]

Local name	*Common name*	*Modification made*	*Calorie content (kcal/ 100g)*		*Weigh per serving*
			Original recipe	*Modified recipe*	
Masak Lemak Labu Kuning	Pumpkin cooked in low fat milk	Substituted coconut milk with natural yoghurt	359	63	85
Pajeri Nenas	Pineapple cooked in yoghurt	1. Substituted coconut milk with natural yoghurt 2. No sugar added 3. Used olive oil	599	80	108
Kacang Buncis Goreng	Fried green beans	1. Reduced quantity of oil used 2. Used olive oil	146	64	74
Masak Lemak Sayur Campur	Mixed vegetables cooked in low fat milk	1. Substituted coconut milk with low fat milk 2. Added soy bean curd (*tempe*)	391	213	101
Masak Lemak Jantung Pisang	Banana flower cooked in low fat milk	1. Substituted coconut milk with low fat milk 2. Added carrot	555	219	56
Pajeri Terung	Brinjal cooked in yoghurt	1. Used brown sugar 2. Reduced quantity of oil used	287	154	65
Tomyam Campur	Mixed tomyam	Used boiling method instead of stir frying	135	55	66
Masak Lemak Nangka	Jackfruit cooked in low fat milk	1. Substituted coconut milk with low fat milk 2. Added celery and tomato	266	94	61
Masak Lemak Telur Itik	Duck egg cooked in low fat milk	Substituted coconut milk with low fat milk	273	96	71
Kari Ayam	Chicken curry	1. No sugar added 2. Substituted coconut milk with natural yoghurt 3. Added carrot and tomato	237	119	83

Table 2. List of cuisines and beverages from the recipe book [Cont'd]

Local name	*Common name*	*Modification made*	*Calorie content (kcal/ 100g)*		*Weigh per serving*
			Original recipe	*Modified recipe*	
Kari Daging	Beef curry	1. Substituted coconut milk with natural yoghurt 2. Reduced quantity of oil used 3. No sugar added	355	182	89
Daging Masak Kicap	Beef cooked in soy sauce	1. Added carrot 2. Reduced quantity of oil used 3. No sugar added	258	119	95
Ayam Masak Kicap	Chicken cooked in soy sauce	1. Reduced quantity of oil used 2. Substituted tomato sauce with fresh tomato	344	111	93
Ayam Masak Sambal	Chicken cooked in chilli paste	1. Roasted the chicken instead of frying 2. Reduced quantity of oil used	383	122	90
Sambal Telur	Egg cooked in chilli paste	1. Reduced quantity of oil used. 2. Added tomato and peas 3. Tomato sauce not used	288	104	64
Masak Lemak Cili Api Ayam	Chicken cooked in low fat milk with bird's eye chilli	1. Substituted coconut milk with low fat milk 2. Added *Ulam Raja*	617	105	96
Masak Lemak Cili Api Daging Salai	Beef cooked in low fat milk with bird's eye chilli	1. Substituted coconut milk with low fat milk 2. Added fern shoots	157	100	80
Ayam Bakar Berempah	Spiced roasted chicken	Roasted the chicken instead of frying	510	227	88
Asam Pedas Daging	Beef cooked in spicy sour sauce	1. Used olive oil 2. Reduced quantity of oil used	268	161	94

Table 2. List of cuisines and beverages from the recipe book [Cont'd]

Local name	*Common name*	*Modification made*	*Calorie content (kcal/ 100g)*		*Weigh per serving*
			Original recipe	*Modified recipe*	
Masak Lemak Cili Api Udang	Prawns cooked in low fat milk with bird's eye chilli	1. Substituted coconut milk with low fat milk 2. Added roasted soy bean curd 3. Added string beans	490	65	53
Udang Masam Manis	Sweet and sour prawn	1. Reduced quantity of flour used 2. Used shallow frying instead of deep frying	306	62	54
Sotong Masak Kicap	Cuttlefish cooked in soy sauce	1. Reduced quantity of oil used 2. Added long beans and spring onions	210	94	57
Sambal Udang	Prawns cooked in chilli paste	1. Used coconut palm sugar 2. Shrimp paste not used	301	129	56
Sambal Sotong	Cuttlefish cooked in chilli paste	1. Used coconut palm sugar 2. Reduced quantity of oil used	275	96	55
Masak Lemak Cili Api Ketam	Crabs cooked in low fat milk with bird's eye chilli	1. Substituted coconut milk with low fat milk 2. Added soy bean curd and tomato	545	103	81
Masak Lemak Sotong	Cuttlefish cooked in low fat milk	1. Substituted coconut milk with low fat milk 2. Added long beans	388	99	56
Kari Sardin	Sardine curry	1. Reduced quantity of oil used 2. Added tomato and peas	318	120	61
Kari Ikan	Mackerel curry	1. Substituted coconut milk with natural yoghurt 2. No sugar added 3. Reduced quantity of oil used 4. Added brinjal	242	76	67

Table 2. List of cuisines and beverages from the recipe book [Cont'd]

Local name	*Common name*	*Modification made*	*Calorie content (kcal/ 100g)*		*Weigh per serving*
			Original recipe	*Modified recipe*	
Masak Lemak Cili Api Tenggiri	Mackerel cooked in low fat milk with bird's eye chilli	1. Substituted coconut milk with low fat milk 2. Added tomato	506	165	95
Sambal Ikan Keli	Catfish cooked in chilli paste	1. Used olive oil 2. Roasted the fish	432	288	68
Asam Pedas Pari	Stingray cooked in spicy sour sauce	Reduced quantity of oil used	435	129	57
Asam Pedas Tenggiri	Mackerel cooked in spicy sour sauce	1. Used olive oil 2. Reduced quantity of oil used	417	221	61
Ikan Selar Bakar Berlada	Roasted yellowstripe scad	1. Roasted the chicken instead of frying 2. Added carrot	507	117	41
Nasi Goreng Cina	Chinese fried rice	1. Substituted usage of oil with small quantity of margerine 2. Added carrot and french beans	442	105	250
Cucur Ikan Bilis	Anchovies fritter	1. Used *atta* flour instead of wheat flour 2. Reduced quantity of oil used	745	261	92
Lempeng Kelapa	Coconut pancake	1. Used atta flour instead of wheat flour 2. Added oats 3. No oil added	272	261	88
Lepat Pisang	Steamed banana packets	1. Reduced quantity of wheat flour used 2. Added more banana	385	133	96
Mee Goreng Mamak	Fried noodles	1. Used chicken breast 2. Reduced quantity of oil used 3. Fish cake not used 4. Added roasted soy bean curd	720	149	134
Nasi Lemak	Rice in low fat milk	Substituted coconut milk with low fat milk	472	144	230

Table 2. List of cuisines and beverages from the recipe book [Cont'd]

Local name	*Common name*	*Modification made*	*Calorie content (kcal/ 100g)*		*Weigh per serving*
			Original recipe	*Modified recipe*	
**Sambal Ikan Bilis*	Anchovies cooked in chilli paste	1. Reduced quantity of oil used 2. Added *petai*	170	178	44
**Kobis Goreng*	Fried cabbage	1. Reduced quantity of oil used 2. Added long beans 3. Added soy bean curd and dried salted prawn	212	305	126
Terung Bakar Berlada	Roasted chillied eggplant	Roasted the brinjal instead of fried	Not applicable	59	61
Buncis Goreng Halia	Stir fried green beans with ginger and beef	Reduced quantity of oil used	Not applicable	150	33
Sup Siew Pak Choy	Pak choi soup	Boiling method	Not applicable	47	59
Kerabu Telur	White egg with salad	Egg yolk not added	Not applicable	295	57
Udang Masak Brokoli	Prawns cooked with broccoli	1. Reduced quantity of oil used 2. Used olive oil	Not applicable	94	78
Botok-botok Tenggiri	Steamed mackerel	Steaming method	Not applicable	151	38
Sup Ikan Merah	Red snapper soup	Boiling method	Not applicable	114	134
Jus Anggur Hitam	Black grape juice	Used low fat milk, fruits and honey	Not applicable	49	250
Jus Brokoli Sedap	Delicious brocolli juice	Used vegetables, fruits and honey	Not applicable	19	250
Jus Buah Campuran	Mixed fruit juice	Used oats, fruits and honey	Not applicable	36	250
Jus Buah Campuran Timun	Mixed fruit with cucumber juice	Used fruits and vegetables	Not applicable	17	250
Jus Epal dan Oat	Apple and oat juice	Used oats, fruits, honey and raisin	Not applicable	37	250

Table 2. List of cuisines and beverages from the recipe book [Cont'd]

Local name	*Common name*	*Modification made*	*Calorie content (kcal/ 100g)*		*Weigh per serving*
			Original recipe	*Modified recipe*	
Jus Epal Lobak Berhalia	Apple, carrot and ginger juice	Used low fat milk, fruits, vegetables and honey	Not applicable	35	250
Jus Hijau	Green juice	Used vegetables and fruits	Not applicable	15	250
Jus Labu Bersusu	Pumpkin juice with milk	Used low fat milk and fruits	Not applicable	12	250
Jus Mangga Oat	Oat and mango juice	Used oats, fruits and raisin	Not applicable	19	250
Jus Sengkuang Cina	Water chestnut juice	Used fruits and vegetables	Not applicable	25	250
Jus Tembikai Susu	Honeydew juice	Used low fat milk and fruits	Not applicable	28	250
Jus Pisang	Banana juice	Used low fat milk, fruits and honey	Not applicable	26	250

*Calories increment after modification was made
'Not applicable' indicates that the recipe was created by nutritionists.

content (96.7%), followed by *Jus Hijau* (96.2%), and *Jus Buah Campuran Timun* (95.8%). These results are consistent with a study carried out by Slavin & Lloyd (2012) that reported various commonly consumed fruits having high moisture content (around 61 to 89%). High ash content is an indicator of high amount of minerals in foods. Ash content of most food samples analysed in this study was <5%, which is the normal range for most food samples (Pallab & Amartya, 2012). In this study, only *Sambal Ikan Bilis* had significantly (p<0.05) high total ash content of >5%. This result is consistent with the reported high amount of mineral content (sodium, potassium, and calcium) in 6-10 g size group of anchovies (Sankar *et al.,* 2013).

Crude protein content

The crude protein content was further categorised into vegetables, meat and poultry, fish, seafood, breakfast and tea-time cuisines, and finally beverages. In the vegetable cuisines category, the amount of protein content (10.1%) was significantly higher (p<0.05) in *Buncis Goreng Halia,* followed by *Masak Lemak Sayur Campur* (6.77%), and *Tomyam Campur* (5.91%) as shown in Table 3. The high protein content in *Buncis Goreng Halia* was due to the meat used in that recipe (NCCFN, 2010). For meat and poultry recipes, *Ayam Bakar Berempah* (30.0%) showed significantly highest (p<0.05) protein content, followed by *Kari Daging* (18.3%) and *Sambal Ayam* (17.6%). As for fish recipes, *Sambal Ikan*

Bilis contained significantly highest (p<0.05) amount of protein (18.4%), followed by *Botok-Botok Tenggiri* (18.1%) and *Asam Pedas Tenggiri* (17.8%). As for seafood cuisines, *Masak Lemak Ketam Cili Api* had significantly highest (p<0.05) protein content (12.3%). The results also showed that cuttlefish recipes such as *Masak Lemak Sotong* had the highest protein content (8.7%), while *Sotong Masak Kicap* and *Sambal Sotong* both had 8.52% protein. However, protein values provided in the MyFCD and Singapore FCD for *Sambal Sotong* were 13.3% and 12.3%, respectively. These values are consistent with those from studies conducted by Varadharajan & Soundarapandian (2014), which found that protein content in cephalothorax of freshwater crab from South East Coast of India was 13.5%. As for breakfast and tea-time recipes, *Lempeng Kelapa* showed significantly highest (p<0.05) amount of protein, which was 9.4%, followed by *Cucur Ikan Bilis* (9.1%), which used *atta* flour. This was because, according to the protein content data from MyFCD, whole wheat flour (*atta* flour) normally used in *Lempeng Kelapa* and *Cucur Ikan Bilis* was moderately high in protein (10.6%). *Jus Tembikai Susu* contained significantly highest (p<0.05) amount of protein (2.5%), while *Jus Mangga Oat* had the lowest protein content (0.2%%). These results are consistent with Slavin & Lloyd (2012)'s study which reported that fruits are low in protein content (0.5 to 1.1%).

Crude fat content

As shown in Table 3, in the vegetable cuisines category, *Pajeri Terung* contained significantly highest (p<0.05) fat content (14.3%), followed by *Masak Lemak Jantung Pisang* (7.2%) and *Pajeri Nenas* (4.6%). In the meat and poultry category, *Ayam Bakar Berempah* showed significantly highest (p<0.05) fat content (8.7%), besides *Kari Ayam* (6.2%) and *Asam Pedas Daging* (6.0%). Among fish dishes, *Asam Pedas Tenggiri* had significantly highest (p<0.05) fat content (15.5%), followed by *Sambal Ikan Bilis* (10.8%) and *Masak Lemak Cili Api Tenggiri* (10.5%). For seafood cuisines, *Sambal Udang* showed significantly highest (p<0.05) amount of fat (9.5%), apart from *Sambal Sotong* (6.0%) and *Sotong Masak Kicap* (5.8%). In the breakfast and tea-time category, *Cucur Ikan Bilis* showed significantly highest (p<0.05) fat content (11.6%), followed by *Mee Goreng* (5.6%) and *Nasi Lemak* (3.8%). Recipes that involved frying in oil as a preparation method may result in high fat content. Nandita (2013) reported that using cooking methods such as steaming, boiling, and baking can reduce fat content in cooked foods. On the contrary, recipes that use frying method have higher fat content than recipes that use boiling method (Dora *et al.*, 2018). However, changing the food preparation method can affect the flavour, texture, appearance, and nutritional quality of the cooked foods (America's Test Kitchen & Crosby, 2012). Meanwhile, for beverages, *Jus Epal Lobak Berhalia, Jus Buah Campuran,* and *Jus Pisang* showed low fat content (less than 0.9%). These results are supported by Slavin & Lloyd (2012)'s study which concluded that fruits contain low amount of fat.

Total carbohydrate content

In the vegetable cuisines category, *Kobis Goreng* had significantly highest (p<0.05) amount of carbohydrates (64.9%). This was due to the low fat and protein contents of this recipe, which affected its carbohydrate content. As for meat and poultry category, *Kerabu Telur* had significantly highest (p<0.05) amount of carbohydrates (64.1%), while *Udang Masak Brokoli* in the seafood cuisines category came second (6.3%). In the fish category, *Sambal Ikan Keli* had

Table 3. Proximate analysis in 48 dishes and 12 juices

Type of cuisines n=60	*Moisture*	*Ash*	*Protein*	*Fat*	*Carbohydrate*	*Calories (kcal/ 100g)*
Vegetable cuisines (mean percentage±*SD*)						
Masak Lemak Labu Kuning	86.4±1.1^{ef}	0.8±0.1^{a}	2.5±0.2^{a}	2.4±0.1^{ab}	7.9±0.9^{b}	63±4^{ab}
Pajeri Nenas	84.9±1.9^{e}	0.8±0.1^{a}	2.1±0.3^{a}	4.6±0.2^{f}	8.3±3.5^{b}	80±7^{bc}
Kobis goreng	26.9±5.5^{a}	0.9±0.1^{ab}	4.0±0.2^{b}	3.3±0.4^{cd}	64.9±5.1^{f}	305±23^{f}
Kacang buncis goreng	87.5±0.3^{ef}	1.2±0.1^{cd}	2.4±0.3^{a}	3.8±0.1^{de}	5.2±0.6^{ab}	64±1^{ab}
Masak Lemak Sayur Campur	50.7±0.2^{b}	1.2±0.1^{cd}	6.8±1.1^{c}	4.0±0.2^{ef}	37.3±1.2^{e}	213±2^{e}
Terung bakar berlada	88.8±0.1^{ef}	1.4±0.0^{d}	1.9±0.4^{a}	4.0±0.1^{def}	4.0±0.5^{ab}	59±1^{a}
Masak Lemak Jantung Pisang	53.0±0.8^{b}	1.2±0.0^{cd}	4.6±0.2^{b}	7.2±0.5^{g}	33.9±1.1^{e}	219±3^{e}
Pajeri Terung	78.1±0.4^{d}	1.3±0.1^{cd}	2.6±0.2^{a}	14.3±0.5^{h}	3.7±0.3^{ab}	154±3^{d}
Tomyam Campur	88.4±0.6^{ef}	1.3±0.2^{cd}	5.9±0.4^{c}	2.8±0.2^{bc}	1.6±0.6^{a}	55±3^{a}
Buncis Goreng Halia	65.7±0.2^{c}	1.4±0.1^{d}	10.1±0.8^{d}	3.7±0.4^{de}	19.1±0.9^{d}	150±2^{d}
Sup Siew Pak Choy	89.3±0.6^{f}	1.1±0.1^{bc}	5.8±0.2^{c}	1.8±0.1^{a}	2.0±1.5^{a}	47±2^{a}
Masak Lemak Nangka	80.2±0.4^{d}	0.9±0.0^{ab}	2.5±0.3^{a}	3.8±0.3^{de}	12.6±0.7^{c}	94±3^{c}
Meat and poultry cuisines (mean percentage±*SD*)						
Masak Lemak Telur Itik	82.5±0.8^{h}	1.6±0.1^{abc}	6.0±0.1^{a}	6.5±0.1^{c}	3.5±0.9^{ab}	96±3^{a}
Kerabu Telur	27.7±0.2^{a}	1.1±0.1^{ab}	5.1±0.5^{a}	2.0±0.1^{c}	64.1±0.5^{d}	295±1^{i}
Kari Ayam	77.0±0.4^{fg}	1.1±0.2^{a}	14.1±0.2^{cd}	6.2±0.1^{c}	1.7±0.5^{a}	119±2^{cde}
Kari Daging	58.2±2.3^{c}	3.0±0.6^{f}	18.3±1.7^{e}	5.4±0.2^{c}	15.1±3.5^{c}	182±6^{cde}
Daging Masak Kicap	75.5±2.0^{ef}	1.8±0.3^{cd}	14.3±2.1^{d}	5.7±0.0^{c}	2.7±0.3^{a}	119±9de
Ayam Masak Kicap	75.6±3.1^{ef}	1.7±0.1^{bc}	11.8±0.1^{bc}	4.0±0.2^{b}	7.0±3.2^{b}	111±13^{bcde}
Ayam Masak Sambal	73.2±0.8^{e}	1.6±0.0^{abc}	17.6±0.2^{e}	4.2±0.2^{b}	3.4±0.8^{ab}	122±3^{e}
Sambal Telur	76.6±2.0^{efg}	2.2±0.3^{de}	5.9±0.6^{a}	3.8±0.2^{b}	11.4±1.2^{c}	104±7^{abc}
Masak Lemak Cili Api Ayam	79.6±0.9^{gh}	1.4±0.0^{abc}	10.8±0.1^{b}	5.8±0.1^{c}	2.4±1.0^{a}	105±4^{abcd}
Masak Lemak Cili Api Daging Salai	79.7±0.0^{gh}	2.3±0.0^{de}	12.4±0.0^{bcd}	5.6±0.1^{c}	0.1±0.1^{a}	100±0^{ab}
Ayam Bakar Berempah	52.2±0.9^{b}	1.8±0.1^{cd}	30.0±1.8^{f}	8.7±1.6^{d}	7.3±1.0^{b}	227±9^{h}
Asam Pedas Daging	65.0±0.7^{d}	2.4±0.1^{e}	12.9±1.0^{bcd}	6.0±0.2^{c}	13.8±1.4^{c}	161±3^{f}

