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Association between frequency of processed and ultra-processed food consumption with lifestyle habits score for cancer prevention among adults in Malaysia

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ABSTRACT

Introduction: Cancer is the second leading cause of mortality globally. Processed and ultra-processed foods are reported to be high in energy, sugar, and unhealthy fats, while low in fibre, vitamins and minerals, that can increase the risk of cancer. **Methods:** A cross-sectional study involving adults aged 18-59 years was conducted in Peninsular Malaysia (n=122). Sociodemographic data were obtained through a questionnaire and lifestyle habits score for cancer prevention was assessed using *MyGenomSihat*©. The food frequency questionnaire used had four groups (i.e., unprocessed or minimally processed foods, processed culinary ingredients, processed foods, and ultra-processed foods) containing 53 food items to evaluate the frequency of food consumption. All data obtained were self-reported by the participants. **Results:** Mean age was 27.6±8.1 years with a majority (76.2%) having household income of less than <RM4,850. The results from *MyGenomSihat*© demonstrated that majority (53.3%) of subjects had average scores between 41-85, which is considered to be satisfactory lifestyle habits. Most Malaysian adults ate ultra-processed foods (32.9%) compared to processed foods (4.9%). However, there were no significant correlations between consumption of both processed and ultra-processed food groups and lifestyle habits score for cancer prevention (r=-0.072, p=0.089 and r=-0.008, p=0.992, respectively). **Conclusion:** Most subjects had satisfactory lifestyle habits scores for cancer prevention. Interventions are needed to improve lifestyle habits among the targeted population for cancer prevention.

Keywords: adult, cancer, *MyGenomSihat*©, NOVA, ultra-processed food

INTRODUCTION

Cancer is the second leading cause of mortality globally (WHO, 2020). By 2040, the incidence of cancer is expected to exceed 27 million cases, which is 50% more than that estimated in 2018 (18.1 million cases) worldwide (WHO, 2020). This rising figure is a growing health concern, and researchers, particularly public health researchers, must pay more attention. Meanwhile, according to statistics from the Malaysia National Cancer Registry Report (2012 – 2016) (National Cancer Institute of Malaysia, 2019), the number of cancers reported increased by 11,731 cases in 5 years from 2012 to 2016 as compared to the number of cases reported from 2007 to 2011 (Azizah *et al.,* 2019). Among the ten common cancers reported, colorectal

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cancer was said to be the second leading cancer in Malaysia after breast cancer, followed by lung cancer, lymphoma, nasopharynx, leukaemia, prostate cancer, liver cancer, cervix cancer, and ovary cancer (Azizah *et al.*, 2019).

The adoption of a healthy lifestyle is known to be associated with a lower incidence of cancer and mortality (Moore *et al.*, 2016). Numerous studies have provided evidence of an association between lifestyle factors and cancer risk. Based on a past research by Roos, Lahti & Rahkonen (2018), there are joint associations between cancer risk and four lifestyle practices – diet, smoking, alcohol consumption, and physical activity; cancer risk is higher when smoking co-occurs with an unhealthy diet or inactive lifestyle. Meanwhile, a study in Malaysia found that most adults have unfavourable lifestyle-related risk factors for cancer, such as being obese, physically inactive, consuming less than five servings of fruits and vegetables per day, and consuming red meat at least once a day or more (Schliemann *et al.*, 2020). Thus, the World Cancer Research Fund (WCRF/AICR) 2018 recommends maintaining a healthy weight and increasing physical activity, especially for those who smoke and drink alcohol. WCRF 2018 also highlighted that a diet high in whole grains, vegetables, fruits, and legumes, but low in fast foods, processed foods, red meat, and alcoholic beverages, is the best dietary strategy for preventing and managing cancer.

Apart from that, many studies have been conducted to examine the relationship between nutritional factors and lifestyle practices for cancer prevention. Consistent results have been obtained, whereby the intake of processed and ultra-processed foods have been shown to increase the risk of getting cancer (Fiolet *et al.*, 2018; Monteiro *et al.*, 2018; Steck & Murphy, 2020). Processed food is a simple product with added sugar, oil, salt, or other ingredients. In contrast, ultra-processed food is a food product manufactured through industrial formulations using five or more components (Monteiro *et al.*, 2019). Thus, to evaluate the impact of ultra-processed food products on many levels, the NOVA system was developed to classify ultra-processed foods according to the purpose and extent of physical, biological, and chemical methods used during the food manufacturing process (Monteiro *et al.*, 2019).

According to a study conducted by Fiolet *et al.* (2018) in France, an increase in processed food intake was directly proportional to the overall risk of cancer and breast cancer for postmenopausal women. The study found a 12% increased risk for all types of cancer and 11% for breast cancer. This applied to 10% of the individual's total diet belonging to processed or ultra-processed food groups, which might be due to dietary changes in people living in urban areas. Diets that include more processed food products tend to be richer in energy, sodium, fat, and sugar, but poor in fibre and various micronutrients (Luiten *et al.*, 2016); thus are associated with increased body mass index (BMI), waist circumference, and the odds of being obese (Rauber *et al.*, 2020). Fazzino *et al.* (2021) also found a more significant weight gain in non-obese individuals after they increased their consumption of ultra-processed foods in a buffet meal for the past twelve months. There is an association between higher consumption of ultra-processed foods and overweight/obesity, a known risk factor for endometrial cancer (Da Silva *et al.*, 2021). Furthermore, most ultra-processed foods, such as dehydrated soups, processed meats, biscuits, and sauces, also have a high salt content. Foods preserved with salt are associated with an increased risk of gastric cancer (Mendonca *et al.*, 2016).