Table 3. Proximate analysis in 48 dishes and 12 juices [Cont'd]

Type of cuisines n=60	*Moisture*	*Ash*	*Protein*	*Fat*	*Carbohydrate*	*Calories (kcal/ 100g)*
Seafood cuisines (mean percentage±*SD*)						
Masak Lemak Cili Api Udang	87.4±0.5^{e}	1.2±0.1^{ab}	5.0±0.8^{b}	3.9±0.2^{a}	2.6±0.6^{b}	65±3^{a}
Udang Masam Manis	88.6±0.8^{e}	0.8±0.0^{a}	2.1±0.1^{a}	4.0±0.1^{a}	4.5±0.9^{b}	62±3^{a}
Udang Masak Brokoli	81.5±0.2^{cd}	1.9±0.0^{c}	4.9±0.5^{b}	5.5±0.2^{b}	6.3±0.6^{d}	94±1^{b}
Sotong Masak Kicap	82.1±0.3^{d}	1.5±0.4^{bc}	8.5±0.2^{c}	5.8±0.3^{bc}	2.1±0.4^{ab}	94±3^{b}
Sambal Udang	76.1±0.6^{a}	3.5±0.2^{d}	8.1±0.7^{c}	9.5±0.3^{d}	2.9±0.8^{bc}	129±2^{d}
Sambal Sotong	80.3±1.2^{bc}	3.3±0.3^{d}	8.5±0.0^{c}	6.0±0.3^{c}	1.8±1.0^{ab}	96±4^{b}
Masak Lemak Cili Api Ketam	79.7±1.0^{b}	1.6±0.2^{bc}	12.3±0.0^{d}	5.5±0.1^{bc}	0.9±0.5^{a}	103±3^{c}
Masak Lemak Sotong	80.5±0.6^{bcd}	1.83±0.0^{c}	8.7±0.1^{c}	5.8±0.1^{bc}	3.3±0.6^{bc}	99±3^{bc}
Fish cuisines (mean percentage±*SD*)						
Kari Sardin	73.0±1.4^{e}	1.5±0.2^{abc}	11.5±0.1^{bc}	3.7±0.2^{ab}	10.4±1.8^{c}	120±6^{b}
Kari Ikan	84.2±1.0^{g}	2.2±0.3^{ef}	6.4±1.0^{a}	4.2±0.2^{b}	3.1±0.5^{ab}	76±4^{a}
Sambal Ikan Bilis	62.4±0.3^{b}	6.6±0.0^{g}	18.4±0.2^{g}	10.8±0.4^{f}	1.8±0.4^{a}	178±2^{f}
Masak Lemak Cili Api Tenggiri	71.0±0.8^{d}	1.0±0.1^{a}	15.7±1.3^{de}	10.5±0.0^{f}	1.8±0.2^{a}	165±2^{e}
Sambal Ikan Keli	38.0±0.5^{a}	1.7±0.3^{cde}	13.4±2.4^{cd}	9.4±0.3^{e}	37.4±1.7^{d}	288±2^{h}
Asam Pedas Pari	74.5±1.0^{e}	2.2±0.3^{f}	13.3±0.2$_{cd}$	7.1±0.1^{d}	2.8±1.0^{a}	129±4^{c}
Asam Pedas Tenggiri	62.0±0.4^{b}	2.1±0.1^{ef}	17.8±0.9^{ef}	15.5±0.1^{g}	2.7±0.7^{a}	221±2^{g}
Ikan Selar Bakar Berlada	73.5±0.4^{e}	1.9±0.1^{def}	9.9±0.9^{b}	3.6±0.4^{a}	11.0±0.9^{c}	117±3^{b}
Botok-Botok Tenggiri	65.5±0.4^{c}	1.2±0.1^{ab}	18.1±1.2^{ef}	3.6±0.1^{a}	11.6±1.0^{c}	151±2^{d}
Sup Ikan Merah	77.6±0.3^{f}	1.4±0.2^{abc}	9.6±0.2^{b}	6.0±0.2^{b}	5.4±0.4^{b}	114±2^{b}

Table 3. Proximate analysis in 48 dishes and 12 juices [Cont'd]

Type of cuisines n=60	*Moisture*	*Ash*	*Protein*	*Fat*	*Carbohydrate*	*Calories (kcal/ 100g)*
Beverages (mean percentage±*SD*)						
Jus Anggur Hitam	87.3±0.2^{a}	0.6±0.0^{i}	2.4±0.3^{f}	0.2±0.0^{b}	9.5±0.4^{h}	49±1^{g}
Jus Brokoli Sedap	95.2±0.2^{f}	0.2±0.0^{ab}	1.2±0.1^{de}	0.1±0.0^{ab}	3.3±0.1^{abc}	19±1^{c}
Jus Buah Campuran	91.2±0.7^{bc}	0.3±0.0^{fg}	0.8±0.1^{bcd}	0.5±0.0^{c}	7.1±0.7^{f}	36±3^{f}
Jus Buah Campuran Timun	95.8±0.5^{fg}	0.2±0.1^{abc}	0.4±0.1^{ab}	0.2±0.0^{ab}	3.5±0.5^{bc}	17±2^{bc}
Jus Epal dan Oat	90.8±0.2^{b}	0.2±0.0^{abcd}	0.9±0.2^{cde}	0.2±0.0^{ab}	7.9±0.1^{f}	37±1^{f}
Jus Epal Lobak Berhalia	91.9±0.7^{c}	0.4±0.0^{gh}	1.3±0.2^{e}	0.8±0.0^{d}	5.6±0.9^{e}	35±3^{f}
Jus Hijau	96.2±0.1^{gh}	0.3±0.0^{cdef}	0.7±0.1^{bc}	0.1±0.0^{a}	2.7±0.1^{ab}	15±0^{ab}
Jus Labu Bersusu	96.7±0.4^{h}	0.3±0.0^{defg}	0.6±0.1^{bc}	0.1±0.0^{a}	2.3±0.3^{a}	12±1^{a}
Jus Mangga Oat	95.3±0.3^{fg}	0.1±0.0^{a}	0.2±0.0^{a}	0.2±0.0^{b}	4.2±0.3^{cd}	19±1^{c}
Jus Pisang	94.3±0.1^{e}	0.2±0.0^{bcde}	1.2±0.2^{de}	0.5±0.0^{c}	3.8±0.3^{bc}	25±1^{d}
Jus Sengkuang Cina	93.2±0.6^{d}	0.3±0.1^{efg}	0.9±0.1^{cde}	0.5±0.1^{c}	5.1±0.5^{de}	28±20^{e}
Jus Tembikai Susu	93.3±0.1^{de}	0.4±0.1^{h}	2.5±0.3^{f}	0.2±0.0^{ab}	3.5±0.5^{bc}	26±1de
Breakfast and tea-time cuisines (mean percentage±*SD*)						
Nasi Goreng Cina	77.3±0.3^{b}	1.0±0.2^{a}	5.8±1.5^{b}	3.7±0.2^{b}	12.2±1.4^{a}	105±1^{a}
Cucur Ikan Bilis	47.4±2.7^{ab}	1.9±0.3^{c}	9.1±0.7^{d}	11.6±0.4^{d}	30.0±1.5^{e}	261±11^{d}
Lempeng Kelapa	37.7±0.6^{a}	1.5±0.0^{b}	9.4±0.3^{d}	3.5±0.1^{b}	47.9±0.3^{f}	261±3^{d}
Lepat Pisang	67.7±0.3^{ab}	1.3±0.0^{ab}	3.3±0.4^{a}	1.7±0.2^{a}	26.2±0.8^{d}	133±1^{b}
Mee Goreng Mamak	67.8±0.5^{b}	2.1±0.0^{c}	7.9±1.4^{cd}	5.6±0.6^{c}	16.6±1.4^{b}	149±3^{c}
Nasi Lemak	67.5±1.5^{b}	1.3±0.1^{b}	6.7±0.4^{bc}	3.8±0.0^{b}	20.8±1.3^{c}	144±6^{bc}

*Different letters ^{abcdefgh} within the same column indicate significant difference (p<0.05)

significantly highest (p<0.05) amount of carbohydrates (37.4%). As for breakfast and tea-time category, *Lempeng Kelapa* showed significantly highest (p<0.05) amount of carbohydrates and this may be due to the *atta* flour and oats used in this recipe (47.9%). MyFCD indicated that carbohydrate contents of *atta* flour and oats were 75.4% and 71.4%, respectively. For juices, total carbohydrate content was not more than 10.0 % and this could be due to the use of vegetables that were low in carbohydrates (MyFCD) such as cucumber, broccoli, pumpkin, water chestnut, carrot, ginger, pennywort, spinach, and celery together with fruits that have been mixed to form healthy juices.

Mineral content

Table 4 shows the mineral content in food samples. Meat cuisines were likely to have high sodium content. *Asam Pedas Daging* contained significantly highest (p<0.05) amount of sodium (554 mg Na/100g). Most processed meat contained variable amount of sodium and are associated with high salt contents (McCance & Widdowson's The Composition of Foods 2014). For potassium (K) content, *Ayam Bakar Berempah* showed significantly highest (p<0.05) content across all categories (471 mg K/100g). Data in the MyFCD showed that chicken breast used in *Ayam Bakar Berempah* contained 313 mg K/100g. *Jus Buah Campuran* (a mixture of fruits such as orange, pineapple and papaya together with oats and honey) contributed to a high amount of calcium (269 mg Ca/100g). Iron (Fe) content was significantly highest (p<0.05) in *Sambal Ikan Bilis* (3.8 mg Fe/100g) within its category compared to the data in MyFCD (1.1 mg Fe/100g). This difference might be due to different sources of anchovies used for these studies as highlighted by Ehigiator & Nwangwu (2011) that nutrient composition and biochemical properties of each organisms are different depending on the parts of animals, season, size, maturity, and natural habitat. In this study, *Sambal Ikan Bilis* has significantly highest (p<0.05) amount of magnesium (Mg) (77 mg Mg/100g) across all categories. Sankar *et al.* (2013), who reported the magnesium content in three different anchovies showed that the magnesium content was between 10 to 30 mg Mg/100g. Finally, *Buncis Goreng Halia* that used beef as one of its ingredient in the recipe had the highest (p<0.05) amount of zinc (Zn) (1.9 mg Zn/100g) compared to other cuisines. By comparing data from this study, it was reported in the International Zinc Nutrition Consultative Group IZiNCG (2004) that the amount of zinc content in beef is within 2.9–4.7 mg Zn/100g.

Fatty acids composition

Based on Table 5, saturated fatty acid (SFA) was the most dominant fatty acid in yoghurt and low-fat dishes, followed by monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). In Table 4, the highest amount of palmitic acid (42.3%) was found in *Masak Lemak Cili Api Ikan Tenggiri*. These results are consistent with Manal's (2009) findings, which reported that Spanish mackerel (*Scomberomorus maculatus*) had high palmitic acid content. Next, the highest content of stearic acid (16.3%) was found in *Masak Lemak Sotong*. Amonrat, Soottawat & Wonnop (2006) reported that palmitic acid and stearic acid are abundant in the head and body of cuttlefish. For MUFA, oleic acid was found in all fourteen cuisines. *Masak Lemak Telur Itik* showed the highest amount of oleic acid (42.5%) and this result is consistent with Vassilia *et al.* (2011)'s study, which showed that duck eggs contain high composition of oleic acid and linoleic acid, compared to saturated and polyunsaturated fatty acids. After oleic acid, palmitoleic

Table 4. Mineral content in 48 dishes and 12 juices

Type of cuisines n=60	*Na*	*K*	*Ca*	*Fe*	*Mg*	*Zn*
Vegetable cuisines (mg/100g)						
Masak Lemak Labu Kuning	33.8±0.4^{a}	212.0±22.6^{cd}	87.2±4.2^{e}	1.3±0.1^{bc}	13.0±1.4^{ab}	0.1±0.0^{a}
Pajeri Nenas	261.8±17.9^{d}	146.0±8.5^{ab}	65.1±1.3^{cd}	1.9±0.1^{d}	21.3±4.7^{b}	0.2±0.1^{ab}
Kobis goreng	196.4±30.9^{cd}	145.3±10.5^{ab}	51.1±0.8^{c}	1.3±0.1^{bcd}	14.9±0.3^{ab}	0.5±0.0^{b}
Kacang buncis goreng	264.0±17.0^{d}	142.0±14.1^{ab}	67.4±10.1^{d}	0.7±0.1^{a}	57.0±4.2^{d}	0.2±0.1^{ab}
Masak Lemak Sayur Campur	218.1±0.6^{cd}	204.5±16.7^{bcd}	107.3±0.9^{f}	1.3±0.2^{bcd}	33.3±0.1^{c}	0.4±0.0^{ab}
Terung bakar berlada	228.4±7.4^{cd}	264.0±11.3^{de}	51.5±1.8^{cd}	1.1±0.2^{ab}	14.0±2.8^{ab}	0.2±0.1^{ab}
Masak Lemak Jantung Pisang	212.7±23.7^{cd}	202 .5±5.4^{bcd}	106.5±4.5^{f}	1.4±0.3^{bcd}	17.2±1.0^{ab}	0.5±0.0^{b}
Pajeri Terung	236.2±8.4^{cd}	314.5±10.3^{ef}	13.8±3.6^{a}	1.6±0.0^{bcd}	20.4±5.2^{b}	0.4±0.1^{ab}
Tomyam Campur	262.6±23.5^{d}	106.4±25.2^{a}	52.7±1.9^{cd}	1.3±0.0^{bc}	19.5±1.3^{ab}	0.4±0.0^{ab}
Buncis Goreng Halia	231.0±20.6^{cd}	350.8±23.0^{f}	33.2±2.1^{b}	1.6±0.1^{bcd}	20.6±0.7^{b}	1.9±0.2^{c}
Sup Siew Pak Choy	192.2±16.7^{c}	145.5±28.8^{ab}	53.3±4.1^{cd}	1.7±0.2^{cd}	9.6±1.6^{a}	0.3±0.1^{ab}
Masak Lemak Nangka	109.4±7.0^{b}	155.1±6.9^{abc}	89.2±3.0^{e}	1.3±0.0^{bcd}	21.9±1.3^{b}	0.4±0.0^{ab}
Meat and poultry cuisines (mg/100g)						
Masak Lemak Telur Itik	295.3±6.7^{c}	251.0±4.2^{abc}	76.5±8.9^{cdef}	1.3±0.1^{a}	12.0±2.8^{a}	0.4±0.1^{abc}
Kerabu Telur	150.0±25.5^{b}	202.0±5.7^{a}	93.0±12.7^{ef}	1.3±0.2^{a}	34.5±7.8^{abc}	0.2±0.0^{a}
Kari Ayam	144.0±11.3^{ab}	230.0±8.5^{ab}	55.3±10.2^{abcd}	1.1±0.1^{a}	15.0±4.2^{ab}	0.4±0.1^{ab}
Kari Daging	49.0±7.1^{a}	300.0±31.1^{bcd}	103.6±15.8^{f}	3.4±0.0^{e}	74.0±11.3^{d}	0.2±0.0^{ab}
Daging Masak Kicap	526.0±59.4^{f}	238.0±14.1^{abc}	60.6±6.4^{bcde}	2.1±0.1^{bcd}	39.0±7.1^{bc}	0.3±0.1^{ab}
Ayam Masak Kicap	426.0±30.8^{de}	204.0±31.1^{a}	58.3±0.5^{bcd}	1.1±0.3^{a}	53.0±1.4^{cd}	0.2±0.1^{a}
Ayam Masak Sambal	368.0±22.6^{cd}	328.0±2.8^{cd}	52.7±0.9^{abcd}	1.6±0.1^{abc}	60.0±14.1^{cd}	1.5±0.2^{d}
Sambal Telur	99.0±18.3^{ab}	230.0±11.3^{ab}	43.0±1.4^{abc}	2.6±0.2^{d}	53.0±4.3^{cd}	0.2±0.0^{a}
Masak Lemak Cili Api Ayam	278.9±15.3^{c}	309.8±64.4^{bcd}	24.2±1.6^{a}	1.3±0.2^{a}	15.6±3.1^{ab}	0.5±0.1^{bc}
Masak Lemak Cili Api Daging Salai	498.9±13.5^{ef}	366.4±7.4^{d}	35.0±3.3^{ab}	2.2±0.3^{cd}	23.4±0.0^{ab}	0.2±0.0^{a}
Ayam Bakar Berempah	277.2±15.6^{c}	471.2±15.5^{e}	47.9±13.1^{abcd}	1.4±0.1^{ab}	24.9±0.1^{ab}	0.7±0.0^{c}
Asam Pedas Daging	553.6±17.8^{f}	178.6±3.9^{a}	79.0±7.1^{def}	2.4±0.0^{d}	22.6±2.1^{ab}	1.7±0.1^{d}

Table 4. Mineral content in 48 dishes and 12 juices [Cont'd]

Type of cuisines n=60	*Na*	*K*	*Ca*	*Fe*	*Mg*	*Zn*
Seafood cuisines (mg/100g)						
Masak Lemak Cili Api Udang	125.4±45.4^{a}	228.0±33.9^{c}	92.2±11.1^{b}	0.9±0.1^{ab}	14.0±2.8^{a}	0.3±0.0^{ab}
Udang Masam Manis	128.0±11.3^{a}	185.0±12.7^{bc}	85.0±4.2^{b}	0.8±0.2^{ab}	68.0±2.8^{c}	0.2±0.1^{ab}
Udang Masak Brokoli	384.0±48.1^{c}	231.0±12.7^{c}	50.5±5.2^{a}	1.2±0.0^{b}	16.0±2.8^{a}	0.5±0.0^{bc}
Sotong Masak Kicap	354.0±8.5^{c}	92.0±2.8^{a}	27.3±7.2^{a}	0.4±0.1^{a}	48.0±2.8^{b}	1.0±0.1^{de}
Sambal Udang	391.0±41.0^{c}	150.0±5.7^{ab}	33.1±7.2^{a}	3.3±0.3^{c}	56.0±8.5^{bc}	0.2±0.0^{a}
Sambal Sotong	229.0±18.4^{ab}	108.0±0.0^{a}	38.6±0.5^{a}	1.0±0.3^{ab}	73.0±7.1^{c}	0.2±0.0^{a}
Masak Lemak Cili Api Ketam	232.7±18.2^{ab}	205.0±12.7^{bc}	100.9±2.9^{b}	0.9±0.1^{ab}	20.0±2.8^{a}	1.0±0.2^{e}
Masak Lemak Sotong	331.2±15.8^{bc}	183.4±21.8^{bc}	76.5±5.1^{b}	1.3±0.0^{b}	28.6±1.1^{a}	0.7±0.1^{cd}
Fish cuisines (mg/100g)						
Kari Sardin	256.0±39.6^{bcd}	167.0±24.0^{a}	140.0±5.7^{d}	1.5±0.1^{ab}	41.0±4.2^{b}	0.5±0.1^{bcd}
Kari Ikan	432.0±16.9^{fg}	252.0±36.8^{bc}	87.6±7.5^{bc}	1.9±0.2^{bc}	52.2±2.3^{b}	0.6±0.1^{bcd}
Sambal Ikan Bilis	90.0±8.5^{a}	341.0±9.9^{d}	233.0±7.1^{e}	3.7±0.3^{d}	77.0±4.2^{c}	0.2±0.0^{a}
Masak Lemak Cili Api Tenggiri	267.1±5.7^{bcd}	193.0±18.4^{ab}	55.5±9.3^{ab}	1.0±0.3^{a}	22.0±5.6^{a}	0.3±0.1^{ab}
Sambal Ikan Keli	294.9±78.6^{cde}	329.6±5.2^{d}	58.9±6.9^{ab}	1.5±0.1^{abc}	23.4±2.1^{a}	0.7±0.0^{d}
Asam Pedas Pari	400.3±10.7^{efg}	314.4±21.6^{cd}	44.9±6.7^{ab}	1.5±0.0^{ab}	26.2±5.1^{a}	0.4±0.0^{abc}
Asam Pedas Tenggiri	439.5±12.9^{g}	300.0±19.7^{cd}	73.1±17.9^{ab}	1.5±0.0^{ab}	24.5±0.4^{a}	0.6±0.1^{bcd}
Ikan Selar Bakar Berlada	313.9±2.2^{def}	206.0±0.1^{ab}	39.9±0.5^{a}	1.7±0.3^{abc}	23.7±0.5^{a}	0.6±0.0^{cd}
Botok-Botok Tenggiri	184.2±4.5^{abc}	313.6±3.5^{cd}	63.5±7.9^{ab}	1.6±0.1^{abc}	20.7±0.2^{a}	0.7±0.1^{cd}
Sup Ikan Merah	169.7±22.4^{ab}	185.4±8.4^{ab}	126.3±26.3^{cd}	2.3±0.1^{c}	15.6 ±0.7^{a}	0.6±0.1^{cd}

Table 4. Mineral content in 48 dishes and 12 juices [Cont'd]

Type of cuisines n=60	*Na*	*K*	*Ca*	*Fe*	*Mg*	*Zn*
Beverages (mg/100g)						
Jus Anggur Hitam	67.0±12.7^{bc}	154.0±11.3^{c}	95.0±15.6^{de}	0.8±0.1^{bc}	33.0±9.9^{b}	0.3±0.0^{abcd}
Jus Brokoli Sedap	28.0±0.0^{a}	251.0±12.7^{d}	58.7±8.0^{bc}	0.3±0.1^{a}	16.0±2.8^{a}	0.3±0.0^{abcde}
Jus Buah Campuran	28.0±2.8^{a}	78.0±14.1^{ab}	268.3±14.4^{g}	0.8±0.1^{bc}	34.0±2.8^{b}	0.4±0.0^{bcde}
Jus Buah Campuran Timun	52.0±16.9^{abc}	42.0±5.7^{a}	54.3±0.1^{bc}	1.0±0.1^{bc}	22.3±2.4^{ab}	0.6±0.2^{de}
Jus Epal dan Oat	34.0±5.7^{ab}	55.0±4.2^{ab}	110.4±8.3^{e}	0.5±0.1^{ab}	21.0±1.4^{ab}	0.5±0.1^{bcde}
Jus Epal Lobak Berhalia	78.0±2.8^{c}	76.0±2.8^{ab}	77.2±7.2^{cd}	0.8±0.1^{bc}	10.3±0.7^{a}	0.3±0.1^{abcde}
Jus Hijau	35.0±1.4^{ab}	56.5±3.5^{ab}	32.4±2.7^{ab}	1.2±0.2^{c}	24.0±2.8^{ab}	0.2±0.0^{ab}
Jus Labu Bersusu	78.0±14.1^{c}	143.0±15.6^{c}	62.6±5.3^{bc}	0.7±0.1^{ab}	16.0±2.8^{a}	0.2±0.0^{abc}
Jus Mangga Oat	26.0±0.0^{a}	74.0±14.1^{ab}	172.6±6.6^{f}	1.2±0.0^{c}	10.0±0.0^{a}	0.6±0.1^{e}
Jus Pisang	35.0±7.1^{ab}	159.0±21.2^{c}	68.4±5.9^{cd}	0.8±0.2^{bc}	22.0±2.8^{ab}	0.5±0.0^{cde}
Jus Sengkuang Cina	29.0±4.2^{a}	254.0±33.9^{d}	10.9±1.1^{a}	0.6±0.1^{ab}	19.0 ±7.1^{ab}	0.3±0.1^{abcde}
Jus Tembikai Susu	45.0±9.9^{abc}	106.0±14.1^{bc}	47.5±1.8^{bc}	0.5±0.1^{ab}	19.4 ±1.9^{ab}	0.1±0.0^{a}
Breakfast and tea-time cuisines (mg/100g)						
Nasi Goreng Cina	216.0±45.3^{a}	129.0±2.0^{ab}	56.1±3.0^{c}	1.4±0.3^{a}	65.0±12.7^{d}	0.2±0.0^{a}
Cucur Ikan Bilis	395.9±8.7^{b}	204.0±12.0^{bc}	36.5±7.3^{b}	2.1±0.0^{b}	42.5±4.6^{cd}	1.2±0.2^{c}
Lempeng Kelapa	234.6±6.1^{a}	269.0±17.0^{cd}	26.6±0.4^{ab}	2.1±0.0^{b}	35.8±9.5^{bc}	1.4±0.0^{c}
Lepat Pisang	191.5±31.8$_{a}$	311.0±59.0^{d}	18.0±0.4^{a}	1.7±0.2^{ab}	16.2±4.0^{abc}	0.4±0.1^{ab}
Mee Goreng Mamak	454.5±61.9^{b}	129.0±6.2^{ab}	55.2±6.1^{c}	1.4±0.1^{a}	13.3±1.3^{ab}	0.4±0.1^{ab}
Nasi Lemak	258.0±4.8^{a}	87.0±7.1^{a}	107.2±0.7^{d}	1.6±0.1^{ab}	8.1±0.3^{a}	0.6±0.1^{b}

* Different letters ^{abcdefg} within the same column indicate significant difference (p<0.05)

Table 5. Fatty acid composition in yoghurt and milk-based cuisines (% from total fatty acids)

Sample (n=14)	*Saturated Fatty Acids (SFA)*					*Monounsaturated Fatty Acids (MUFA)*			*Polyunsaturated Fatty Acids (PUFA)*		
	Palmitic C 16	*Stearic C 18*	*Miristic C 14*	*Lauric C 12*	*Total SFA*	*Oleic C 18:1n9c*	*Palmitoleic C 16:1*	*Total MUFA*	*α-linolenic C 18:3n3*	*Linoleic C 18:2n6c*	*Total PUFA*
Masak Lemak Sotong	37.2±0.1	16.3±0.1	7.1± 0.1	4.6±0.1	71.8±0.6	14.9±0.1	1.3±0.0	16.8±0.2	0.9±0.0	2.8±0.1	3.7±0.1
Pajeri Nenas	20.2±0.9	3.9±0.0	7.5±0.0	17.0±0.1	56.3±1.2	31.7±0.2	1.1±0.0	33.0±0.2	0.6±0.0	10.2±0.1	10.8±0.1
Masak Lemak Cili Api Udang	24.4±0.0	8.9±0.0	3.0±0.0	2.8±0.0	41.5±0.1	24.2±0.0	0.7±0.0	24.9±0.1	4.1±0.0	29.5±00.0	33.7±0.0
Masak Lemak Cili Api Ayam	29.9±0.1	8.9±1.4	2.5±1.2	0.5±0.0	46.1±2.8	31.1±0.2	5.3±0.0	37.5±0.3	1.5±0.0	14.1±0.1	16.0±0.1
Masak Lemak Nangka	35.9±0.2	12.7±0.2	4.6±0.1	2.3±0.1	59.2±0.7	25.2±0.3	1.6±0.03	27.2±0.3	1.1±0.0	10.5±0.1	11.5±0.1
Nasi Lemak	32.0±0.1	9.8±0.0	5.4±0.0	1.6±0.0	53.3±0.2	27.8±0.0	1.9±0.0	30.6±0.1	0.6±0.0	11.8±0.0	15.0±0.1
Masak Lemak Sayur Campur	34.7±0.4	6.3±0.1	1.8±0.1	0.9±0.1	46.4±1.1	35.7±0.4	0.5±0.0	36.2±0.4	1.5±0.0	16.0±0.1	17.4±0.2
Masak Lemak Cili Api Ketam	27.8±0.2	9.0±0.0	3.8±0.1	1.7±0.1	42.4±0.4	26.5±0.0	1.4±0.1	27.9±0.1	3.5±0.0	26.2±0.1	29.7±0.1
Masak Lemak Cili Api Ikan Tenggiri	42.3±0.2	15.1±0.2	4.7±0.2	0.9±0.1	66.9±0.8	23.1±0.2	6.4±0.0	31.4±0.4	0.5±0.0	1.2±0.0	1.7±0.1
Masak Lemak Labu Kuning	38.1±0.1	9.9±0.2	9.3±0.2	5.43±0.1	71.2±1.2	20.6±0.3	2.01±0.1	23.3±0.5	0.9±0.1	4.6±0.5	5.5±0.2
Masak Lemak Telur Itik	27.6±0.0	7.7±0.0	1.5±0.0	0.6±0.0	38.6±0.1	42.5±0.1	2.16±0.0	45.1±0.1	0.9±0.0	10.2±0.0	15.8±0.1
Masak Lemak Jantung Pisang	32.1±0.3	10.3±0.1	6.2±0.1	7.30±0.1	64.5±0.8	20.6±0.1	0.93±0.0	22.2±0.2	2.6±00.0	6.0±0.1	13.4±0.1
Masak Lemak Cili Api Daging Salai	35.2±0.1	13.5±0.1	6.2±0.0	2.1±0.0	61.6±0.3	26.9±0.0	2.5±0.0	30.4±0.1	0.8±0.0	7.4±0.0	8.2±0.1
Kari Daging	27.5±0.0	4.4±0.04	7.3±0.0	16.6±0.0	63.4±0.3	28.1±0.2	0.3±0.0	29.0±0.3	0.3±0.1	7.5±0.0	7.8±0.1

Values are expressed as mean±*SD* from duplicate determinations

acid was the second highest amount of monounsaturated fatty acids in the analysed cuisines. The highest amount of palmitoleic acid (6.4%) was found in *Masak Lemak Cili Api Ikan Tenggiri*. For polyunsaturated fatty acids, *Masak Lemak Udang Cili Api* showed the highest amount of α-linolenic acid (4.11%) and linoleic acid (29.5%). Abdullah *et al.* (2009) showed that total polyunsaturated fatty acids in two types of fresh shrimp were the highest (42% and 35%, respectively). Trans-fatty acid could only be found in small amounts (0.19%) in *Nasi Lemak* and 0.06% for both *Masak Lemak Telur Itik* and *Kari Daging*.

All these new findings will be added to the database of the Malaysian Food Composition. However, the limitation of this study was that only foods and beverages in the recipe book were analysed. Future research should consider collecting and analysing food samples from across Malaysia.

CONCLUSION

Based on the nutrient content obtained from each food and beverage in this study, overweight and obesity problems among respondents could be overcome by implementing the recommended modifications made during meal preparations. The food analysis carried out gave reliable results on the determination of nutrient contents in the recipes. Food analysis results showed that out of 48 types of dishes and 12 types of healthy beverages, *Sambal Ikan Bilis* showed the highest amount of ash, protein, iron and magnesium; while protein, fat, sodium and potassium contents were highest in *Ayam Bakar Berempah* ($p<0.05$). Saturated fatty acids were found abundant in *Masak Lemak Sotong*, *Masak Lemak Labu Kuning,* and *Masak Lemak Cili Api Ikan Tenggiri,* while the lowest saturated fatty acids could be found in *Masak Lemak Telur Itik*. Monounsaturated fatty acids were found highest in *Masak Lemak Telur Itik,* while the lowest amount was present in *Masak Lemak Sotong*. Besides, the highest amount of polyunsaturated fatty acids was shown in *Masak Lemak Cili Api Udang,* while the lowest was in *Masak Lemak Cili Api Ikan Tenggiri*. The results of this study will make the recipe book more meaningful as the food analysis found most of the modified recipes showed lower caloric content compared to the original recipes. Reducing calories and fat in foods to an equally liked preference may be a strategy to improve one's dietary intake when preparing these foods. This approach takes the burden off the individuals in making healthier choices.

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Author's contributions

NA, conducted the study and compiled the data, data analysis and interpretation, prepared the draft of the manuscript; WNNWN and WM, assisted in the study and reviewed the manuscript; HH, conceptualised and designed the study, provided advice on data analysis and its interpretation, and reviewed the manuscript; RAT, conceptualised and designed the study, provided advice on data analysis and its interpretation, and reviewed the manuscript.

Conflict of interest

Authors declare no conflict of interest.

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Calcium and iron intakes of adolescents in Malaysia and their relationships with body mass index (BMI): Findings from the Adolescent Nutrition Survey 2017

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Abstract

Introduction: Adolescent health is a priority considering they represent the future generation. Data from the Adolescent Nutrition Survey 2017 were analysed to determine the prevalence of micronutrient inadequacy, particularly calcium and iron, and the relationship with body mass index (BMI) among Malaysian secondary school students. **Methods:** This cross-sectional study included students aged 13 to 17 years old studying at public and private schools. Twenty-four hours dietary recall via face-to-face interview was conducted by trained nutritionists to obtain data on dietary intake. For nutritional status, BMI-for-age *z*-score (BAZ) was analysed using WHO Anthroplus software. **Results:** From 999 respondents, 449 were boys and 550 were girls. Overall findings indicated that both boys and girls had inadequate intakes of calcium and iron in their daily diet. Mean intakes of calcium (695.7±463.2 mg/day) and iron (23.4±21.0 mg/day) were higher among boys aged 16 to 17 years old. Mean intake of iron were higher among the older age groups. Majority of the respondents (boys: 94%; girls: 97%) did not meet the recommended nutrient intake (RNI) for calcium and more than half (boys: 50%; girl: 80%) did not achieve the RNI for iron. Current findings also found significant positive but weak correlations between calcium (r=0.112, p=0.001) and iron (r=0.084, p=0.008) intakes with BMI-for-age. **Conclusion:** BMI-for-age was related to calcium and iron intakes among secondary school students in Malaysia. Thus, intervention strategies should focus on early screening and nutrition education on food choices of high calcium and high iron contents, including iron supplementation programmes, if needed.

Keywords: adolescents, calcium, iron, intake, Malaysia

INTRODUCTION

Adolescence is the transition period from childhood to adulthood with immense growth in opportunities, trials and challenges. The World Health Organization (WHO) (2014) defines an adolescent as any person between the ages of 10 and 19 years. Due to their rapid biological and psychological changes, adolescents are frequently perceived as a nutritionally vulnerable group (WHO, 2014). Adolescent health is

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to be prioritised as they are the future generation of the world and their health status is important for the well-being of each community (Patton *et al.*, 2016).

Calcium is important for bone health. Due to intensive bone and muscular development, there is an increased need for calcium among adolescents (Mouratidou *et al.*, 2013). Apart from bone and muscular development, hormonal changes and growth during puberty also encourage more mineral use, thus requiring higher intake of minerals such as calcium (Mesias *et al.*, 2013). However, most children and adolescents throughout the world are unable to meet the recommended calcium intake.

Another mineral essential for growth and development during adolescence is iron. Iron stored during growth is important for haemoglobin and blood circulation. Among boys, adequate intake of iron is necessary to sustain the rapid gain in muscle mass and blood volume. Although girls gain muscle and blood volume slower than boys, adolescent girls remain in need of high iron intake to support menstrual losses as well as for growth. Deficiency in iron among children and adolescents can lead to anaemia, which can cause impairment in physical growth, mental and motor development, as well as learning capacity (Lassi *et al.*, 2017). Iron deficiency among adolescents is recognised as a significant nutritional problem worldwide, across both developing and developed nations (Finkelstein *et al.*, 2018).

Food intake among adolescents in many developing countries nowadays comprises primarily of foods high in fat, sugar and salt content such as fast foods, processed foods, sweets and sugar-sweetened drinks. Conversely, their diet is frequently low in fibre-rich foods including whole grains, fruits and vegetables or legumes (Hassapidou *et al.*, 2006). In a past study involving 165 adolescents between 12-19 years old from a rural community in Sabah, Malaysia (Foo *et al.*, 2004), almost 98% of subjects did not meet the Malaysian Recommended Dietary Allowance (RDA) level (<9mg/day) for dietary iron intake (NCCFN, 2017). Another study in Malaysia also revealed that 80% and possibly more children achieved the Recommended Nutrient Intake (RNI) for almost all nutrients, except for calcium and vitamin D (Poh *et al.*, 2013). About 794 diet histories from adolescents aged 13 years old were analysed in The MyHEART cohort study (Majid *et al.*, 2016) in Peninsular Malaysia, which was aimed to investigate the dietary intakes of Malaysian adolescents. The study showed that both males and females consumed inadequate intakes of energy, vitamin D, and calcium. It also showed that females consumed inadequate levels of iron (<100%) (Majid *et al.*, 2016).

Healthy eating habits during adolescence are the foundation for optimum health in adulthood. Previous findings have reported an association between body weight and iron deficiency among adolescents (Hutchinson, 2016; Aigner *et al.*, 2014, Moayer *et al.*, 2006). Meanwhile, another study found no significant association between calcium and body weight management (Shapses, Heshka & Heymsfield, 2004).

Therefore, this study was undertaken to determine the prevalence of inadequate micronutrient intake, particularly calcium and iron intakes, among Malaysian adolescents, and to investigate the relationships between calcium and iron intakes with body mass index (BMI) using data from the Adolescent Nutrition Survey (ANS) (IPH, 2017).

MATERIALS AND METHODS

Study setting and sample size

The optimum sample size required for the ANS 2017 was 30,496 respondents based on the single proportion formula

and stratification of the 16 states in Malaysia (including Federal Territory of Kuala Lumpur, Putrajaya, and Labuan) (IPH, 2017). Based on students' enrolment data, as well as the schools registered with the Ministry of Education Malaysia, there were 7,926 primary schools and 2,688 secondary schools were included in the sampling frame. The schools were inclusive of national schools, vernacular schools, and private schools. From this sampling frame, 311 schools were selected based on random selection to participate in ANS 2017.

Study design and participants

The Adolescent Nutrition Survey was a cross-sectional study involved adolescents aged 10 to 17 years old, attending public and private schools in Malaysia. However, only data of respondents aged 13 to 17 years old were analysed for this manuscript as this age group was more likely to have low calcium and iron consumption (IPH, 2017). Data collection commenced from 26th March to 3rd May 2017 (IPH, 2017).

Ethics approval and consent to participate

Ethical approval was obtained from the Ministry of Health, Research and Ethics Committee (NMRR No: NMRR–16–698–30042). Approval was also granted by the Ministry of Education officials at the State and District levels, as well as from the respective selected schools.

Sampling procedure

A multistage stratified cluster sampling was used to ensure a nationwide representative sample of adolescents aged 13 to 17 years old. Students were selected based on random sampling method and invited to participate in the survey. Informed consent from the students involved and parental consent was obtained before the survey was conducted. However, from the 311 qualified schools participated in this survey, only 999 respondents who completed dietary recall and sociodemographic characteristics were being analysed.

Anthropometric measurements

BMI-for-age *z*-scores (BAZ) of adolescents were identified based on the WHO Growth Reference 2007 (WHO, 2007). Trained research assistants conducted the anthropometric measurements. Weight and height measurements were obtained based on standard methods using a SECA weighing scale (SECA Clara 803, Germany) and a SECA stadiometer (SECA 217, Germany), recorded to the nearest 0.5 kg and 0.1 cm, respectively. The measurements were taken twice and average readings were calculated and recorded. Weight, height and age of children were processed using the WHO AnthroPlus software to calculate standard scores (*z*-scores) for BMI-for-age, which is an indicator for thinness.