The growth of the food and beverages industry in Asian countries, such as Malaysia, Thailand, the Philippines, and India, have contributed to increased access to processed and ultra-processed foods in these countries (Baker & Friel 2016). According to Habib *et al.* (2011), the Malaysian population is seen to slowly adapt to a Westernised diet, characterised by its high calories, highly processed and refined foods with high sugar, fat, and salt content, but low in fruits and vegetables. This is an unhealthy transition as a Westernised diet contributes to obesity-related diseases and co-morbidities such as cardiovascular diseases and cancer (Rakhra *et al.*, 2020). Hence, this study aimed to determine the association between the frequency of processed and ultra-processed food consumption and lifestyle habits score using *MyGenomSihat*© for cancer prevention among adults in Malaysia.

MATERIALS & METHODS

Study design and sampling

This cross-sectional study was conducted from July 2020 to March 2021 in Peninsular Malaysia, mainly in the states of Perak, Selangor, and Kuala Lumpur Federal Territory. A convenience sampling technique was used to select samples from the community in these locations, representing the urban and suburban areas. The inclusion criteria were individuals aged 18-59 years old who had Internet access and no history of being diagnosed with any cancer. Subjects with chronic health problems, such as diabetes, high blood pressure, hyperlipidemia, cancer, or kidney disease, were excluded from this study. Questionnaires were given via an online platform to eligible subjects. Sample size was calculated using the Cochran formula (1963) with an expected proportion of 0.404 (Ali *et al.*, 2019) and a precision of 0.1. The estimated sample size was 116 subjects. This study was reviewed and approved by the Universiti Kebangsaan Malaysia Medical Research Ethics Committee (JEP-2020-534). The subjects were recruited for the study after obtaining written informed consent.

Study instrument

Data were obtained via a self-administered online questionnaire. Sociodemographic data included name, age, residence, phone number, email address, gender, marital status, ethnicity, education level, occupation, and monthly household income. Monthly household income was classified based on the country's income threshold of the Bottom 40% (B40), Middle 40% (M40), and Top 20% (T20) (DOSM 2020). Self-reported weight (kg) and height (m) were used in the study and are considered reasonably accurate (Qin *et al.,* 2018). The classification of BMI was based on WHO (1998) cut-offs of <18.5kg/m^2 (underweight), 18.5-24.9kg/m^2 (normal), 25.0-29.9kg/m^2 (overweight), and ≥30.0kg/m^2 (obesity).

The score for lifestyle habits was collected using a questionnaire named *MyGenomSihat*©, which has been validated for use in Malaysia. It was initially adapted from the assessment of lifestyle habits for cancer prevention that had been widely used worldwide to provide health information and encourage the public to change towards healthy lifestyle habits (Nur Shafiqah, 2019). This questionnaire consisted of 9 domains: fruits and vegetables intake, unhealthy food intake, physical activity, cervical cancer examination, breast/testicular self-examination, hepatitis B virus (HBV) and human papillomavirus (HPV) vaccination, BMI, and tobacco intake. The total score was 125; categorised into three groups – good lifestyle habits (0-40), satisfactory lifestyle habits (41-85), and bad lifestyle habits (86-125).

Food frequency questionnaire (FFQ)

Fifty-three food items included in the questionnaire were based on the comparison between the NOVA food classification system by Monterio *et al.* (2016) and past studies in Malaysia (Ali *et al.* 2019; Kasim *et al.* 2018; Shyam *et al.* 2020). The NOVA classifies foods into four distinct groups - Group 1 (unprocessed food) is natural or minimally processed food where the inedible parts are removed and there are no added substances; Group 2 (processed culinary ingredients) is foods that are preserved by additives and usually have to undergo processes like pressing and refining; Group 3 (processed food) includes various preservation and cooking by adding processed culinary ingredients to increase its durability; and Group 4 (ultra-processed food) usually contains more than five added substances and is formulated industrially, which is hyper-palatable and ready to eat/drink/heat.

Twelve selected subjects were asked to pre-test the questionnaire. For each item, respondents needed to tick boxes on how many times they consumed the food per serving in a day, week, or month, and the frequency was presented in row percentages. This pre-test process aimed to test the suitability of food items added and the understanding and acceptance of study subjects towards the FFQ. We also explained aspects to look out for during the pre-test. For example, during the pre-test session, a subject expressed that he did not understand the difference between alcoholic beverages under Groups 3 and 4. So, the researchers took those comments into account and noted three examples of alcoholic beverages for each group in the FFQ so that subjects can choose and distinguish them more easily. Researchers also provided knowledge and information on why alcoholic beverages in Group 3 differed from Group 4. There were study subjects who provided recommendations to include foods like *bihun, kuey teow, laksa,* and *yee mee*, along with yellow noodles, as most Malaysians often consume these foods. Similarly, other food items, such as chocolate drinks, biscuits, as well as health and beauty supplements also created some confusion among the study subjects. As a solution, examples of food brands often found in Malaysia were mentioned to facilitate the process of answering this questionnaire.