Assessment of dietary intake

Dietary assessment in the Adolescent Nutrition Survey 2017 involved habitual food intake assessment using food frequency questionnaire and 24-hour dietary recall. However, this study only reports the outcomes from the 24-hour dietary recalls. The assessment of dietary intake was done by trained nutritionists using a single 24-hour dietary recall via face-to-face interview in the national language (Hassapidou *et al.*, 2006, Mouratidou *et al.*, 2013). Data were gathered using a standard form with the aid of food album and explorative questions to allow respondents to recall consumed foods and beverages. Nutrient intakes were determined from the diet recalls using the Nutritionist Pro software (Axxya Systems, Stafford, TX USA), based principally on the Malaysian Food Composition (Tee *et al.,* 1997). Mean intakes of calcium and iron were derived from the analysis. However, sources of calcium and iron from foods

were not determined in this study, and iron intake was not differentiated between haem and non-haem iron.

Data processing and analysis

Data were analysed using SPSS version 21. Sample weight was calculated based on every student's dietary recall record and was adjusted for varying probabilities of selection, as well as for study non-responses. Complex samples analysis was performed in the statistical analysis and was conducted at 95% confidence interval. For analysis purposes, respondents were assigned according to age groups based on RNI for Malaysia 2017 (NCCFN 2017). Thus, data of calcium and iron intakes were interpreted based on these age group classifications.

RESULTS

Sociodemographic characteristics

Sociodemographic characteristics of the adolescents are shown in Table 1. Overall, 999 respondents completed data collection for dietary intake and sociodemographic characteristics. A large percentage of the respondents was from the age group of 15 to 17 years old, with slightly higher proportion of female (55.1%) students, from urban areas (53.8%), and Malays (66.6%).

Comparison of calcium and iron intakes by sex, strata and ethnicity

In Table 2, from Mann-Whitney U test and Kruskal-Wallis test, there were statistically significant differences for calcium and iron intakes

Table 1. Socio-demographic characteristics of subjects

Characteristics	*Age group*		*Total population*
	13-14 years n (%)	*15-17 years* n (%)	n (%)
Sex			
Boys	190 (42.3)	259 (57.7)	449 (44.9)
Girls	236 (42.9)	314 (57.1)	550 (55.1)
Strata			
Urban	223 (41.5)	314 (58.5)	537 (53.8)
Rural	203 (43.9)	259 (56.1)	462 (46.2)
Ethnicity			
Malay	291 (43.8)	374 (56.2)	665 (66.6)
Chinese	51 (36.2)	90 (63.8)	141 (14.1)
Indian	21 (48.8)	22 (51.2)	43 (4.3)
Others	55 (40.4)	81 (59.6)	136 (13.6)
Bumiputeras			
Others	8 (57.1)	6 (42.9)	14 (1.4)
BMI categories			
Thinness	27 (43.5)	35 (56.5)	62 (6.2)
Normal	303 (40.7)	442 (59.3)	745 (74.6)
Overweight	61 (48.6)	59 (49.2)	120 (12.0)
Obese	35 (48.6)	37 (51.4)	72 (7.2)

Table 2. Comparison of calcium and iron intakes by sex, strata and ethnic groups

Variable	*Calcium (mg)*	*Z-statistics*	*p-value*[a]	χ^2 *statistics (df)*	*p-value*[b]	*Iron (mg)*	*Z-statistics*	*p-value*[a]	χ^2 *statistics (df)*	*p-value*[b]
Sex		-5.479	<0.001***				-7.270	<0.001***		
Male	555.35					573. 45				
Female	454.81					440.04				
Strata		-2.676	0.007**				-1.651	0.099		
Urban	522.66					513.98				
Rural	473.66					483.75				
Ethnic				3.132	0.536				6.204	0.184
Malays	502.81					506.35				
Chinese	498.23					468.66				
Indian	551.30					421.47				
Other Bumiputeras	468.98					523.15				
Others	528.00					530.54				

[a]Mann-Whitney U test
[b]Kruskal-Wallis test
**p<0.01, ***p<0.001 indicate statistical significance

Table 3. Percentage of adolescents according to RNI achievements in calcium intake and iron intake

Gender	*Age group (years old)*	*Calcium n (%)*		*Iron n (%)*	
		Did not achieve RNI n (%)	*Achieved RNI n (%)*	*Did not achieve RNI n (%)*	*Achieved RNI n (%)*
Boys	13-14	183 (96.3)	7 (3.7)	85 (44.7)	105 (55.3)
	15-17	243 (93.8)	16 (6.2)	139 (53.7)	120 (46.3)
Girls	13-14	232 (98.3)	4 (1.7)	123 (52.1)	113 (47.9)
	15-17	305 (97.1)	9 (2.9)	294 (93.6)	20 (6.4)

between sex and strata. However, there was no statistically significant difference in calcium intake (χ^2=3.13, p=0.536) and iron intake (χ^2=6.20, p=0.184) between ethnic groups.

Proportion of adolescents meeting the RNI

Table 3 outlines the percentage of adolescents achieving the RNI for calcium and iron intakes in a day. Overall, most respondents did not achieve the RNI for calcium intake and more than half of them did not reach the RNI for iron intake. The results showed that 96.3% boys and 98.3% girls aged 13 to 14 years old did not achieve the RNI for calcium, respectively; while 93.8% boys and 97.1% girls aged 15 to 17 years old did not achieve the RNI for calcium. For iron intake, 44.7% boys and 53.5% girls aged 13 to 14 years old did not achieve the RNI for iron; while 52.1 % boys and 93.6% girls aged 15 to 17 years old did not achieve the RNI for iron. Besides, this study also revealed that the proportion of girls who failed to achieve the RNI for both calcium and iron intakes were higher than boys.

Correlation between calcium and iron with BMI-for-age

Although most respondents did not meet the RNI for calcium and iron intakes, this study found a significant, positive but weak correlation between calcium (r=0.112, p=0.001) and iron (r=0.084, p=0.008) intakes with BMI-for-age as presented in Table 4.

DISCUSSION

The present study highlighted the inadequacy of calcium and iron intakes among Malaysian adolescents. Despite rapid socio-economic growth, Malaysia faces the double burden of malnutrition (Ihab *et al.*, 2013), with low intakes of these two nutrients among the adolescents. Calcium need increases

Table 4. Correlations between calcium and iron intakes with BMI-for-age z-scores

Mineral	*BMI-for-age z-scores*	
	r	*p*†
Calcium (mg/day)	0.112	0.001
Iron (mg/day)	0.084	0.008**

† Pearson Correlation test

** p<0.01 indicates statistical significance

with age due to intensive bone and muscular progress (Mesias *et al.*, 2011). It is therefore crucial to ensure sufficient calcium intake throughout the growing age to ensure optimum peak bone mass and to prevent osteoporosis in adulthood (Mouratidou *et al.*, 2013). Unfortunately, findings from the current study showed that more than 90% of adolescents did not meet the calcium RNI, thereby presenting a public health risk.

The findings from this study are consistent with earlier reports (Im *et al.*, 2014; de Assumpcao *et al.*, 2016), which revealed that most children and adolescents in Korea and in Iran failed to meet the recommended calcium intake (1300 mg/day) (WHO, 2006). Therefore, it is encouraged to provide a diet with adequate nutrient composition and sufficient calcium allowance for maximum bone mass growth (Im *et al.*, 2014).

Findings from this study also showed that adolescents consumed insufficient iron. As compared with this study, findings from of an earlier local study by Foo *et al.,* (2004) among rural communities in Sabah, Malaysia showed unsatisfactory dietary iron intake among adolescents aged 12 to 19 years, with 98% of subjects not meeting the Malaysian RDA level for iron [14.4 μmol/L (male), 10.3 μmol/L (female)]. In another local study, about 91% of female subjects had dietary iron intake below two-thirds of the RDA level compared to a smaller proportion among male adolescents (68%) (Foo *et al.*, 2004). Another finding from this current study was the lower mean calcium intake among adolescents living in the rural area, which is in line with another local study that suggested it was a common occurrence in rural areas (Foo *et al.*, 2004).

This is probably due to females having menses and not eating appropriately according to their requirements. Mesias *et al.* (2013) justified that adolescence is a period of time when iron needs are optimised in line with increases in blood volume and growth of muscle mass. Hence, lack of iron intake may lead to iron deficiency and adversely affect body physiology. Adverse effects related to iron deficiency are more likely to occur especially among female adolescents, athletes, and those who limit their meat intake (Mesias *et al.*, 2013). The current findings also showed that there were significant differences in calcium and iron intakes between boys and girls. These findings are similar with a study from China that also showed significant gender differences (Wang *et al.*, 2017).

There is a possibility of calcium absorption being interfered by high protein intake, as a previous finding mentioned that adequacy of high-quality protein intake may impact calcium absorption (Calvez *et al.,* 2012). Low bioavailability of iron might also be related to the intake of vitamin C. However, a study from the Kansas, USA mentioned most epidemiology studies were unable to show an association between vitamin C intake and iron absorption status (Cook & Reddy, 2001). Furthermore, iron deficiency anaemia is known as a significant factor contributing to micronutrient malnutrition in Malaysia (Teegarden, 2003).

This study showed a positive correlation between calcium and iron intakes with BMI -for-age z-score. This indicates the contribution of these minerals towards BMI. Although there is no evidence available to support the association between calcium intake and BMI, dietary calcium seems to be related to energy metabolism, where it is suggested to play a substantial role in contributing and reducing the incidence of obesity (Teegarden, 2003). However, this may be due to the effect of nutrient density, that might be influenced by total energy intake rather than the micronutrient itself. No local study has revealed any association between BMI and iron intake. Nevertheless, a foreign study which took place in a rural

area in North West Ethiopia among adolescent schoolgirls, found that BMI-for-age [*AOR*=3.2; 95%CI (1.43-7.05)] was among the predictors of anaemia (Mengistu *et al.*, 2019).

Adolescents should be trained, supported and encouraged to eat healthy foods and a balanced diet, as well as to practise a healthy lifestyle to promote optimal calcium consumption and iron absorption. Findings from the current study indicate the importance of nutrition education and promotion in school environment or via school programmes to ensure that messages on balanced and healthy diet reach the adolescent group. Nutrition education and promotion on high calcium and high iron foods can enhance the knowledge and awareness on healthy nutrition practices among adolescents (Xie *et al.*, 2003).

The present study is among the few large-scale population-based studies that have been conducted in Malaysia. The data represent all students attending schools in Malaysia and the outcomes can be used to enhance health programmes, focusing particularly on nutrition, for adolescents.

Limitation of the study

This study did not encompass major food sources of calcium and iron intakes of adolescents. Besides, factors contributing to the inadequacy of calcium and iron intakes among adolescents were not determined in this study. Therefore, this information should be included in future studies to help Malaysian adolescents achieve calcium and iron recommendations.

CONCLUSION

The findings confirmed that Malaysian adolescents have inadequate calcium and iron intakes. The percentage of adolescents meeting the RNI for both calcium and iron was alarmingly high. There were significant associations between calcium and iron intakes with BMI. These results indicate that messages promoting balanced nutrition are not reaching the adolescents. The level of knowledge and awareness on good nutrition should be emphasised. Thus, intervention strategies should focus on early screening and nutrition education on food sources of high calcium and high iron contents, including iron supplementation, if needed.

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Authors' contributions

SMS, involved in collating the data, preparation of the draft manuscript, and reviewed the manuscript; RAT was responsible for the concept of the project development, prepared the draft of the manuscript, involved with interpretation of data analysis, and reviewed the manuscript; RS, principal investigator, conceptualised, responsible for the project development, supervised the project's progress, and reviewed the manuscript; RA, MP, NSAA assisted in the preparation of the manuscript, reviewed and approved the final manuscript. NIW and MAO conducted the data analysis and interpretation of the data, and reviewed the manuscript.

Availability of data and materials

Data will be available upon request from the corresponding author.

Conflict of interests

The authors declare that they have no competing interests.

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Healthier pineapple tart pastry using oleogel-based solid fat replacement

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ABSTRACT

Introduction: Pineapple tarts are a commonly consumed Southeast Asian pastry made using solid fats like butter and palm shortening. These solid fats predominantly contain high amounts of saturated fats which have been implicated in negative health effects. However, solid fats impart important textural properties in pastry formation and is not easy to replace. To overcome this challenge, a concept to enhance the nutritional value whilst maintaining the textural properties of pineapple tart pastry formed the basis of this study. **Methods:** This short study explored the use of "healthy" avocado-olive oil-based oleogels structured with food-grade ethylcellulose (EC), monoglycerides (MG) or its combination (EC-MG) as solid fat replacements to butter and palm shortening. The textural properties of the pastry dough and tart were determined using a texture analyser, while the nutritional content of the pastries was compared. **Results:** The firmness of pastry dough decreased in the order: EC >> EC-MG > butter ~ MG ~ shortening, while tart hardness decreased: EC > shortening ~ butter > MG > EC-MG. The combination EC-MG oleogel had positive effects on the textural properties by improving the dough workability and reducing the tart hardness compared to EC. Remarkably, the oleogel tart pastries had up to 70% less saturated fat compared to the butter or palm shortening pastries. **Conclusion:** This study confirms the ability to create healthier pastries whilst maintaining its texture.

Keywords: oleogels, fat mimetics, ethylcellulose, monoglyceride, pastry

INTRODUCTION

Pineapple tarts are a popular pineapple jam-filled pastry commonly consumed in Southeast Asia. Like many pastries, these tarts are often made using solid fats such as butter and vegetable shortening to impart desirable texture, mouthfeel and structure. Consequently, these snacks usually contain high saturated fat content (Yeo *et al.*, 2020), which is undesirable as diets rich in saturated fats have been associated with increased risk of cardiovascular diseases and the development of other health complications (Tan *et al.*, 2018). Therefore, there is an opportunity to modify pastry recipes to produce healthier alternatives without compromising the palatability of the product (Guinard *et al.*, 2020).

In our study, cardio-protective oils rich in monounsaturated fatty acids and phytochemicals, such as avocado and olive oil (Dreher & Davenport, 2013; Gorzynik-Debicka *et al.*, 2018), were used to negate the ill effects of

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saturated fats, which are extensively used in pastry manufacturing. However, liquid oils do not possess the physical properties of solid fats, thus limiting their applications in pastry. To tackle this problem, we proposed structuring these plant oils into oil gels (oleogels) that could behave as solid fats in pastry application (Rogers *et al.*, 2014).

Food gels like jellies, jams and tofu are typically made by the entrapment of water by various gelling agents. These are known as hydrogels. Replacing the continuous water phase by oil leads to the formation of oil gels or oleogels. In general, the liquid oil is entrapped through physical interactions within supramolecular structures or polymeric networks formed by structuring agents (Co & Marangoni, 2018). These structuring agents can include small molecules like monoglycerides or polysaccharides such as ethylcellulose (Maya Davidovich-Pinhas, 2019). Furthermore, from our previous work, ethylcellulose is able to reduce postprandial lipemia (Tan *et al.*, 2018), while monoglyceride is already present in many food products as emulsifiers (Valoppi *et al.*, 2017).

Therefore, to explore the potential replacement of saturated fats with healthy oils in pastry, we investigated the effect of substituting butter or palm shortening with avocado-olive oil oleogels structured by ethylcellulose (EC) or monoglyceride (MG) on pastry dough and pineapple tart texture. As different oil gelling agents have distinct properties, we also examined the combination of both structuring agents (EC-MG). Finally, nutritional comparisons of the various pineapple tart pastries were made.

MATERIALS AND METHODS

Materials and baking ingredients

Extra virgin avocado oil (Grove Avocado Oil Ltd, New Zealand), pure olive oil (Naturel, Lam Soon Pte Ltd, Singapore), unsalted Danish butter (RedMan, Phoon Huat Sdn Bhd, Malaysia), and refined palm fat shortening (RedMan, Phoon Huat Sdn Bhd, Malaysia) were obtained from a local supermarket. Ethylcellulose was obtained from Dow Wolff Cellulosics GmbH (ETHOCEL™ 45 premium, viscosity 45 cP, Germany). Commercial food-grade mixture of monoglycerides was kindly donated by Palsgaard Asia-Pacific Pte Ltd (DMG 0093, Singapore). The monoglyceride powder contained > 90% monoglycerides and had maximum free glycerol, free fatty acids, and iodine values of 1.0%, 1.5%, and 2 g/100 g powder, respectively from the technical data sheet provided by the manufacturer. Analysis by other workers showed that the DMG 0093 MG powder was composed of ~38% 1-mono-stearoyl-*rac*-glycerol and ~54% 1-mono-palmitoyl-glycerol (López-Martínez *et al.*, 2014).

The remaining baking ingredients, also obtained from a local supermarket, included condensed milk (Milkmaid, F&N Foods Pte Ltd, Singapore), skim milk powder (NTUC Fairprice Co-operative, Singapore), eggs (Seng Choon, Singapore), plain flour (RedMan, Phoon Huat Sdn Bhd, Malaysia), and vanilla essence (RedMan, Phoon Huat Sdn Bhd, Malaysia).

Preparation of oleogels

From preliminary works done to optimise the sensory profile, a 1-part avocado oil to 1.1-part olive oil was mixed to form an oil blend. To prepare the oleogels, ethylcellulose and/or monoglyceride powders were added to the blended avocado-olive oil under constant stirring and heated at 150°C (or 80°C for the samples containing only monoglyceride) for 10 minutes to achieve full dissolution. Three oleogel compositions were made containing 12% (w/w) ethylcellulose (EC), 12% (w/w) monoglyceride (MG), or 6% (w/w) ethylcellulose with 6% (w/w) monoglyceride (EC-MG). The mixtures were then poured into containers and stored at ~23°C to set.

Preparation of pastry dough

The tart-making was adapted from a traditional recipe. The pastry formulation included 130 g plain flour, 110 g butter/shortening/oleogel, 30 g condensed milk, 20 g skim milk powder, 15 g egg yolks, 10 g egg white, and 1 g vanilla essence. Condensed milk and butter/shortening/oleogel were creamed together for 5 minutes under low speed using a stand mixer. Eggs and vanilla essence were added and mixed for 1 minute. Plain flour and skim milk powder were then added and mixed for 1 minute to form a smooth dough. In total, five different types of pastry doughs were prepared (butter, shortening, EC, MG and EC-MG).

Mechanical properties of pastry dough and tarts

Texture analyses were conducted using a TA.XT plus texture analyser (Stable Micro Systems Ltd, Surrey, England) equipped with a 30 kg load cell. All tests were performed at 22±1°C. To measure dough firmness and stickiness, 20 g each of the various pastry doughs was first shaped in a round aluminium tart shell (4.5 cm top diameter x 2 cm bottom diameter x 2 cm height). The shaped dough was then penetrated to 75% depth using a ½ inch stainless steel spherical probe (P/0.5S, Stable Micro Systems, Surrey, UK) at a test speed of 1 mm/s. The maximum force required for 75% penetration and to lift the probe was defined as firmness and stickiness, respectively.