Statistical analysis

All statistical analysis was conducted using IBM SPSS Statistics for Windows version 25.0 (IBM Corp, Armonk, New York, USA). The variable of food groups was categorised using the NOVA food classification system by Monterio *et al.* (2016). The total score for lifestyle habits was categorised into three groups, followed by univariate analysis. Normality of the data was assessed by referring to the Kolmogorov-Smirnov test. Descriptive tests were used to analyse all variables such as sociodemographic data, lifestyle habits scores for cancer prevention (*MyGenomSihat*™), and food intake frequency. Chi-square test was used to compare the frequency between lifestyle habits scores for cancer prevention. Besides, Spearman's rho correlation test was also conducted to determine the correlation between lifestyle habits score and food intake frequency.

RESULTS

Sociodemographic background

Table 1 presents the sociodemographic data of the subjects. A total of 122 adults aged 18-59 years from various states in Malaysia were included in this study. Participants' average age was 27.6±8.1 years with 25.4% (n=31) males

and 74.6% (*n*=91) females. The majority of the subjects were single (68.9%), Malays (79.5%), had a Bachelor's degree (60.7%), and a household income of not more than RM4,850, belonging to the B40household income group. One-third of the subjects lived in Perak (36.9%), Selangor and Kuala Lumpur (32%), and other cities (31.1%), respectively.

Evaluation of lifestyle habits score for cancer prevention (*MyGenomSihat*©)

Table 2 shows the lifestyle habits score levels for cancer prevention using *MyGenomSihat*©. It was shown that more than half of the subjects (53.3%, *n*=65) were at a satisfactory level of risk (moderate), followed by low level of risk (45.9%) and high level of risk (0.8%).

Table 1. Participants' sociodemographic characteristics

Characteristic	*n*	*%*	*Mean±SD*
Age (year)			27.6±8.1
Gender			
Men	31	25.4	
Women	91	74.6	
Status			
Single	84	68.9	
Married	38	31.1	
Ethnicity			
Malay	97	79.5	
Chinese	16	13.1	
Indian	6	4.9	
Others	3	2.5	
Religion			
Islam	100	82.0	
Buddha	14	11.5	
Hindu	5	4.1	
Christian	3	2.5	
State			
Perak	45	36.9	
Selangor	29	23.8	
Wilayah Persekutuan	10	8.2	
Others	38	31.1	
Education level			
Secondary level	21	17.2	
Diploma	10	8.2	
Degree	74	60.7	
Doctor of Philosophy	9	7.4	
Others	8	6.6	
Occupation			
Government employment	13	10.7	
Private	24	19.7	
Businessman	7	5.7	
Student	65	53.3	
Does not work	13	10.7	
Household income[†]			
B40 (<RM 4,850)	93	76.2	
M40 (RM 4,850 – RM 10,959)	26	21.3	
T20 (>RM 10,959)	3	2.5	

[†]Bottom 40% (B40), middle 40% (M40), and top 20% (T20) based on DOSM (2020)

Table 2. Evaluation of lifestyle habits score for cancer prevention (*MyGenomSihat*™)

Lifestyle habits score for cancer prevention (*MyGenomSihat*™)	*Mean score*	*% (n)*
Low (0 - 40)	29.6	45.9 (56)
Medium (41 - 85)	46.8	53.3 (65)
High (86 - 125)	92.0	0.8 (1)

Frequency of intake of food items from Group 3 (processed foods) and Group 4 (ultra-processed foods)

Table 3 shows the frequency of food intake from Group 3 (processed foods) and Group 4 (ultra-processed foods) among Malaysian adults. Food items from Groups 3 and 4 were analysed as evidence suggested that these groups may increase cancer risk via their carcinogenic compounds or obesogenic properties (Fiolet *et al.*, 2018; Kliemann *et al.*, 2022). For Group 3 items, 68% of respondents rarely/never consumed salted, dried, cured, or smoked meat/ fish/poultry, followed by salted or sugared nuts and seeds (62.3%), canned fruits and vegetables (58.2%), cheese (45.1%), canned food (43.4%), and lastly unpackaged fresh bread (39.3%). Meanwhile, the most frequently consumed food item from Group 3 was unpackaged fresh bread, with 24 subjects (19.7%) consuming it 2-3 times a week and seven subjects (5.7%) consuming it 4-6 times a week. This was followed by other items such as cheese, canned food, salted or sugared nuts and seeds, canned fruits and vegetables, and smoked meat. Most respondents never consumed or only consumed processed foods 1-4 times a month.

For Group 4 (ultra-processed food) items, it was shown that majority (83.6%) of respondents rarely or never consumed health and slimming products. Other things that were rarely or never consumed by most of the respondents included candies (50.8%), margarine and other spreads (40.2%), energy bars or drinks (77%), flavoured milk drinks (40.2%), fruit drinks (41.0%), pasta (50.0%), pizza (45.1%), sausages or hotdogs (45.1%), and breakfast cereals (41.8%). This study also showed a high consumption frequency of cocoa drinks and packaged bread and buns among Malaysian adults, whereby 31.1% and 42.6% consumed them 2-3 times weekly, respectively.

Association between lifestyle habits score for cancer prevention and frequency of processed and ultra-processed food intake

The determination of the association between food groups and lifestyle habits score for cancer prevention is shown in Table 4. Based on the findings, there were no significant correlations (p<0.050) obtained from the Spearman's rho correlation test for lifestyle habits score with both food groups (processed and ultra-processed foods).

DISCUSSION

Our study was the first to explore the association between the frequency of processed and ultra-processed food consumption and lifestyle habits score for cancer prevention among Malaysian adults. This study found that Malaysian adults had medium/satisfactory lifestyle practice scores and a significant difference between lifestyle habits for cervical cancer screening, breast self-examination, and HPV vaccination. This might be due to the study subjects being predominantly females. Although there was a high prevalence of breast self-examination among Malaysian women,

Table 3. Frequency of intake of food items from Group 3 and Group 4

Items	*Frequency of intake, n (%)*				
	> 1 time a day	*4-6 times a week*	*2-3 times a week*	*1-4 times a month*	*Rarely/ Never*
Group 3 (Processed food items)					
Canned fruits and vegetables	1 (0.8)	2 (1.6)	9 (7.4)	29 (32.0)	71 (58.2)
Salted or sugared nuts and seeds	1 (0.8)	3 (2.5)	13 (10.7)	29 (23.8)	76 (62.3)
Salted, dried, cured, or smoked meat / fish / poultry	2 (1.6)	3 (2.5)	5 (4.1)	29 (23.8)	83 (68.0)
Canned food (sardine, tuna and others)	1 (0.8)	1 (0.8)	6 (4.9)	61 (50.0)	53 (43.4)
Cheese	1 (0.8)	1 (0.8)	10 (8.2)	55 (45.1)	55 (45.1)
Unpackaged fresh breads	2 (1.6)	7 (5.7)	24 (19.7)	41 (33.6)	48 (39.3)
Group 4 (Ultra-processed food items)					
Sweet or savoury packaged snacks	0 (0.0)	3 (2.5)	22 (18.0)	55 (45.1)	42 (34.4)
Ice-cream	0 (0.0)	3 (2.5)	21 (17.2)	66 (54.1)	32 (26.2)
Chocolate	0 (0.0)	2 (1.6)	15 (12.3)	65 (53.3)	40 (32.8)
Candies	1 (0.8)	3 (2.5)	15 (12.3)	41 (33.6)	62 (50.8)
Carbonated soft drinks	0 (0.0)	1 (0.8)	11 (9.0)	56 (45.9)	54 (44.3)
Packaged breads and buns	4 (3.3)	11 (9.0)	52 (42.6)	38 (31.1)	17 (13.9)
Margarines and other spreads	4 (3.3)	5 (4.1)	21 (17.2)	43 (35.2)	49 (40.2)
Cookies (biscuits)	2 (1.6)	11 (9.0)	41 (33.6)	44 (36.1)	24 (19.7)
Pastries or cakes	1 (0.8)	0 (0.0)	12 (9.8)	68 (55.7)	41 (33.6)
Energy bars or drinks	1 (0.8)	3 (2.5)	5 (4.1)	19 (15.6)	94 (77.0)
Flavoured milk drinks	2 (1.6)	2 (1.6)	22 (18.0)	47 (38.5)	49 (40.2)
Cocoa drinks	5 (4.1)	9 (7.4)	38 (31.1)	37 (30.3)	33 (27.0)
Fruit drinks	4 (3.3)	3 (2.5)	18 (14.8)	47 (38.5)	50 (41.0)
Fries	1 (0.8)	2 (1.6)	18 (14.8)	72 (59.0)	29 (23.8)
Yellow noodles/*laksa*/ *kuey teow* /*yee mee*	1 (0.8)	3 (2.5)	48 (39.3)	55 (45.1)	15 (12.3)
Pasta	1 (0.8)	1 (0.8)	7 (5.7)	52 (42.6)	61 (50.0)
Fish ball and fish cakes	1 (0.8)	3 (2.5)	29 (23.8)	60 (49.2)	29 (23.8)
Instant noodles	1 (0.8)	7 (5.7)	23 (18.9)	60 (49.2)	31 (25.4)
Desserts	1 (0.8)	3 (2.5)	23 (18.9)	48 (39.3)	47 (38.5)
Pizza	1 (0.8)	1 (0.8)	5 (4.1)	53 (44.3)	55 (45.1)
Sausages/hotdogs	1 (0.8)		12 (9.8)	45 (44.3)	55 (45.1)
Burgers	1 (0.8)	1 (0.8)	12 (9.8)	71 (58.2)	37 (30.3)
Poultry/meat nuggets	1 (0.8)	2 (1.6)	15 (12.3)	73 (59.8)	31 (25.4)
Vegetable/meat/poultry stocks or cube	5 (4.1)	4 (3.3)	18 (14.8)	54 (44.3)	41 (33.6)
Breakfast cereals	1 (0.8)	2 (1.6)	20 (16.4)	48 (39.3)	51 (41.8)
Health and slimming products	8 (6.6)	1 (0.8)	7 (5.7)	4 (3.3)	102 (83.6)

Table 4. Determination of the association between food groups and lifestyle habits score for cancer prevention

Food group	*Lifestyle habits score for cancer prevention*	
	Correlation coefficient, r_s	*p-value*[†]
Group 3 (processed food)	-0.072	0.892
Group 4 (ultra-processed food)	-0.089	0.992