To measure tart hardness, 20 g each of the various pastry doughs was first shaped in a round aluminium tart shell (4.5 cm top diameter x 2 cm bottom diameter x 2 cm height) and baked in a pre-heated oven at 160℃ for 27 minutes and allowed to cool. Each tart was removed from the tart shell and compressed to 50% displacement using a 75 mm flat, circular compression plate (P/75, Stable Micro Systems, Surrey, UK) at a test speed of 1 mm/s. The peak force during compression was defined as hardness, while the total amount of energy per second required to break the tart (as measured by total area under the curve) was defined as the work of failure.

All textural parameters were evaluated using the Texture Expert software (version 6.1). Six replicates were prepared and measured for each of the pastry dough and tart.

Nutritional analysis

The nutritional data was compiled using nutritional information provided on the ingredients packaging and calculated based on the proportions used in the recipe. As the monoglycerides used were saturated fats, they were assumed to have similar nutritional content to lipids (9 kcal/g). As the nutritional information for ethylcellulose is currently not known, it was assumed to behave like cellulose fibre.

Statistical analysis

Statistical analysis was conducted using R v. 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). A one-way analysis of variance (ANOVA) was performed for texture measurements to determine if the mean values of measured parameters differed significantly with formulation. The significance was established using Tukey HSD (honest significant difference) post-hoc tests. A probability level of $p<0.05$ was considered significant. All values were expressed as mean ± 1 standard deviation.

RESULTS AND DISCUSSION

Mechanical properties of the pastry doughs and tarts

The various pastry doughs' firmness and stickiness values are presented in Figure 1. Notably, the trends for dough firmness and stickiness values were similar in the order: EC >> EC-MG > butter ~ MG ~ shortening. The firmness of the dough reflects the maximum force needed to break intermolecular

interactions for plastic deformation to occur and indicates its ease of workability. Dough stickiness relates to the adhesion between the dough and the spherical probe due to the formation of molecular interactions between the dough and probe surface (Dobraszczyk, 1997). EC dough was significantly firmer and stickier than all other doughs, reflecting its hard texture and difficulty to work with. At the other spectrum, shortening dough had the lowest

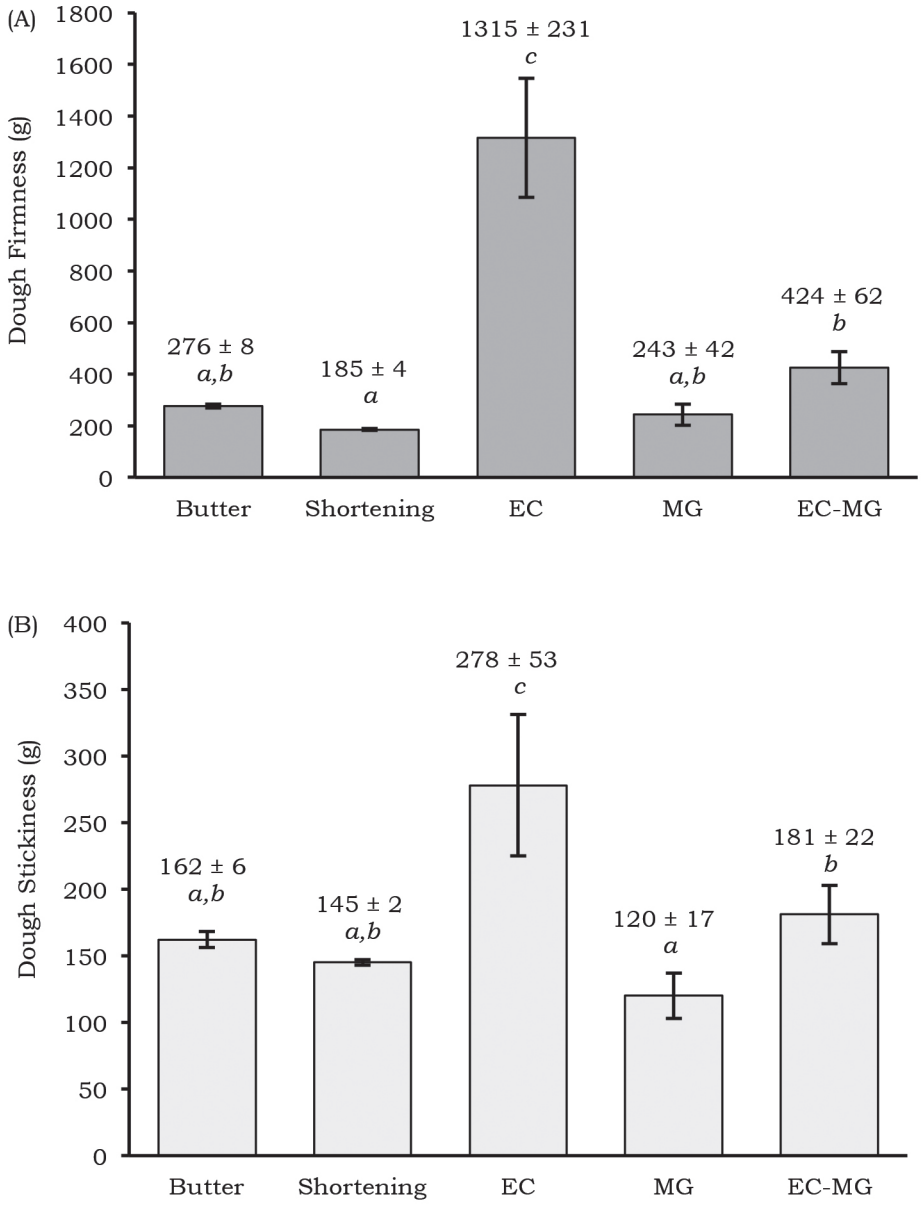

Figure 1. Texture profile analyses on firmness (A) and stickiness (B) of pastry dough made using butter, palm shortening, ethylcellulose oleogel (EC), monoglyceride oleogel (MG) or mixed oleogel (EC-MG). Data expressed as mean ± 1 standard deviation (n=6). Data points connected by the same letter are not significantly different from each other (p>0.05)

firmness reflecting its soft consistency. The firmness and stickiness of MG dough were not significantly different to butter dough and they had similar workability. EC-MG dough had intermediate firmness and stickiness to EC dough and MG dough. Previous studies demonstrated that monoglycerides had a plasticising effect on ethylcellulose (Davidovich-Pinhas, Barbut & Marangoni, 2015). In this study, both MG and EC-MG oleogels were comparable in texture to softened

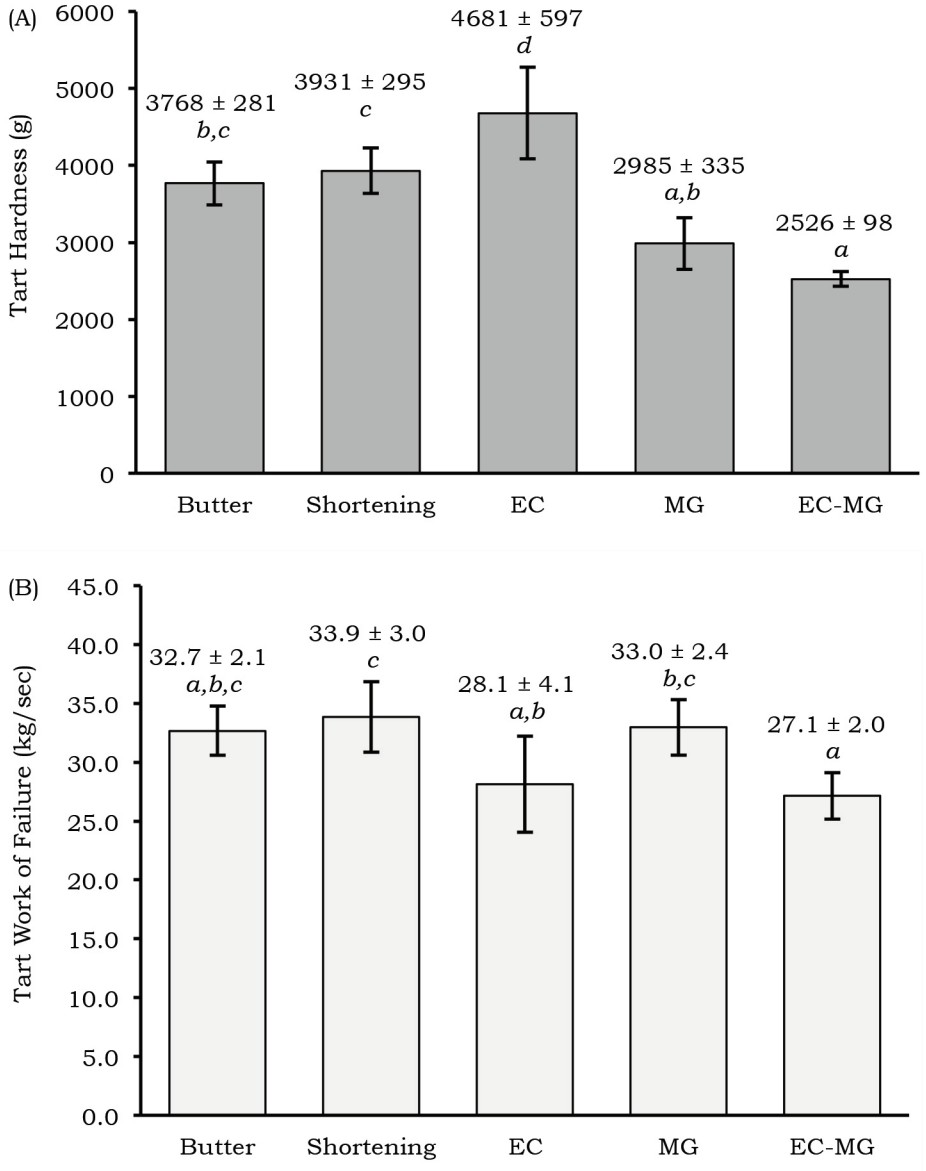

Figure 2. Texture profile analyses on hardness (A) and work of failure (B) of pastry tart made using butter, palm shortening, ethylcellulose oleogel (EC), monoglyceride oleogel (MG) or mixed oleogel (EC-MG). Data expressed as mean ± 1 standard deviation (n=6). Data points connected by the same letter are not significantly different from each other (p>0.05)

Table 1. Nutritional comparisons, calculated from the nutritional data of ingredients, between uncooked pineapple tart pastry made using ethylcellulose oleogel (EC), monoglyceride oleogel (MG), mixed oleogel (EC-MG), butter or palm shortening

Nutritional parameters	*EC*	*MG*	*EC-MG*	*Butter*	*Palm shortening*
Serving size (g)	100	100	100	100	100
Energy (kcal)	486	523	504	460	523
Protein (g)	8.3	8.3	8.3	8.6	8.3
Carbohydrate (g)	38.4	38.4	38.4	38.5	38.4
Total fat (g)	33.2	37.3	35.2	30.7	37.3
Saturated fat (g)	5.1	9.3	7.2	18.3	16.6

butter, so it was unsurprising that their respective doughs were comparable.

From Figure 2, there were significant differences in the hardness of the oleogel tart samples compared to butter and shortening controls. Tart hardness was in the order: EC > shortening ~ butter > MG > EC-MG. The work of failure was more equivocal, with only EC-MG and shortening differing significantly. Although EC tart was relatively hard, its work of failure was relatively low compared to butter and shortening tarts as EC tart fractured easily. Despite the comparable work of failure, the MG tart's hardness was significantly lower than the butter and shortening tarts. Interestingly, while EC tarts were considerably harder than MG tarts, EC-MG tarts had the lowest hardness and work of failure values. Overall, there was no significant difference in the dough and tart texture between MG and butter. Although EC was not able to mimic the properties of butter, combining EC and MG improved the pastry dough and tart texture.

Nutritional comparisons between the tart pastries

As calculated from the ingredients, the nutritional data between the uncooked tart pastries made using oleogels, butter or palm shortening are shown in Table 1. The protein and carbohydrate contents of the pastries were similar as only the lipid profile was altered. The energy content of the pastries decreased in the order: shortening ~ MG > EC-MG > EC > butter, mirroring its total fat content. However, the pastries made using the oleogels had markedly lower saturated fat content – up to 70% reduction – compared to the pastries made using butter or palm shortening. Furthermore, the avocado-olive oil blend contains healthful phytochemicals with anti-inflammatory and anti-atherogenic properties (Dreher & Davenport, 2013; Gorzynik-Debicka *et al.*, 2018). Additionally, as we noted from our previous work, ethylcellulose is able to reduce postprandial lipemia (Tan *et al.*, 2018). Thus, we speculate that the ethylcellulose oleogels (EC and EC-MG) are further able to restrict lipid absorption, consequently reducing the effective energy content of the pastries. Based on the nutritional and textural data, the EC-MG oleogel enabled both the nutritional benefits of the ethylcellulose oleogel and the textural properties of the monoglyceride oleogel to be harnessed. Further work is needed to fine-tune the balance between both oleogelators.

CONCLUSION

This short study evaluated the effects of replacing butter and palm shortening with potentially health-promoting avocado-olive oil-based oleogels structured with EC, MG and EC-MG on pineapple pastry dough and tart texture. While EC led to brittle pastry dough and tart,

MG oleogel led to relatively comparable properties to butter and shortening. The combination EC-MG oleogel had positive effects on textural properties, reducing the dough and tart's brittleness, and improving the workability of the dough compared to EC. The EC-MG oleogel may also deliver the nutritional benefits of EC oleogels. Importantly, the oleogel pineapple tart pastries had up to 70% less saturated fat compared to the butter or palm shortening tart pastries. The monounsaturated fatty acid-rich avocado and olive oils may further confer cardio-protective effects. This brings us a step closer to the paradigm where pastries not only bring us delight but also could enhance our health.

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Authors' contributions

SYJS, conceptualised and designed the experiments, analysed the data and prepared the manuscript; KXW, performed the experiments and collated the data, analysed the data and prepared the manuscript; CJH, conceptualised and designed the experiments, reviewed the manuscript.

Conflict of interest

All authors declare no conflict of interest.

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Validity and reliability of plant-based culinary nutrition model questionnaire for fitness among sports science students

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ABSTRACT

Introduction: There is an emerging focus on plant-based foods that have the added advantage of being nutritious without side effects. Besides, its preparation with nutritional awareness and culinary skills could be an effective solution for improving personal fitness. This study aimed to determine validity and reliability of a questionnaire for the development of a plant-based culinary nutrition model for fitness among sports science students. **Methods:** This study employed exploratory sequential mixed method design and was carried out in two phases. Phase 1 employed a qualitative design utilising modified Delphi method to determine content validity index (CVI) of the questionnaire, while phase 2 was a quantitative design using Cronbach's alpha statistical analysis to evaluate reliability of the instrument. The questionnaire consisted of the need for plant-based culinary nutrition model (Section A) and the model's components (Section B). Thirteen expert panels from diverse expertise in sports nutrition and 30 sports science students took part. **Results:** Phase 1 resulted in development of a questionnaire where Section A and Section B scored CVI of 0.834 and 1.000, respectively. For phase 2, Cronbach's alpha score for reliability of the questionnaire was 0.836 with a total of 15 items. The Cronbach's alpha score for questionnaire items from Sections A and B were 0.709 and 0.832, respectively. **Conclusion:** The questionnaire developed in this study is validated and is considered reliable for use as a significant tool for plant-based culinary nutrition among sports science students.

Keywords: plant-based nutrition, culinary, health and fitness, sports science students

INTRODUCTION

Previous literature findings revealed that plant-based whole foods have the added benefits of being entirely healthy without side effects (Evans, 2015). Generally, plant-based diets are rich in carbohydrates, fibre, micronutrients, phytochemicals, and antioxidants. However, they contain lower calories, protein, vitamin B12, n-3 fats, calcium, and iodine. Hence, meal planning and management of food choices become crucial for the achievement of recommended energy and nutrient intakes. In addition, a well-planned plant-based diet can provide adequate amounts of all known nutrients for fitness and sports performance (Burke & Hawley, 2018; Hever, 2016). Data indicated that all

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essential and non-essential amino acids can be supplied by plant food sources alone as long as a variety of foods is consumed and the energy intake to maintain fitness is adequate (Hever, 2016; Venderley & Campbell, 2006).

A plant-based diet facilitates high intake of carbohydrate and protein, while at the same time maintains prolonged exercise, recovery, and repeated performance (PCRM, 2014; McAnulty, Holden & Keith, 2001). Some of the popular plant-based diets being practised world-wide are lacto-vegetarian, ovo-lacto vegetarian, vegan, and raw vegan. Vegan diet which emphasises a complete removal of animal-based products from diet is also gaining popularity among the public and younger generations (Wirnitzer, 2018).

While healthy eating behaviour is being touted, similar emphasis is still lacking when it comes to instilling culinary skills from young. Fundamental culinary skills are choosing, preparing, and consuming foods prepared from 'scratch' (Caraher, 2015). Culinary skills are not only for professional chefs, but they are also practical techniques that everyone should master to maintain health and fitness. The merging of nutrition and food science with culinary skills has resulted in healthy eating behaviours due to increased confidence and nutrition awareness (Condrasky & Hegler, 2010).

Plant-based culinary nutrition model is a model that includes both the fundamentals of nutrition and the basics of culinary skills to manage fitness. It was pursued to compliment various efforts and strategies taken by the Ministry of Health in managing fitness of the nation in general and sports science students specifically. Under these circumstances, a plant-based culinary nutrition model may be useful as a guidance for sport science students in managing their fitness better through plant-based nutritional knowledge and culinary skills. Besides that, there is a need for a plant-based culinary nutrition model for fitness due to the awareness, practicality and safety of consuming plant-based diets for sustainability, health, and ethical reasons. Many athletes and experts in the health and fitness industry or sports are receptive towards plant-based diet for fitness and performance, and have thus adopted plant-based lifestyle (Burke & Hawley, 2018). In the local scenario, a plant-based culinary nutrition model for fitness is perhaps anticipated to guide sports science students with a healthier diet preference for optimum fitness. Therefore, the validity and reliability of a suitable questionnaire play an important role in developing a useful plant-based culinary nutrition model.

The aim of testing for validity is to determine if the instrument measures what it was intended to measure, and whether it is usable (Oluwatayo, 2012). Validity is also viewed as an indication of the extent to which a research conclusion reflects reality (White & McBurney, 2012); whereas reliability is referred as dependability and replicability, striving for accuracy and comprehensiveness of a survey instrument. One of the ways to test reliability is through Cronbach's alpha. It measures internal consistency reliability based on the average inter-item correlation of an instrument (Koonce & Kelly, 2014).

While validity is about the capacity of an instrument to quantify the properties of the construct (Barton, Wrieden & Anderson, 2011), reliability is the consistency of a measure. Hence, the questionnaire needs to be validated and tested for reliability in order to be a reliable data collection tool for the development of plant-based culinary nutrition-related studies. Thus, the aim of this study was to determine the validity and reliability of the survey instrument developed for data collection. These valuable data will measure the need and significance of developing a plant-based culinary nutrition

model for fitness among sports science students.

MATERIALS AND METHODS

Study design

This study employed a mixed method exploratory sequential design which consisted of two phases. Phase 1 was qualitative data collection that utilised the modified Delphi technique, whereas phase 2 was quantitative data collection. In phase 1, the reporting of interview with expert panel members used the Consolidated Criteria for Reporting Qualitative Research (COREQ) as a guideline (Tong, Sainsbury & Craig, 2018). It is a formal reporting checklist for in-depth interviews and focus groups. The reporting of expert panel's interview in the current research was based on three main domains in COREQ. These included researcher or research team and reflexivity, study design, and analysis and findings. Meanwhile, content validity ratio (CVR) was used for quantitative assessment of the content validity (Ayre & Scally, 2014). This was done by simplifying the information into CVR Worksheet that calculated the CVR for an item and content validity index (CVI) value for the overall instrument (Lawshe, 1975).

In phase 2, there were four research questions to answer: (1) how far experts perceive the importance of having a plant-based culinary nutrition model for fitness; (2) what are the components of plant-based culinary nutrition model for fitness; (3) what is the consensus level of experts on components in plant-based culinary nutrition model; and (4) what is the process of plant-based culinary nutrition model development. These were answered through responses from the expert panel, whereas the survey items were built from consensus by the experts.

Participants

For phase 1 of the study, participants were a panel of experts chosen based on purposive sampling. The panel of 13 experts identified and interviewed were subject matter experts in the area of sports nutrition and dietetics, coaching, athletics, academicians, sports scientists, and a locally and internationally renowned Emeritus Professor in Nutritional Sciences. Of these, three experts were practising a plant-based diet in their daily life. There are no strict rules on the number of experts required for this study (Thangaratinam & Redman, 2005).

The recruitment was conducted through personal contacts of supervisors and web search. Official email invitation was sent to the experts as well. This email also served as a consent to be interviewed at expert's time and venue of convenience without any obligation or monetary gain. Confidentiality was maintained throughout recruitment, interview process, and data analysis. The replies from experts were via emails and text messaging. Table 1 shows the demographic information of expert panels.

Phase 2 involved the recruitment of sports science students from a local public university and data collection on reliability. These students were between 19-25 years old pursuing various degree programmes in Sports Science. They were selected as the subjects for phase 2 of the study in order to equip sports science students with an alternative diet option with relevant culinary skills applicable for managing fitness. Samples were calculated based on a flat rule of thumb in which a single number is recommended for any situation. For the current research, a flat rule of thumb by Browne (1995), which is a sample size of 30 was adopted (PASS Sample Size Software, 2019). This research was conducted with the approval of the University of Malaya Research Ethics Committee (UM.TNC2/UMREC-431). The participants provided their written informed consent.

Development of instrument by using Delphi technique

The research instrument, a questionnaire, was developed based on the modified Delphi method (Thangaratinam & Redman, 2005). It was appropriate because it involved face-to-face and individual interview with each expert. It also used open-ended questions that allowed flow of opinions, ideas, suggestions, and experience sharing. External experts were engaged during validation process. Data were collected thru in-depth interviews by using open-ended questions: (1) what do you know about plant-based diet; (2) what is your opinion on the research that shows plant-based culinary nutrition is able to manage fitness; (3) should plant-based culinary nutrition be introduced as a course or programme in the current sports nutrition curriculum at higher learning institutions; (4) what components of plant-based culinary nutrition should be included into the plant-based culinary nutrition model for fitness.

Development of questionnaire

The qualitative data were analysed by using modified Delphi for three rounds. The interview with expert panel was recorded, transcribed verbatim, and coded manually based on Saldana's coding methods (Onwuegbuzie, Frels & Hwang, 2016). A consistent approach to coding is important to ensure the credibility of data analysed. There were four steps in the qualitative data analysis. The first step was basically transcribing each interview, whereas the second step involved the transfer of the completed transcription data onto Microsoft Excel sheet and analysed to view the similarities or differences between expert views. The third step was initial coding in which the code was either a word or phrase that represented aspects of a research question. They were examined to identify patterns and frequency. The last step was the assigning of labels to research questions in the form of anchor codes. Under each anchor code, there was a compilation of initial codes used to generate categories.

Categories would have larger viewpoints which were then condensed further into Themes 2 and further narrowed into Themes 3. Themes were used to guide the development of the

Table 1. Demographic information for expert panels based on COREQ checklist

Experts	*Expertise*
1	Dietitian and assistant director (sports performance) at National Sports Institute, Malaysia
2	Sports nutrition lecturer
3	Director, Division of research and innovation, Sports Institute
4	Head of Food service department, Sports Council
5	Executive chef, Sports Council
6	Sports medicine specialist
7	Sports coach and tower runner
8	Endurance sports coach
9	Endurance sports coach
10	Head of postgraduate studies, Faculty of Sports Science & Recreation
11	Plant-based sports scientist
12	Emeritus Professor in nutrition
13	Director of plant-based nutrition education

survey instrument, which eventually answered the research questions. Based on the analysis of themes and also literature review, it was possible to generate items for the questionnaire and eventually a questionnaire draft based on a 5-point Likert scale (1=disagree; 2=slightly disagree; 3=neither agree nor disagree; 4=agree; 5=strongly agree) (Simon & White, 2016). There were two parts and a total of 20 items in the questionnaire draft. Part 1 was the demographics of the respondents. Part 2 had Section A with 12 items about the need to develop a plant-based culinary nutrition model for fitness, and Section B about the components in the model, which consisted of 8 components.

Generation of items

The drafted questionnaire was distributed to six experts for feedback, as well as face and content validity analysis. The standard number of experts required for Delphi studies is still ambiguous (Hsu, 2007). However, in the present study, the selection of six experts was based on their willingness to participate in the following rounds. They were requested to provide comments and suggestions to improve the instrument.

Experts had to fill in an Expert Validation form, which was adapted from Survey/Interview Validation Rubric for Expert Panel (VREP) (Simon & White, 2016). Experts had to score each criterion with a range of 1=not acceptable (major modifications needed); 2=below expectations (some modifications needed); 3=meets expectations (no modifications needed but could be improved with minor changes); and 4=exceeds expectations.

Content validity

Additionally, the six experts were required to complete the questionnaire. Content validity is basically the constancy of content made by experts based on their experience, knowledge, and wisdom in respective fields. Items that were given a response of 3=neither agree nor disagree; 4=agree; and 5=strongly agree were chosen for the calculation of CVR and CVI. Items with the scores of 1=disagree and 2=slightly disagree were not considered for CVR and CVI calculation. CVI is the mean of CVR values of the retained items (Lawshe, 1975). Data collected were gathered and computed. An approach developed by Lawshe in 1975 was employed in this research. It was simplified into CVR Worksheet that calculated the CVR for an item and a CVI value for the overall instrument. The worksheet was based on the following formula:

$$CVR = \frac{n_e - \left(\frac{N}{2}\right)}{\frac{N}{2}} \quad (1)$$

Where n_e is the number of panelists indicating "essential" and N is the total number of panelists.

CVR score ensures the content is important and most accurate. The questions were designed in the best way to gauge the need of this model and the components that should go into the model.

$$CVI = \frac{Number\ of\ experts\ who\ give\ 3\ or\ 4\ to\ this\ item}{Total\ number\ of\ experts} \quad (2)$$

Statistical analysis

Internal consistency of the questionnaire was assessed to ensure reliability and it was measured by using the Cronbach's alpha. Results of reliability were expressed as mean ± standard deviation. Furthermore, data collected from the respondents were statistically analysed using the Statistical Package for Social Sciences (SPSS) version 23 (IBM SPSS New York, United States, 2019).

RESULTS AND DISCUSSION

The aim of this research was to determine the validity and reliability of

a questionnaire for the development of a plant-based culinary nutrition model for fitness among sports science students. Based on data interpretation by the 13 experts, a questionnaire with 20 items was drafted. The questionnaire drafted was administered to six selected experts in round two for review, feedback, and suggestions. In this round, the redundant items were removed. All items were made into statements and language was corrected structurally. The redundant items were merged and there was one item that was completely removed. In round three, questionnaire was refined and re-sent to the six experts for final review and comments. After modification, the total number of items was 15. Experts were satisfied with the items in the questionnaire and approved it for pilot study.

Table 2 shows the validation of the questionnaire by experts. The criteria for validation were clarity, wordiness, negative wording, overlapping response, balance, use of jargon, appropriateness of responses listed, use of the English Language, and application to praxis. For criteria with scores 3 and 4, there were no changes required to be made on the questionnaire. Significant feedback from experts were important to make the questionnaire credible and subsequently contribute to a valuable research process. All six experts responded to the expert validation form and this indicated the responses for overall questionnaire, which was face validity.

Table 3 shows the opinion of experts on items of the questionnaire and amendments made after round two. It was found that the items were clear, no wordiness, negative wording or jargons. The level of English language was appropriate. Besides that, items that were related to respondents' lifestyle and statements were unbiased. At round three, the experts reviewed the drafted questionnaire as a final step before data collection from respondents. Table 4 presents the experts' concluding comments. At this round, the validated questionnaire had a total of 15 items. The need to develop a plant-based culinary nutrition model for fitness (Part I, Section A) had eight items. However, one out of the eight components in the model (Part II, Section B) was removed due to lack of consensus. Specifically, plant-based Culinary Art (food presentation) was not endorsed by five experts. Therefore, a total of seven items for the components in the model were retained in the survey. The items retained in the final questionnaire are shown in Table 5. Experts' comments were recorded and changes were made accordingly. Some of the changes were standardisation of items from questions into statements, removal of redundant statements or merging of statements, and more precise phrases were added to the instructions in the questionnaire to be more approachable while at the same time ensured that humanising element was present.

CVR and CVI were based on expert advice and the score remained high. Table 5 shows the CVR of questionnaire items**.** The score of CVR was 0.667 and above for each item, and this was accepted as valid. Only one item was removed from Section B due to lack of consensus among experts. Apart from that, phase 1 of the study resulted in the development of a questionnaire, which included the needs and components, respectively. It had two sections and a total of 15 questionnaire items. Each section scored a CVI of 1.000 and 0.834, respectively. CVI value was based on CVR calculation of each item in the questionnaire survey.

Reliability analysis is a measure to reflect the construct of the questionnaire items' consistency. Cronbach's alpha is the most common measure used to measure internal consistency reliability (Field, 2009). A reliable instrument should be able to produce similar data from similar respondents in the future. There are varying suggestions to the

Table 2. Validation of expert panels on the questionnaire (*n*=6)

No	*Criteria*	*Operational definitions*	*Number in agreement*	*Validation by six experts*
1	Clarity	The questions are direct and specific. Only one question is asked at a time. The participants can understand what is being asked. There are no double-barrelled questions (two questions in one).	6	Statements are clear
2	Wordiness	Questions are concise. There are no unnecessary words.	5	No wordiness
3	Negative wording	Questions are asked using the affirmative (e.g., Instead of asking, "Which methods are not used?", the researcher asks, "Which methods *are* used?")	6	No negative wording
4	Overlapping responses	All possibilities are considered. There are no ambiguous questions.	5	No ambiguity
5	Balance	The questions are unbiased and do not lead the participants to a response. The questions are asked using a neutral tone.	6	Questions are balanced
6	Use of jargon	The terms used are understandable by the target population. There are no clichés or hyperbole in the wording of the questions.	6	No jargon used
7	Appropriateness of responses listed	The choices listed allow participants to respond appropriately. The responses apply to all situations or offer a way for those to respond with unique situations.	6	Responses listed are appropriate
8	Use of English language	The use of English Language is basic and appropriate. All acronyms are defined.	5	English Language level is appropriate
9	Application to praxis	The questions asked to relate to the daily practices or expertise of the potential participants.	6	Statements relate to participants' lifestyle

Table 3. The final comments of expert panels and amendments (*n*=6)

Expert Panel	*Comments*	*Amendment*
Expert 1	The "note" on page 2 defines a plant-based diet - then the first question on page 3 asks the respondents to comment on a definition - I wonder how much the response to the question on page 3 will be biased by the definition already given on page 2?	No further comments Re-worded: Culinary is an art of cooking, nutrition is food science. Both culinary and nutrition principles learning should be combined for better physical fitness management
	Section A, Q3 - this statement a slight re-wording required	No reason just overlooked. Changed highlight to blue
	Section A, Q8 - is there any reason this row is highlighted in green rather than blue (as per the other rows above)	
	Your survey is looking great!	
Expert 2	This looks good to me!	None
Expert 3	I still have my reservation on your definition. You may need to say like milk, eggs which applies to vegetarians if different categories. If not the definition of plant-based diet is not having any animal products. They can be vegan	A plant-based diet is an eating pattern that is dominated by minimally processed plant foods including whole grains, fruits, vegetables, legumes, nuts and seeds, perhaps, with limited animal-based products (dairy or eggs) or no animal-based products at all. Also commonly known as vegetarian or vegan diet lifestyle in Malaysia
Expert 4	Part II: Section A, No. 3, to add: …physical fitness to athletic performance and sports No 5: …related to performance	Culinary is an art of cooking, nutrition is food science. Both culinary and nutrition principles learning should be combined for better physical fitness management No changes because this study is not probing into various sports performances.
Expert 5	1. Page 1: Highlighted - Perhaps you can use a more approachable/humanising phrases. E.g., thank you for participating in this questionnaire/survey etc. 2. page 2: Age: Perhaps you could use age range for better quantitative data analysis on the demographic. But if you only have a very small gap of the age group then leave it as it is. 3. Page 3: Again, the use of the term respondent. And you may want to add a description of the items. E.g. The statement below … Please rate/response … (Please refer to Section B instruction)	Thank you for completing this survey. We greatly value the information you have provided. Your responses will contribute to the development of plant-based culinary nutrition model for fitness No changes. Sports science students are bachelor's degree students with age range between 19-25years old The list below contains views on the need to develop a plant-based culinary nutrition model for fitness. Please mark the range that you think best reflect the need.
Expert 6	All ok! Good to go	None

Table 4. Items retained in the questionnaire

Section	*Items retained in the questionnaire*
A. The need for a plant-based culinary nutrition model	Responses 1. Plant-based diet is about consuming food from plants and fruits ONLY (NO animal-based food products or ingredients included). 2. Plant-based diet is an environment friendly diet 3. Culinary is an art of cooking, nutrition is food science. Both culinary and nutrition principles learning should be combined for better physical fitness management 4. Plant-based culinary nutrition knowledge encourages healthier cooking and eating lifestyle 5. Culinary skill is a life skill; Sports Science students should learn various ways of cooking to prepare healthy meals 6. Plant-based culinary nutrition for fitness should be taught in schools 7. The development of a model for plant-based culinary nutrition for fitness will provide standard guidelines for Sports Science students to practice plant based culinary nutrition lifestyle 8. Plant-based culinary nutrition should be introduced as a program in the current sports nutrition course at higher learning institutions
B. Components	1. Fundamentals of nutrition (plant-based macronutrients and micronutrients) 2. Setting up a culinary nutrition kitchen (kitchen tools, equipment, safety and hygiene) 3. Plant-based menu planning based on nutritional needs 4. Plant-based ingredients substitutions 5. Plant-based shopping list 6. Plant-based cooking methods 7. Culinary herb and spices

acceptable variance for reliability of between 0.60 (60%) to 0.70 (70%) (Field, 2009). Reliability was measured based on pilot study. The reliability was high for both Section A and Section B. The overall instrument reliability score based on Cronbach's alpha was also high, making it a reliable instrument. As a result, the pilot study provided affirmative reliability scores of the instrument developed, a positive representation of the population about the need to develop a plant-based culinary nutrition model for fitness, and acceptance of the components suggested for the model. The validity and the reliability of the questionnaire were good overall. This questionnaire can therefore be used for data collection to assess the need to develop a plant-based culinary nutrition model for fitness and the culinary components required in the model.

The finalised questionnaire for this research had 15 items with two parts. Part 1 was about demography, whereas Part 2 had two sections. There were eight statements in Section A and seven statements in Section B. For the quantitative part, the validity and reliability of the questionnaire included quantitative and qualitative face validity, quantitative and qualitative content validity, construct validity, and reliability of the tool assessed.

Table 5. The content validity ratio (CVR) of questionnaire items

No	*Statements*	*CVR*	*Accepted or not accepted*	*Finalised statements*
Part II – A: The need to develop a plant-based culinary nutrition model for fitness				
1	Plant-based diet is about consuming 100% plants and fruits only (NO animal-based products or ingredients)	0.667	Accepted	Plant-based diet is about consuming food from plants and fruits. (Minimal animal-based products or ingredients might be included)
2	Plant-based diet is an environment friendly diet	1.000	Accepted	Plant-based diet is an environment friendly diet
3	Culinary is an art of cooking. Nutrition is food science. Both Culinary and Nutrition should be learnt together for health and fitness	0.667	Accepted	Culinary is an art of cooking. Nutrition is food science. Both culinary and nutrition principles learning should be combined for better physical fitness management
4	Plant-based culinary nutrition knowledge encourages healthy eating	0.667	Accepted	Plant-based culinary nutrition knowledge encourages healthier cooking and eating lifestyle
5	In order to cook healthy food, one must have plant-based nutrition knowledge?	0.667	Accepted, merged with item 8	
6	Cooking is a life skill; everyone should learn various ways of cooking	0.667	Accepted	Culinary skill is a life skill; Sports Science students should learn various ways of cooking to prepare healthy meals
7	Plant-based culinary nutrition for fitness should be taught in school	0.667	Accepted	Plant-based culinary nutrition for fitness should be taught in school
8	Personal fitness can be managed through plant-based culinary nutrition	0.667	Accepted, merged with item 5	
9	Do you agree that plant-based nutrition education for fitness should be supported by culinary skills?	0.667	Accepted, merged with item 6	
10	To what extent do you agree that culinary skills is a must-learn skill for sports science students?	0.667	Accepted, merged with item 11	
11	To what extent do you agree with the development of a model for plant-based culinary nutrition to provide standard guidelines?	1.000	Accepted, merged with item 10	The development of a model for plant-based culinary nutrition for fitness will provide standard guidelines for Sports Science students to practise plant-based culinary nutrition lifestyle

Table 5. The content validity ratio (CVR) of questionnaire items [Cont'd]

No	*Statements*	*CVR*	*Accepted or not accepted*	*Finalised statements*
12	Plant-based culinary nutrition should be introduced as a programme in the current sports nutrition curriculum at higher learning institutions	0.667	Accepted	Plant-based culinary nutrition should be introduced as a programme in the current sports nutrition course at higher learning institutions
Part II – B: Components in the model				
1	Fundamentals of nutrition (whole food, plant-based macronutrients and micronutrients)	1.000	Accepted	Fundamentals of nutrition (plant-based macronutrients and micronutrients)
2	Setting up a culinary nutrition kitchen (kitchen tools, equipment, safety and hygiene)	1.000	Accepted	Setting up a culinary nutrition kitchen (kitchen tools, equipment, safety and hygiene)
3	Whole food plant-based menu planning based on nutritional needs	0.667	Accepted	Plant-based menu planning based on nutritional needs
4	Plant-based ingredient substitutions	1.000	Accepted	Plant-based ingredient substitutions
5	Whole food plant-based shopping list	1.000	Accepted	Plant-based shopping List
6	Plant-based cooking methods	1.000	Accepted	Plant-based cooking methods
7	Culinary herb and spices	1.000	Accepted	Culinary herb and spices
8	Plant-based culinary art (food presentation)	0.333	Not accepted	

Reliability was achieved through pilot test. For this research, Cronbach's alpha score of above 0.7 was considered reliable. This was based on George & Mallery (2003) and the rule of thumb for alpha coefficients by Gliem & Glim (2003). The analysis was run on Section A and Section B of the questionnaire separately. The questionnaire items from Section A scored 0.709 and Section B had a score of 0.832. The 15 items had a mean score of between 3.800 and 4.430 each, and the standard deviation was between 0.568 and 0.964. Accordingly, the internal consistency of the entire 15-item instrument was demonstrated by Cronbach's alpha at 0.836. However, no item was deleted to improve the score as the overall reliability of the instrument was 0.836, which was considered acceptable. The results obtained were consistent with the findings from Kliemann *et al.* (2016). The authors found that the reliability of the updated version of the general nutrition knowledge questionnaire was greater than 0.7. This indicated that their updated scale had good reliability. Besides that, Eshaghi *et al.* (2020) reported that the Persian version of questionnaire was acceptable because it scored more than 0.80.