[†]No significant value (p<0.050) with Spearman's Rho correlation test
Both variables were treated as ordinal data to qualify for Spearman's test. The frequency of food intake levels were in ordinal form (never/rarely, occasionally, often, frequently, always) in replacement of rarely/never, 1-4 times a month, 2-3 times a week, 4-6 times a week, and >1 time a day, respectively. For lifestyle levels, the ordinal form were good, medium/satisfactory, and bad lifestyle habits

there is still a need to encourage breast self-examination among women because breast cancer is one of the most common cancer sites (34.1%) suffered by women (Malaysia National Cancer Registration Report 2012-2016). Furthermore, a previous study has shown the opposite result where most female subjects did not practise breast self-examination despite most of them having heard about it (Paruchuri *et al.*, 2021).

Regarding HPV vaccination, a study from Lei *et al.* (2020) showed that the cumulative incidence of cervical cancer was higher (94 cases per 100,000 persons) among those who had not been vaccinated as compared to those who had been vaccinated (47 cases per 100,000 persons). Besides, HBV is the first example of a cancer-preventive vaccine in humans that can prevent hepatocellular carcinoma (HCC) from childhood to early adulthood. However, our findings were like the previous study, whereby the uptake of HBV vaccination was low (Omotowo *et al.*, 2018).

Our findings were consistent with a previous study conducted in Kuala Nerus, Terengganu (Ali *et al.*, 2019), where ultra-processed foods (Group 4) had the second highest food intake frequency among Malaysian adults. Malaysians are prone to eating unhealthy diets containing high calories, fat, sugar, and salt. Besides, this study found that the five most consumed ultra-processed foods were packaged bread and buns, biscuits, cocoa drinks, instant noodles, and yellow noodles, *laksa*, *kuey teow* or *yee mee*. Our results were in line with previous studies for noodles, cream crackers, and white bread as the most consumed food items (Ali *et al.,* 2019). In addition, our findings also supported the results from the Malaysian Adult Nutrition Survey (2014) (IPH, 2014), which reported that chocolate or malt drinks were one of the most frequently consumed food items among Malaysian adults (Kasim *et al.*, 2018). Meanwhile, this study demonstrated that breakfast cereals were a less popular item, which is inconsistent with previous studies showing that breakfast cereals were the most frequently consumed food item among adults and university students in Malaysia (Shyam *et al.*, 2020).

We found no significant correlations (p<0.050) between lifestyle habits score for cancer prevention and both food groups (processed and ultra-processed foods). This finding was inconsistent with a previous study by Fiolet *et al.* (2018), in which ultra-processed food intake was associated with higher overall cancer risk. For every 10% increment in the proportion of ultra-processed foods in the diet, there was a 12% higher risk for prevalent cancer and an 11% increased risk for breast cancer (Fiolet *et al.*, 2018). Another study also reported that higher consumption of

ultra-processed foods was strongly associated with an increased risk of all-cause mortality, including cancer (Rico-Campà *et al.*, 2019). This study is essential in collecting information on which food groups, according to the NOVA classification system, are most often consumed in Malaysia and encourages the implementation of this system in observing the frequency of food intake, focusing on processed and ultra-processed foods.

However, this study has its limitations. The cross-sectional design was only carried out at one point in time and thus, is unsuitable for studying temporal relationships. Besides, the food frequency questionnaire did not allow the explanation of energy intake or grams of food weight or nutritional status of individuals. This study may not be generalised to all Malaysians due to the small sample size and limited coverage areas in Malaysia. Most studies on the consumption of ultra-processed foods are mainly concerned with the product's calories, macronutrients, micronutrients, fibre, and added salt and sugar contents (Ali *et al.*, 2019; WCRF/AICR, 2018). Therefore, more research is needed in the future to identify the association between processed and ultra-processed food intake with lifestyle habits score for cancer prevention.

CONCLUSION

In conclusion, most subjects had a satisfactory level lifestyle habits score for cancer prevention (*MyGenomSihat*©) Fruits and vegetables intake, breast (women)/testicular (men) self-examination, HBV and HPV vaccination, and tobacco intake were found to have significant differences on lifestyle habits score for cancer prevention (*MyGenomSihat*©). Most Malaysian adults regularly consumed ultra-processed foods (Group 4), followed by processed foods (Group 3). Although ultra-processed food was categorised as the most frequent food consumed by subjects compared to processed food, this study showed no correlations between lifestyle habits scores for cancer prevention in both food groups (processed and ultra-processed). Further studies are needed to confirm these results in larger populations and to clarify whether high consumption of ultra-processed foods will affect health.

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Authors' contributions

Sharif R, led the conception and initial design of the study, conducted the analysis and interpretation of data, drafted the manuscript, and revised content based on feedback; Ramli NR and Mohammad NMA, assisted with data collection, data analysis, writing and revising of the manuscript; and all authors: read and approved the final manuscript.

Conflict of interest

None declared.