This model basically imparts culinary skills with plant-based nutrition knowledge. The model has two parts with seven components: (a)

Fundamentals of nutrition (plant-based macronutrients and micronutrients), (b) Setting up a culinary nutrition kitchen, (c) Plant-based menu planning, (d) Plant-based ingredients substitutions, (e) Plant-based shopping list, (f) Plant-based cooking methods, and (g) Culinary herbs and spices. The fundamentals of nutrition and culinary skills should be applied in daily food consumption to maintain fitness. This research has adopted the power plate as part of its model, which is a guidance for a balanced plant-based food consumption daily. The Power Plate was created by the experts at Physicians Committee for Responsible Medicine (PCRM, 2014). Power plate for fitness features vegetables, fruits, whole grains and legumes, which are essential for health management. All seven components listed had CVR and CVI value of 1 unanimously, which indicated maximum consensus for each component. Content analysis was done, then followed by CVR and CVI of the questionnaire items. The findings proposed that the questionnaire for a plant-based culinary nutrition model for fitness among sports science students was a valid and reliable survey instrument for data collection.

Strengths and limitations

This was a mixed method research, which is a new contemporary wave in science methodology (Petrovic, Koprivica & Bokan, 2017). The integration of qualitative and quantitative methods provide an innovative way that enables researcher to gain opinions from experts to understand the complex and sensitive issues in sports nutrition. Plant-based culinary nutrition model is related to real health and fitness issues among athletes. Optimistically, this research will engage people and policy makers to improve the fitness level of athletes and possibly the nation. However, the study population was limited to sports science students in Klang Valley. As a result, they only represent a small part of the Malaysian sports science student population.

CONCLUSION

The questionnaire for a plant-based culinary nutrition model for fitness among sports science students was validated and tested for reliability. Cronbach's alpha score was 0.836 with a total of 15 items. The questionnaire items from both Sections A and B were statistically reliable, with a score of 0.709 and 0.832, respectively. Hence, a questionnaire on a plant-based culinary nutrition model for fitness among sports science students that is reliable for data collection has been developed.

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Authors' contributions

BBM, principal investigator, conceptualised the study, prepared the draft of the manuscript; AZAR and SHH, advised on data analysis and interpretation, and reviewed the study design. All authors reviewed the manuscript and approved the final version of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interests.

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Clinical utility of low branched-chain amino acid modular diets in patients with isovaleric aciduria and maple syrup urine disease

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ABSTRACT

Introduction: Modular diets (MDs) with low amount of offending amino acids have been developed using locally available food ingredients as alternatives to commercial formulas for the treatment of branched-chain organic acidurias (BCOAs). Herein, we conducted a clinical investigation of MDs in patients with BCOAs. **Methods:** Modular diet A (MDA), with low leucine was produced for maple syrup urine disease (MSUD), and modular diet B (MDB) products, MDB-1, -2, -3, and -4, with low leucine, valine, methionine and threonine were made for isovaleric aciduria (IVA)/methylmalonic aciduria (MMA)/propionic aciduria (PA). Children aged 4-18 years, with MSUD, IVA, PA or MMA were invited to participate in the study. The research subjects switched from metabolic formula protocol to modular diet protocol. They were followed-up at 0, 1, 2, 4, and 6 months. Clinical efficacies of MDs were determined by completion of study, compliance to MDs, clinical outcomes and complications, and parental satisfaction. **Results:** Six children (2 MSUD and 4 IVA) participated and completed the study. Compliance to MDA was 100% in MSUD subjects with G-tube feeding, while compliance to MDB varied among self-fed individuals with IVA. One subject with MSUD was clinically stable throughout the study, while the other experienced metabolic instability. All IVA individuals showed clinical and laboratory stability during the study. One MSUD and three IVA families preferred the metabolic formula, whereas the other IVA family reported no preference and the other MSUD subject preferred MDs. **Conclusion:** We provided a proof of concept in developing modular diets for BCOAs, and showed favourable outcomes when using MDs in IVA and varying clinical benefits in MSUD.

Keywords: branched-chain organic acidurias, isovaleric aciduria, maple syrup urine disease, metabolic disorders, modular diet

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INTRODUCTION

Branched-chain organic acidurias (BCOAs) are a group of disorders resulted from deficiencies of branched-chain amino acids (BCAAs) catabolism enzymes. The most common disorders of this group are maple syrup urine disease (MSUD), isovaleric aciduria (IVA), propionic aciduria (PA), and methylmalonic aciduria (MMA). These disorders are characterized by ketoacidosis and acute/progressive neurological involvement, caused by accumulation of toxic metabolites proximal to the metabolic block (Leonard & Morris, 2000; de Baulny, Vici & Wendel, 2012; Saudubray *et al.*, 2002).

During an acute metabolic crisis, dialysis or haemofiltration are often performed in order to remove the toxic metabolites and prevent severe permanent brain damage and death (de Baulny *et al.*, 2012; Saudubray *et al.*, 2002; Zand *et al.*, 2008). For long-term treatment, dietary management is mandatory. The primary goals of dietary management include the promotion of growth and development, prevention of the accumulation of toxic metabolites, enhancement of the excretion of harmful substances accumulated, administration of co-factors, and close monitoring for nutritional deficiencies (Manoli & Venditti, 2016). Low protein diets in combination with disease-specific amino acid-free medical formulas, known as metabolic formulas, are recommended to stabilize the metabolic conditions, and support normal growth and neurodevelopment (Knerr *et al.*, 2012; de Baulny *et al.*, 2012; Yannicelli, 2006). The metabolic formulas are manufactured in powder form in which crystalline amino acids voiding of the offending amino acids are used as protein sources. These formulas are enriched with vitamins, minerals, and some with fatty acids, as well as additional sources of energy (Marriage, 2010; Strauss *et al.*, 2010).

Currently, these metabolic formulas are not registered under the Thai Food and Drug Administration (FDA) due to a lack of interest by the companies as a result of small market size and expenses related to the registration process. In addition, the cost of treatment using these metabolic foods is not covered by any health schemes. The metabolic formulas currently used in Thailand have been donated from a single production company. There has been periodic shortage of these medical foods, which contribute to suboptimal treatment in patients. Direct import of these products by the families is problematic due to custom issues and its status as non-registered medical products. While the availability of medical products and periodic shortage are still unresolved, an alternative measure needs to be developed for temporary resolution.

Modular diets (MDs) contain single or multiple nutrients which are combined to fulfil a specified need in medical treatment (Davis & Baker, 1996). The use of MDs allows flexible mixing ratio; therefore, one food ingredient module can be combined with another. Food ingredients are available in general markets, over the counter or by prescription. Examples of carbohydrate modules are starch, polysaccharides, glucose polymers, mono- and disaccharides. Medium-chained triglycerides (MCT) and long-chained triglycerides (LCT) oil can be used as fat sources (Davis & Baker, 1996).

We previously published an article on the development of food products using fish maw and roasted sunflower kernel for patients with branched-chain organic aciduria (Chatvuttinun *et al.,* 2018). Five products were successfully developed: Formula I, low leucine diet for MSUD

and Formulas II-V, low leucine and valine diet made for IVA, PA, and MMA. Herein, we conducted a clinical study of MDs in children with branched-chain organic acidurias using these previously developed food products, and evaluated their clinical efficacies.

MATERIALS AND METHODS

Subject recruitment and study design

Eligible subjects were children with MSUD, IVA, PA, or MMA, aged 4-18 years who were followed-up at Pediatric Outpatient Clinic, Ramathibodi Hospital. Exclusion criteria included (i) in serious metabolic illness; (ii) have cardiac or kidney disease related to the underlying metabolic disorder; (iii) have a history of multiple (>2) hospitalisations related to their metabolic disorder during one year prior to entering the study; and (iv) subject withdrawal. Parents of the research subjects were required to provide written informed consents. The study was conducted following an approval from the Ramathibodi Hospital Institutional Review Board (ID 09-57-25:MURA2014/492S1; MURA2020/1058).

This study was an outpatient-based, single-arm trial lasting a period of six months (March-August 2015). Patients' demographic data including age, sex, underlying diseases and severity, onset of diseases, feeding history, current clinical status, and baseline dietary treatments were reviewed.

Preparation of modular diets (MDs)

For the convenience of monitoring, we reassigned the MDs in this study into two major formulas - MDA for MSUD and MDB for IVA, PA and MMA. The MDA (previously named as Formula I) contained low leucine, whereas the MDB contained low leucine, valine, isoleucine, methionine, and threonine. The MDs were produced using fried fish maw (Tawan Fish Maw, Tawan Produce Co., Ltd., Samutprakarn, Thailand) and roasted sunflower kernels (Flower Food Ltd., Bangkok, Thailand), following the established protocols (Chatvuttinun *et al.,* 2018). These food items were selected because they contained minimum/low ratio of leucine to protein and were produced under systematic farming in which the quality could be controlled and natural variation was minimal. The fish maw was obtained from a farmed fish called "Pla Swai" *(Pangasius hypophthalmus)*, while sunflower kernel *(Helianthus annuus)* was harvested from a sunflower farm, followed by sorting and roasting. Both raw materials were preserved in dried/roasted and dried/fried forms, which made them available all year round. These products are commercially and widely available worldwide. The production of MDs was carried out at a facility available at the Institute of Nutrition, Mahidol University.

Given that all eligible MSUD subjects were on G-tube, only mixed powder form of MDA was prepared. For those self-fed IVA/MMA/PA patients, four types of MDB products were made available for the patients to choose, namely MDB-1: rice sprinkle powder, MDB-2: bouillon cube for stir-frying, MDB-3: instant cocoa drink, and MDB-4: chocolate snack bar (Figure 1A). The ingredients, nutrient composition, and branched-chain amino acid profiles of MDA and the four MDBs were described in an earlier publication. The MDB-1, MDB-2, MDB-3, MDB-4 were formerly named as Formulas II, III, IV, V, respectively (Chatvuttinun *et al.,* 2018).

We informally performed a simple sensory testing among four adult volunteers and three eligible research subjects by giving them a bite try of modular products, then modified the recipes to improve their texture, taste, and flavour as needed. After achieving

the final recipes, we performed quality analysis of all products including macronutrients, amino acid profiles, and water activity. The MDs produced were also proven to be void of chemical toxicity and were microbiologically safe for food grade. Each product provided 500-600 kcal per 100 g edible portion. Energy sources of the MDs were mainly from carbohydrate and fat, similar to the metabolic formulas (BCAD2/LMD). However, MDA and MDB as compared to metabolic formulas, contained higher distributive energy from fat (46-67% vs. 18-46%), and less distributive energy from carbohydrate (24-46% vs. 41-56%). Energy distribution from protein source was lower in the MDs as compared to metabolic formulas at 5.8-86% vs. 13-23% (Chatvuttinun *et al.,* 2018).

We then calculated energy requirement for each patient, following the Holliday-Segar equation and standard nutritional recommendations for severe MSUD, IVA, MMA, and PA (Barshop, 2006; Wappner & Gibson, 2006). Baseline nutritional intake of each patient was taken into account for preparation of MD-nutrients to be provided. Total protein intake for patients aged 4-8 and 8-15 years were 1.5-2.0 and 1-1.2 g/kg/day, respectively (Wappner & Gibson, 2006). For MSUD and IVA, leucine was selected as the primary offending amino acid for the calculation of amino acid allowance per day. The daily leucine allowance was 500-750 mg/day for MSUD and 650-1,500 mg/day for IVA because leucine tolerance is known to be higher in individuals with IVA than MSUD (Marriage, 2010; Wappner & Gibson, 2006). As for MMA and PA patients, valine was chosen as the primary for calculation of maximum daily allowance of offending amino acids, leading to the following assumptions – 700-1600 mg of valine, 600-1300 mg of isoleucine, 500-1200 mg of threonine, and 250-800 mg of methionine. Each MD product was packaged into a cling wrapped foil sachet to suit individual nutritional requirement. Upon completion of the packaging, microbial contamination was analysed following published protocols (Chatvuttinun *et al.,* 2018).

Metabolic formula protocol, modular diet protocol, and prescription of modular diets

Metabolic formula (MF) protocol involved medical formula plus "baseline-low protein diet" (baseline-LPD) that the subjects regularly consumed prior to study entry. During the study, subjects were asked to switch from MF-protocol to MD-protocol, which included MDs plus adjusted type and amount of natural low protein diet (adjusted-LPD) following the recommendations by researchers. The amounts of MDs and adjusted-LPD to be consumed per day were calculated for each subject, based on individual body weight, weight-for-height, and baseline amino acid tolerance according to the widely used standard treatment for amino acid disorders. As cooked rice is a basic diet for most Thai people, we started by calculating energy, protein, and leucine intakes from the amount of cooked rice the participating subjects usually consumed and negotiated this to a realistic amount acceptable for them in order to keep the amount of offending amino acids from exceeding the maximum allowance.

The study subjects were asked to continue their routines including school activities. They were asked to visit the research team at 0, 1, 2, 4, and 6 months for clinical and dietary assessments, checking of compliance to MDs, and for blood tests. A 1 to 2-month supply of MDs was provided to the subjects at each clinic visit. Telephone communication was used as extra follow-ups when necessary. Individuals with IVA were invited to choose specific products, MDB-1 and MDB-2, during

the first month of the study. In order to provide new choices and to motivate the children's appetite, MDB-3 and MDB-4 were offered as alternatives at the end of the first month and during following visits.

Dietary assessment and compliance monitoring

Parents were asked to record the amount of MDs and adjusted-LPD consumed by their children daily. Additionally, the parents prepared a three-consecutive day food record prior to each clinic visit. They were required to bring the left-over MDs to the clinic, which allowed the researchers to assess and confirm individual compliance to MDs. Compliance to MDs was defined as percentage of the amount of MDs consumed as compared to the amount prescribed.

Clinical assessment

At every clinic visit, anthropometric characteristics including weight, height, head circumference, mid arm circumference, and triceps and biceps skinfolds were measured following standard methods. Data on weight, height, length were analysed and expressed as weight-for-age z-scores (WAZ), height (length)-for-age z-scores (HAZ), and weight-for-height (length) z-scores (WHZ), using the WHO (2006) for the growth standards (for 0-5 years old) and WHO (2007) for the growth reference (for children 5 years and above) (de Onis *et al.*, 2007), and WHO AnthroPlus software version 1.0.4 (available at http://www.who.int/growthref/tools/en/). WAZ or HAZ <-2 indicated underweight or stunting, respectively. Plasma amino acid profiles (fasting), electrolytes (including TCO_2 content), ammonia, albumin, pre-albumin, blood urea nitrogen, and complete blood count were investigated.

To monitor for metabolic illness and patient safety, information on adverse clinical symptoms, hospital admission, dietary adjustment, and other additional treatments were recorded. Standard sick day and/or emergency protocols were activated when there was medical illness or metabolic crisis (Wappner & Gipson, 2006). The sick day protocols included home-based close monitoring, reduction of intact protein by 50-100% for 24-48 hours, increase of fluid hydration, and adding non-protein energy sources such as fruit juices (Wappner & Gipson, 2006). Once the acute illness/crisis was over, MD-protocol was resumed. If the patient's metabolic condition became uncontrolled, defined as having ≥2 hospitalisations during the study period, the MD-protocol would be terminated for that particular individual. In addition, the subjects continued taking vitamin and mineral supplements at the same dosages that they have received prior to study entry.

Analysis of clinical utility

Clinical utility of MDs was determined using descriptive analysis, by (i) complete vs. drop-out from the study; (ii) compliance to MDs; (iii) clinical outcomes and complications as evidenced by nutritional status, clinical and metabolic instability, hospitalisations, and adverse laboratory changes; and (iv) parental satisfaction. Parents of subjects were asked, at the end of the study, to express their overall preference on MD-protocol vs. MF-protocol (if they preferred continuation of MD-protocol or MF-protocol). Statistical analysis was not employed due to small number of research subjects.

RESULTS

Patient characteristics and MD prescription

There were nine eligible subjects

including one MMA, three MSUD and five IVA patients. Two patients (MSUD and IVA) declined to take part in the study because long distance travelling and frequent clinic visits were required. The MMA patient was excluded due to having complicated chronic kidney disease. As a result, a total of six subjects including two MSUD and four IVA patients participated in the study. Their age ranged between 4.0-17.3 years. All research subjects had severe neonatal onset of the diseases (Table 1).

Energy, nutrient, and BCAA intakes at baseline and during the study are shown in Table 1. Each individual was equally prescribed to obtain 490 kcal/day from three meals of adjusted-LPDs and the remaining daily calories from MDs at all visits until the end of the study. The caloric intake from MDs ranged 52-66% of their daily prescribed calories.

Drop-out, compliance to modular diets, and product acceptability

All participating subjects completed the study with no drop-outs. Compliance to MDA product was 100% in MSUD subjects. As for IVA self-fed subjects, after one month (M1) of trial, the compliance to MDB-1 and MDB-2 ranged 0-70% and 20-60%, respectively. With the additional choice of MDB-3, the compliance of most subjects increased to 45-90% at the end of the second month (M2) and subsequently to 40-100% during the fourth and sixth months (M4 and M6) of the study (Figure 1B).

Clinical outcomes and complications

All IVA subjects reported that they were able to continue their daily activities as usual during the study, including attending school and play, whereas those with MSUD (with severe psychomotor delay) stayed at home as

Table 1. Demographic data of the study patients

Characteristic	*Pt. 1*	*Pt. 2*	*Pt. 3*	*Pt. 4*	*Pt. 5*	*Pt. 6*
Underlying disorder	MSUD	MSUD	IVA	IVA	IVA	IVA
Sex	male	female	male	male	male	female
Age of onset	5 d.	7 d.	11 d.	3 d.	8 d.	4 d.
Age at diagnosis	2 m.	2 m.	14 d.	10 d.	4 m.	6 d.
Age at entry (years)	17.3	4.4	4.6	9.7	9.0	4.0
Current clinical status						
Psychomotor delay	severe	severe	no	mild	mild	mild
Other manifestation	seizure	seizure	ADHD	ADHD	ADHD	no
Baseline daily dietary intake						
Route of delivery	G-tube	G-tube	oral	oral	oral	oral
Diet	BCAD2 & LPD	BCAD2 & LPD	LMD & LPD	LMD & LPD	LMD & LPD	LMD & LPD
Calories (kcal)	1819	1355	1197	953	991	878
Protein (g)	51.1	22.8	25.8	24.2	30.8	28.2
Leucine (mg)	1374	735	1191	1100	1804	1200
Leucine (mg/kg)	34.4	49.0	59.3	44.9	50.8	86.3

Notes: ADHD, attention deficit and hyperactivity disorder; G-tube, gastrostomy tube; IVA, isovaleric acidemia; LPD, low protein diet; MSUD, maple syrup urine disease

they did previously. During the study, all but one subject (P2), reported no vomiting, gastrointestinal discomfort, or any illnesses. One patient (P1) became neurologically more alert, energetic, and communicative as compared to his baseline. Subject P2, at 2.5 months of the study, developed frequent vomiting whilst having negative urine ketone, normal blood sugar and electrolytes profiles (TCO_2 20, anion gap 10). This patient was managed at home, following the sick day protocols, with extra juices and calories, which made the symptoms subside within 48 hours. At 4.5 months of the study, the patient had episodic vomiting with hypokalemia and mild metabolic acidosis (K 2.9, TCO_2 12, anion gap 17) without ketonuria, necessitating hospitalisation for fluid rehydration for 48 hours. An additional 230 calories per day (300 mL of orange juice, apple, and banana) was provided to this patient, yielding no further adverse events during the rest of the study.

Figure 1C shows that in all subjects but one (P2), the comparison of WAZ between pre- and post-study entry were: normal in three subjects (P3: Z-score of 1.1/1.2 for pre- and post-study; P4:-1.6/-1 and P6: -0.2/-0.4); remained underweight in one (P1: Z-score of -3.05/-3.12); remained overweight in the other (P5: Z-score of 1.6/1.8). The WAZ of P2 was negatively changed from normal to underweight (Z-score -0.8/-

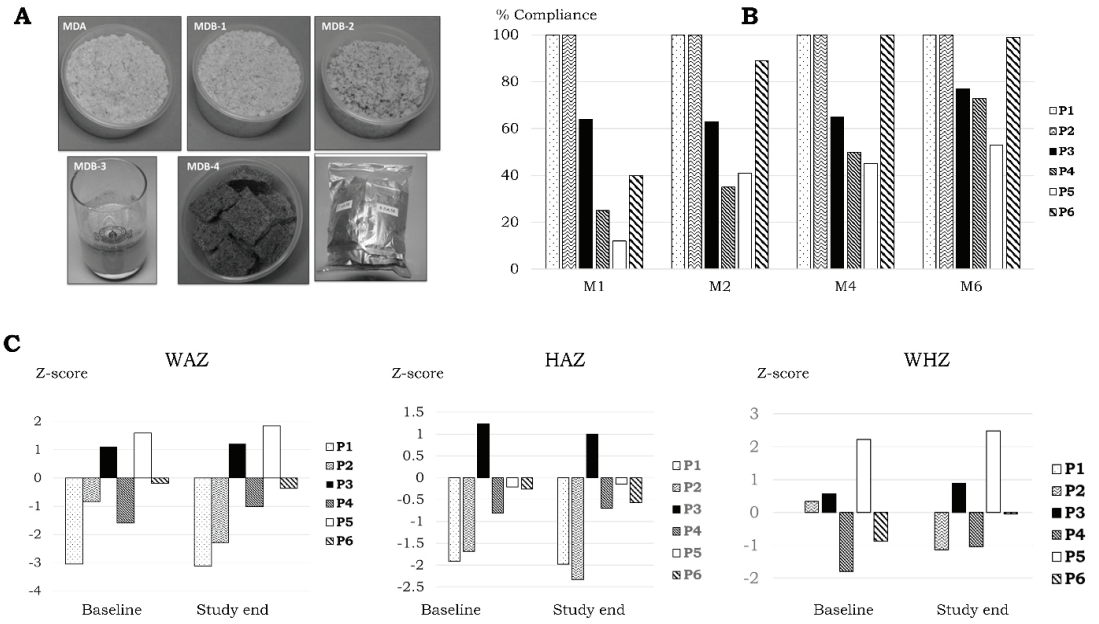

Figure 1. Modular diets, Compliance, and Growth outcomes. **A)** Five MD products including MDA, mixed powder for MSUD; MDB-1: rice sprinkle powder, MDB-2: bouillon cube for stir-frying, MDB-3: instant cocoa drink, and MDB-4: chocolate snack bar for IVA/PA/MMA. Each MD product was prepared and packaged into clean wrapped foil sachets. **B)** Compliance to modular diets shown in percentage of the amount of MD consumed as compared to the amount prescribed. **C)** Growth outcomes. Noted growth changes of the patients as expressed by weight-for-age z-scores (WAZ), height (length)-for-age z-scores (HAZ), and weight-for-height (length) z-scores (WHZ). M (month) represents time point: M0, the start of the study; M1, M2, M4 and M6 represent the end of the 1st, 2nd, 4th and 6th month of the study, in order. P1-6 represent patients 1-6 in respective order.

2.2). HAZ remained unaffected in all, but one (P2), as follows: P1: Z-score of -1.9/-2.0 for pre- and post-study; P2: -1.8/-2.3; P3 1.2/1; P4: -0.9/-0.7; P5: -0.2/-0.1; and P6: -0.2/-0.6). As for WHZ, it was unaltered for all subjects: normal in four individuals (P2: 0.3/-1.1; P3:0.6/0.9; P4: -1.8/-1.1 and P6: -0.9/-0.1); overweight in another (P5: 2.1/2.5; Figure 1C). Mid arm circumference, triceps and biceps skinfolds increased in all subjects as predicted for well growing children (data not shown).

Haematocrit, serum TCO_2 content, albumin, and pre-albumin were within normal ranges throughout the study (Figure 2). Subjects with MSUD showed low blood urea nitrogen (BUN) values (<7 mg/dL) prior to and throughout the study, probably due to more restricted protein intake, as compared to normal BUN levels among IVA individuals. Plasma leucine level was periodically within the acceptable range in one MSUD subject (P1), but persistently high in the other (P2; Figure 3). All IVA subjects had normal plasma amino acid profiles before and during the study (Figure 4).

Parental satisfaction

Three out of four IVA families (P3, P4 and P5) reported preferences for the MF-protocol as the protocol provided flexibility for their children to consume more natural foods with intact protein at school, in addition to drinking LMD

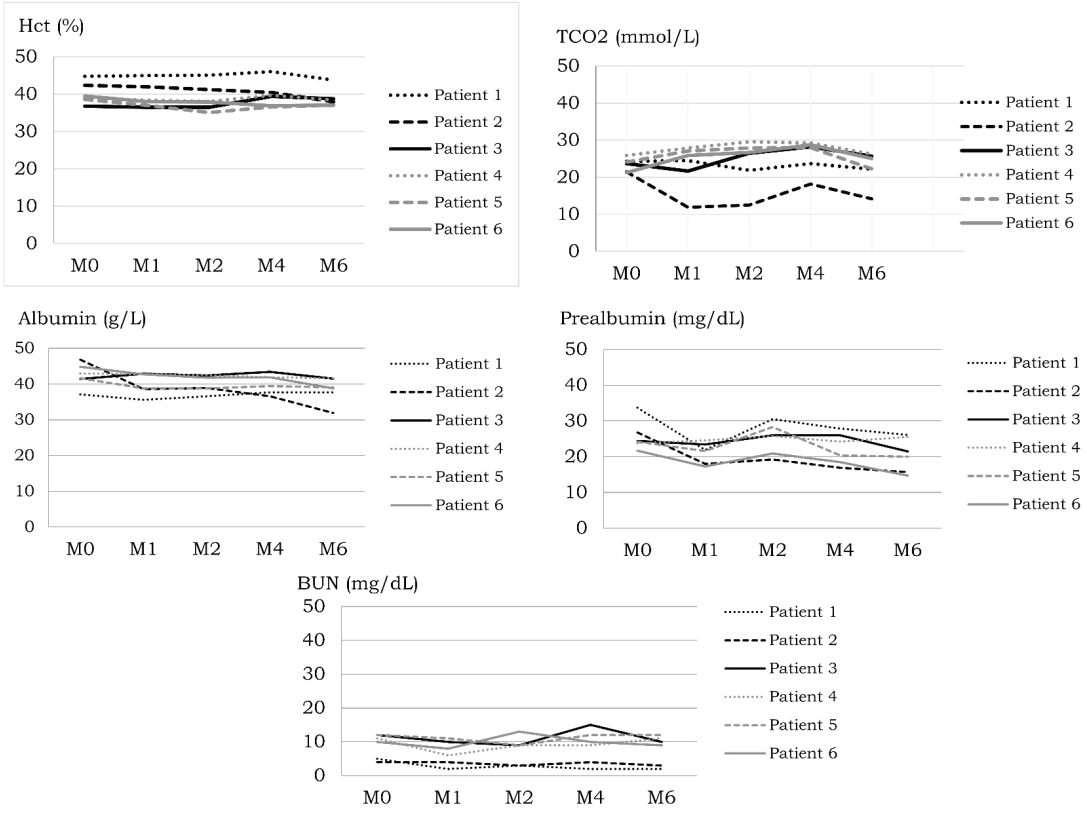

Figure 2. Haematological and biochemical parameters of the patients receiving modular diets. M (month) represents time point: M0, the start of the study; M1, M2, M4 and M6 represent the end of the 1st, 2nd, 4th and 6th month of the study, in order.

milk. The other IVA family (P6) reported no preference to either diet protocols. One MSUD family (P1) preferred the MD-protocol because their child appeared healthier and more energetic, as compared to baseline treatment. The other MSUD family (P2) preferred MF-protocol because their child was more clinically stable with no illness/weight loss whilst on MF-protocol.

DISCUSSION

We demonstrated the clinical utility of MDs, using locally available raw materials, namely dry fish maw and roasted sunflower kernels, in patients with MSUD and IVA. The distributive energy of MDs developed was higher from fat and less from carbohydrate, compared to MF. It was not our intention to increase fat intake for caloric requirement, but to maintain the texture and shape of the products. Therefore, we suggest partial replacement of fat module with carbohydrate (dextrin) module in the future. Protein quality of the MDs in this study was considered as "good", based on the standard definition of amino acid scores. "Good protein quality" is achieved when the percentage of each essential amino acid consumed by each individual is ≥70% of the recommended amount.

The raw materials, namely dried fish maw and sunflower kernels, are widely available at supermarkets and on E-commerce websites. The easiest products to be made are mixed powder product or rice sprinkle powder (MDA and MDB-1), followed by bouillon cubes for stir-frying (MDB-2), and instant cocoa drink (MDB-3). These products can be simply cooked at home using a regular blender, following the established recipes. The chocolate snack bar (MDB-4) was the most difficult to prepare at home because of its time-consuming feature and difficulty in mixing the baking chocolate to other ingredients. To avoid complicated microbial testing steps and its related expenses, the recipes for these MDs need to be given to the families so that they can prepare the MDs correctly at home, on a daily basis.

For self-fed children, product acceptability was a major concern especially when they were forced to switch from their routine diet (metabolic formula) to new products. Therefore, the new products must be competitive in

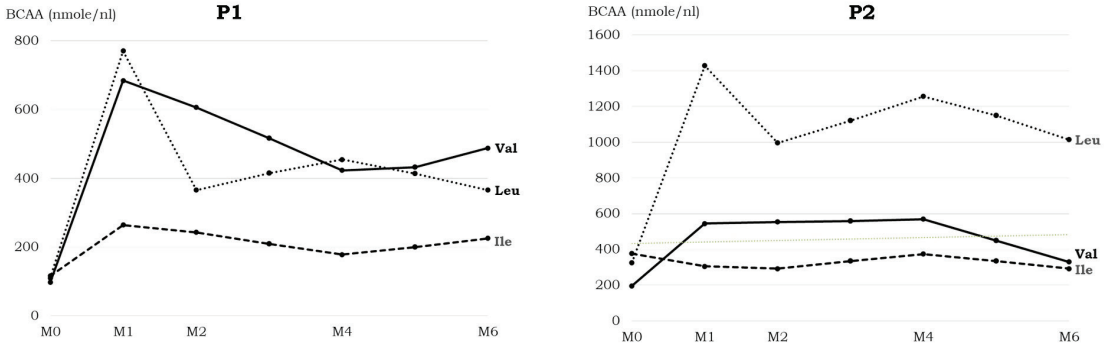

Figure 3. Branched-chain amino acids (BCAA) levels of patients with MSUD. Goal levels of BAAA in MSUD are: Leu and Ile 150-300 μmol/L (or nmole/ml), and Val at least two-fold plasma leucine concentration. M (month) represents time point: M0, the start of the study; M1, M2, M4 and M6 represent the end of the 1st, 2nd, 4th and 6th month of the study, in order.

terms of texture, flavour, and taste. In this study, the compliance to MDs was quite low during the first month of the trial, but subsequently improved when patients became more familiar with the MD products. We observed that MDB-3: instant cocoa drink was the most acceptable among all self-fed children, with 100% compliance in three out of four patients at the 4th month of the study (Figure 1B). The reason for this is probably due to its liquid texture that is similar to a drinkable metabolic formula. As for chocolate snack bar, two out of four self-fed patients preferred the snack bar to the instant cocoa drink, whereas another showed no preference, and the other totally declined the snack bar. Anecdotally, we found that strong encouragement by parents was another key success factor to increase compliance to these MDs.

Clinical outcomes of MDB in IVA were highly favourable since there were no adverse events throughout the study. Despite incomplete compliance to MDs by some IVA subjects, all of them showed normal or unaltered growth parameters, haematologic and biochemical profiles, possibly due to the wide range of safety margin of leucine intake in IVA. Metabolic ketoacidosis in children with IVA often occurs during infancy and early childhood, but the episodes usually decrease with age (Castorina *et al.,* 2008; Dercksen *et al.,* 2012; Martin-Herhandez *et al.,* 2009). This may be one of the reasons that we

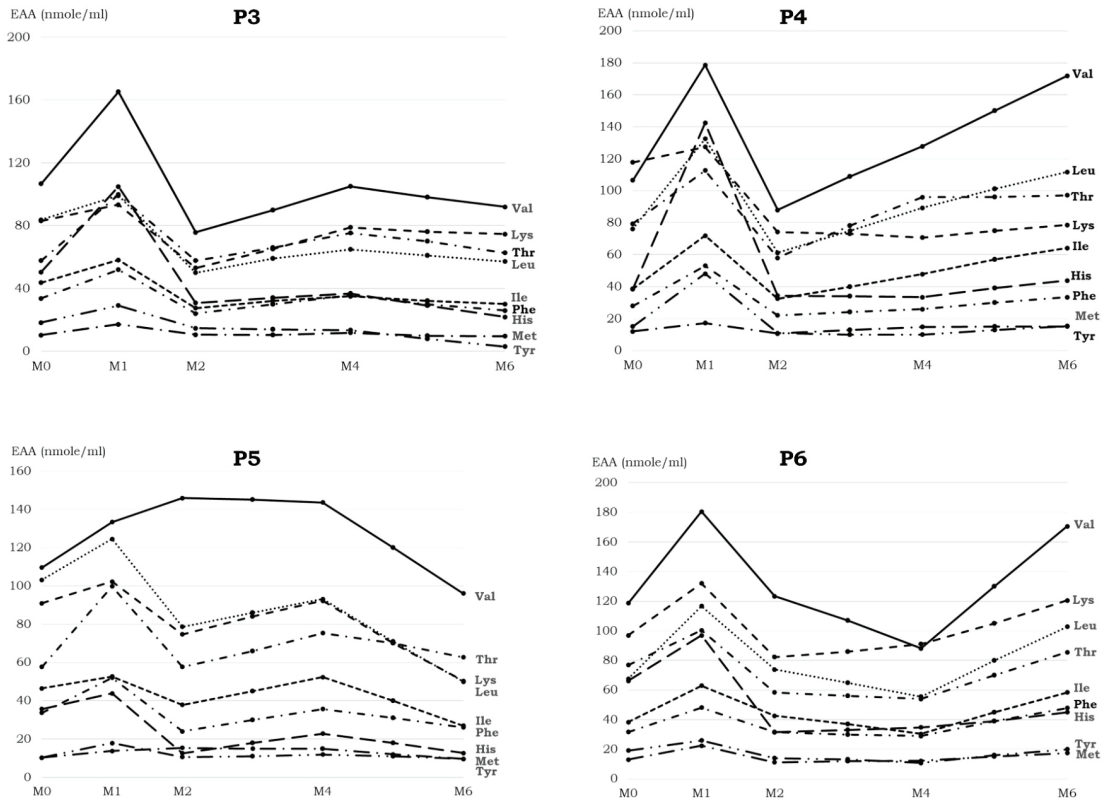

Figure 4. Plasma levels of essential amino acids (EAA) of patients with IVA. Normal range for plasma amino acids are: Val 88-275; Leu 52-149; Ile 25-76; Lys 56-168; Thr 12-138; Phe 41-84; His 8-43; Met 7-42; Tyr 5-24 nmole/ml

did not see metabolic attack in our IVA children who did not adhere to dietary protocols during the study. Clinical variation of IVA with the same mutation in a homogeneous population has been demonstrated, suggesting that multiple factors, such as poor diet compliance, delayed diagnosis and treatment, epigenetic factors, and yet unidentified factors, may be involved in the variability of manifestations (Dercksen *et al.,* 2012). It has been suggested that there is no reliable metabolomic markers for monitoring the prescribed treatment in patients with IVA during the time of well-being (Vockley & Ensenauer, 2006). Unsatisfactory diet compliance in IVA could lead to chronic intoxication of abnormal organic acid accumulation, leading to psychomotor delay and neurobehavioral problems including attention deficit, learning disability, and intellectual disability (Castorina *et al.,* 2008).

As for MSUD, the MD-protocol yielded impressive clinical outcomes in one patient (P1) but was unsuccessful or partially effective in the other (P2). MSUD is generally considered more severe and much more difficult to control and therefore requires more stringent dietary management than IVA. The adverse outcomes in this subject (P2) were likely due to several factors including (i) relatively lower caloric and protein intakes during the study; (ii) poorer absorption of amino acids from natural foods (MDs) than from metabolic formulas; and (iii) lower bioavailability of MDs relative to metabolic formulas.

We did not include children aged under 4 years in the study because younger children with these disorders are more vulnerable to metabolic decompensation. Given the impressive results of MDBs for IVA, they may be considered as an alternative in the management of younger IVA patients, if necessary. Theoretically, the MDBs developed in this study can be used for MMA and PA as the amounts of valine, isoleucine, threonine, and methionine calculated were within the allowable ranges. However, further clinical investigation is required.

The development of MDs to be used as substitutes for semi-elemental products and MF have been described, with varying degree of success depending on the conditions. Perhaps the most successful examples are MDs for chronic diarrhoea and malabsorption (Kolacek *et al.,* 1996), hepatic and renal failure (Bell *et al.,* 1987), and burn (Bell & Wyatt, 1986). The main reason of the development of a MD for the aforementioned disorders was the very high cost of commercial formulas. There has been scarce data on using MDs for organic acidurias, as Kabra reported no clinical success in using modified Indian diet for organic aciduria patients (Kabra, 2002). Although our MDs are not perfect substitutes for metabolic formulas, they can however be used as alternatives during severe shortage and for geographical areas lacking metabolic formulas.

The advantages of the MDs developed in this study included local availability, good protein quality, simple preparation, proven food safety, low cost, and proven clinical efficacy (for IVA). Limitations of this study were small sample size, incomplete compliance to MDs, incomplete metabolomic data such as plasma essential fatty acids, and absence of micronutrient data (vitamins and minerals).

CONCLUSION

We have provided a proof of concept in developing modular diets for BCOAs using locally available raw materials with low cost and simple preparation, with favourable outcomes in clinical utility among IVA and varying clinical

benefits in MSUD. A large cohort of clinical study using the developed modular diets in patients with organic acidurias is required. A modular diet can be used as an alternative during the long-time shortage of metabolic formulas in resource-limited communities.

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Authors' contributions

SC, conducted the study and wrote the manuscript draft; DW, involved in the conceptualization and design of the study, supervised data analysis, and wrote the manuscript; VC, supervised modular diet formation and production; OD and TT, provided patient care and clinical data; US and NC, involved in study design and advice on patient care.

Conflict of interest

The authors declare no conflict of interest.

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