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REVIEW

Potential risk of stunting in children under five years living by the riverside: A systematic review

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ABSTRACT

Introduction: Stunting, or linear growth failure, is defined by a height-for-age z-score of below -2SD according to WHO growth standard. Stunting can have short-, medium-, and long-term consequences. Rivers have an important role in human life. In several riverside areas, households still depend on the river for their livelihoods and health. This study aimed to explore the prevalence of stunting in children living by the riverside and its related factors. **Methods:** Epidemiological studies published from PUBMED, MEDLINE via EBSCOHost, Science Direct, ProQuest, and Research Gate databases were systematically searched. The publication period was not restricted. Only open-access and English articles were examined. **Results:** A total of 20 from 1200 studies were reviewed. The prevalence of stunting ranged from 20% to 48.3%. The other outcomes besides stunting were wasting and underweight status. There were 83 risk factors studied, and the most studied variables were age, gender, diarrhoea, water source, parent's education, immunisation, and inappropriate complementary feeding practices (6 to 13 studies). Household water sources from rivers and economic status were consistently correlated with stunting. Majority of the risk factors studied were related to nutrition. From the environmental aspect, the most studied risk factors were water sources and sanitation. **Conclusion:** Children living by the riverside face a significant risk of stunting attributed to the consistent correlation between household water sources from rivers and economic status, affecting various aspects of daily life beyond drinking water. Future research is needed to examine the impact of environmental factors and the behaviours of riverside communities.

Keywords: children, risk factor, river, riverside, stunting

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INTRODUCTION

Stunting is the failure to achieve height growth, measured by a height-for-age z-score (HAZ) of <-2 standard deviation (SD) according to the World Health Organization (WHO) growth standards (Prawirohartono & Press, 2021; Vonaesch *et al.*, 2018). In children, it is associated with many factors, including socioeconomic factors, dietary intake, recurrent infections, maternal nutritional status, infectious diseases, micronutrient deficiencies, and the environment (Stevens, Finucane, & Paciorek, 2016; WHO, 2018a).

Growth impairment can occur even in the foetus (Prendergast & Humphrey, 2014). The average length-for-age z-score (LAZ) for a baby in developing countries is -0.5 and will continue to decrease, even reaching -2.0 at the age of 18-24 months (Victora *et al.*, 2010). However, growth impairment can continue after the age of 24 months when socio-economic influences (Alderman & Headey, 2018; Rajpal *et al.*, 2020), education and home environment such as the use of a latrine or toilet can have a great effect between the ages of 24-59 months (Alderman & Headey, 2018; de Onis & Branca, 2016).

The disruption of growth of a child's height can have short-term (Olofin *et al.*, 2013), medium-term (Nguyen *et al.*, 2021; Prendergast & Humphrey, 2014) and long-term consequences (De Lucia *et al.*, 2018; Prendergast & Humphrey, 2014). In the short term, stunting can increase morbidity and mortality from infectious diseases, especially pneumonia and diarrhoea (Prendergast & Humphrey, 2014). The medium-term impact is related to child development such as cognitive abilities, education, and child behaviour (Cheung & Ashorn, 2010; Nguyen *et al.*, 2021; Prendergast & Humphrey, 2014). Metabolic syndrome, usually associated with excess nutrition, is more common in adults who were stunted in early childhood than in those with normal growth (Victora *et al.*, 2008).

Globally in 2016, as many as 22.9% or 154.8 million children under the age of 5 years suffered from stunting. In the same year, as many as 87 million stunted children lived in Asia, 59 million in Africa, and 6 million in Latin America and the Caribbean. Five sub-regions with child stunting rates that exceed 30% are West Africa (31.4%), Central Africa (32.5%), East Africa (36.7%), South Asia (34.1%), and Oceania (38.3%; excluding Australia and New Zealand) (WHO, 2018a). In 2018, around 22% of children under five worldwide were stunted. A 40% reduction in the number of stunted children is the global target for 2025 (Lissauer & Carroll, 2021).

Rivers have an important role in life. Although not in all countries, households still depend on natural capital for their livelihoods and health, including riverside areas (Ricketts *et al.*, 2017). The river is a source of life used by a community for water resource, recreation, irrigation, and transportation (Ikhsan *et al.*, 2021). In certain areas, the environmental health conditions of people living by the riverside do not meet WHO standards (Shinta *et al.*, 2020). This is due to the lack of adequate sanitation facilities (Rahmadani & Ridlo, 2020) and disposal of solid and liquid waste (Bartram & Ballance, 1996; Pratama, *et al.*, 2020; Verbyla *et al.*, 2021). Additionally, people still defecate in public places and throw their trash into rivers (Shinta *et al.*, 2020; Zahtamal *et al.*, 2020). These situations will certainly have an impact on the health status of people living by the riverside, especially children.

A systematic literature review explored the prevalence of stunting and its risk factors in children who live by the riverside. Thus, the questions posed for this review were "what is the magnitude of the stunting problem in children

under five who live by the riverside" and "what are the risk factors associated with stunting?"

MATERIALS AND METHODS

A review protocol was developed following the preferred reporting items for systematic review and meta-analysis PRISMA 2020 (Page *et al.*, 2021). Literature searches were conducted in the following databases: PUBMED, MEDLINE via EBSCOHost, Science Direct, ProQuest, and ResearchGate. The publication period was not restricted. Eligibility was limited to peer-reviewed scientific articles published in the English language and open-access articles. Review articles, conference proceedings, book chapters, thesis dissertations, case reports, and all non–English language materials were excluded. The search used the following terms: ("children under five" OR child* OR preschool OR toddler*) AND ("Riverside" OR river* OR watershed*) AND ("risk factor" OR risk OR "determinant") AND (Stunt* OR "growth disorders" OR "growth impairment" OR "growth failure" OR "growth faltering"). Another search strategy was used for the databases by limiting the number of Boolean connectors, such as "children under five" AND ("risk factor" OR determinant) AND "river" AND "stunting".

The reviews included all epidemiological studies without being limited to the study design. The last literature search was 10th November, 2022. Data were organised according to the author, year of publication, country or national setting, sample size, study design, stunting risk factor, and outcomes. The eligible studies had to match the inclusion and exclusion criteria according to the PEO (Population, Exposure, and Outcome) approach (Table 1) (Munn *et al.*, 2018). Qualitative studies and studies which had similar results from the same author(s) were excluded. The EndNote X9 reference manager program filtered duplicate studies and excluded research protocol or review studies.

Table 1. The PEO Criteria for inclusion studies

Parameter	*Inclusion criteria*
Population	Children under five living at riverside
Exposure	Stunting risk factor or determinant
Outcome	Stunting
Study design	Epidemiological study

RESULTS

A total of 1200 studies from five databases were retrieved, while 39 and 56 studies were excluded because of duplication and research protocol or review studies, respectively. The next stage was filtering articles based on title, abstract and keywords. A total of 1054 articles were excluded because of they did not meet the specified inclusion criteria. Articles were excluded at this stage because they did not include the author's name (usually in large-scale or global study and collaborator authors), the outcome studied was not stunting (even though the study's sample was children under five years old), the location was not in a riverside area, and the sample was children aged more than 5 years. The number of studies that were assessed in full text were 51, but only 20 met the inclusion criteria (Figure 1).

Table 2 shows the summary of studies included in this review. The studies' publication year range was from 2006 to 2022. Geographically, most of the research was conducted in African countries; studies were also conducted in several countries in South America, South Asia, and Southeast Asia. Cross-sectional study was the most common

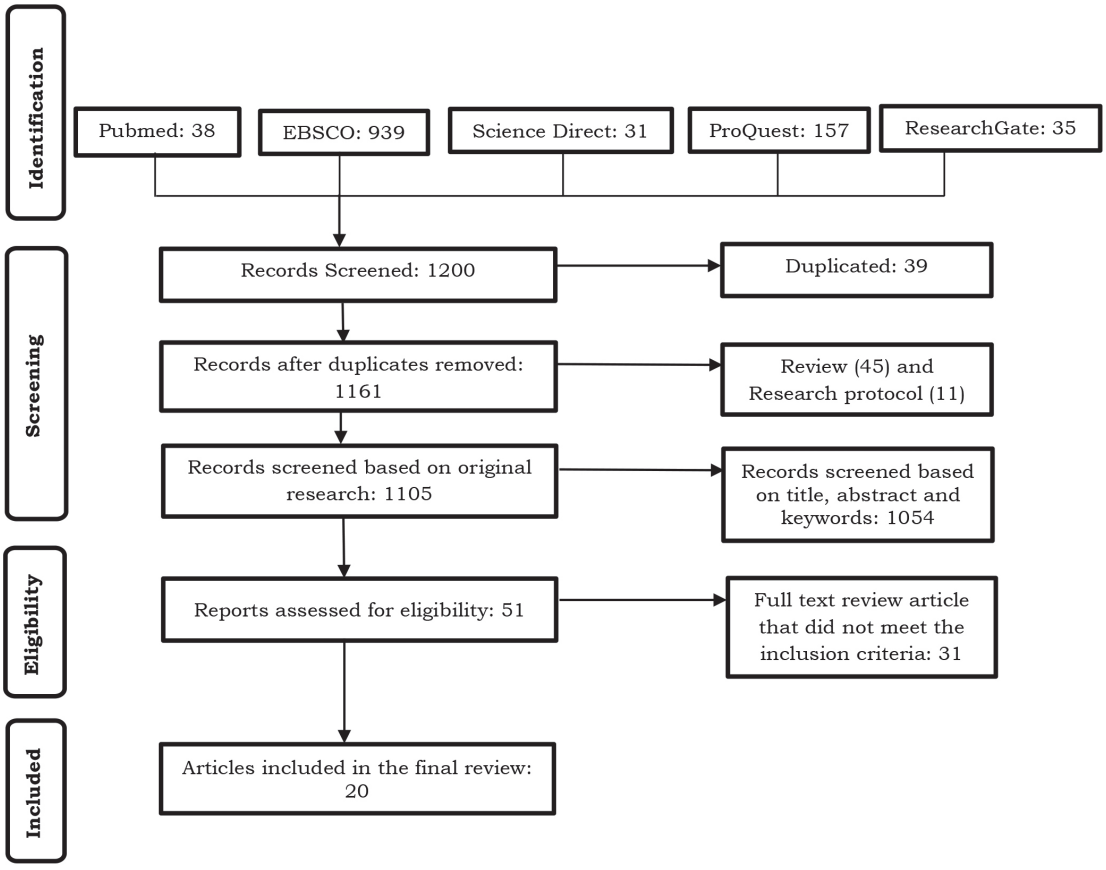

Figure 1. PRISMA flowchart and selection process

study design. However, two cohort studies and one spatial study were discovered. This review included the space-time analysis study because it compared the prevalence of stunting by the riverside and other areas.

The prevalence of stunting ranged from 20% to 48.3%. The other outcomes shown besides stunting were wasting and underweight status. There were 83 risk factors studied, with the most studied variables being age, gender, diarrhoea, water source, parent's education, immunisation, and inappropriate complementary feeding practices (6 to 13 studies). Table 3 describes the stunting risk factors studied (from the most studied to the least).

Household water sources from rivers and economic status were consistently correlated with stunting. Majority of the risk factors studied were related to nutrition, such as food diversity, breastfeeding and complementary food, as well as socio-demographic factors. From the environmental aspect, the most studied risk factors were water sources and sanitation. Figure 2 compares the significant and insignificant factors for stunting in children under five.

In Figure 2, socioeconomic class and water sources were consistently related to stunting. Although economic status and water sources had not been studied much, they need to be considered as important risk factors for

Table 2. Summary of studies

Author	*Title*	*Country*	*Design study*	*Sample size*	*Result*
Kinyoki *et al.*, 2016	Assessing comorbidity and correlates of wasting and stunting among children in Somalia using cross-sectional household surveys: 2007 to 2010	Somalia	Cross-sectional study	73,778 children under five years	• In geographical variation, a high risk of all forms of malnutrition in the southern regions, especially around the two main rivers of Juba and Shebelle, compared to the Northern regions of Somalia. • Prevalence of stunting 31%, underweight 58%, and wasting 21%. • (+): diarrhoea, acute respiratory infection, female child, child age, age of mother, household size, number under 5, female household head, high carbohydrate foods, high protein foods, fats, fruits, and vegetables.
Wachukwu-Chikodi & Sonye, 2022	Assessment of Complementary Feeding Practices and Nutritional Status of Children (0-24 months) in Akuku Toru Local Government Area of Rivers State, Nigeria	Akuku Toru Local Government Area of Rivers State, Nigeria	Descriptive Cross-sectional study	210 infants 0–24 months	• Prevalence of stunting: 30%, underweight 39%, and wasting 31%. • There was a significant association between inappropriate complementary feeding practices and under-nutritional status of infants. • This study also found that children were at high risk of malnutrition when introduced to inappropriate complementary feeding practices such as complementary feeding at age of 0–3 months and not receiving meal diversity.
Fernandes, de Castro & Sartorelli, 2017	Associated factors of malnutrition among African children under five years old, Bom Jesus, Angola	Bom Jesus, Angola	Cross-sectional study	742 children under 5 years	• Prevalence of stunting: 22%, underweight 13%, and wasting 7%. • (+): Child's age, male child, source of water from river or lake, parasite expulsion, and ear infection (-): number of father's children, fathers living with another family, parent's age, father's ethnicity, number of parent's children, mother's occupation, electricity, mother education level, number of mother's children, number of siblings under 5 years old.

Table 2. Summary of studies (continued)

Author	*Title*	*Country*	*Design study*	*Sample size*	*Result*
Udoh & Amodu, 2016	Complementary feeding practices among mothers and nutritional status of infants in Akpabuyo Area, Cross River State Nigeria	Akpabuyo Area, Cross River State Nigeria	Cross-sectional study	330 pairs of mothers and children aged 6-11 months	• Prevalence of stunting 24.6%, underweight 33.3%, and 26.4% wasting. • (+): Children who did not receive the minimum feeding frequency were more likely to be stunted than their peers who received the minimum feeding frequency and child's age. (-): milk feeding frequency for non-breastfed child, hand washing with soap, diarrhoea in last 2 weeks, vitamin, minerals, supplements, hospital medicine offered in the previous 7 days. Child was sick in the previous 1 month, mother's age, mother's occupation, place of work, mother's income, number of people in the household, parity, child's gender, and birth order of child.
Rukambile *et al.*, 2020	Determinants of diarrhoeal diseases and height-for-age Z-scores in children under five years of age in rural central Tanzania	Tanzania	Kohort study	493 children under five years of age	• The proportion of stunted children among the 24-34; 35-45; and 46-56 months age groups were 47.3%, 48.3%, and 33.3%, respectively. • The mean of diarrhoea incident was 2.3 with range of 0-16 incidents over 24 months • The source of water and animal kept inside the house overnight significantly correlated with both diarrhoea and stunting. • (+): language group of head of household, handwashing method in running water, hanging utensils after washing.
Guptan *et al.*, 2007	Early introduction of water and complementary feeding and nutritional status of children in Northern Senegal	Nothern Senegal	Cross-sectional study	374 children aged 6-23 months	• Prevalence of stunting was 20% and wasting 16%. • (+): age, source of water not from river/ pond. River or pond as the primary source of drinking water was also associated with recent diarrhoea.

Table 2. Summary of studies (continued)

Author	*Title*	*Country*	*Design study*	*Sample size*	*Result*
Kinyoki *et al.*, 2016	Environmental predictors of stunting among children under-five in Somalia: cross-sectional studies from 2007 to 2010	Somalia	Spatial – time study	Data from household nutritional surveys in Somalia from 2007 to 2010 with a total of 1,066 clusters covering 73,778 children aged 6-59 months	• Overall, the distribution of stunting in Somalia suggested substantial spatial heterogeneity with prevalence consistently higher in the regions of the South-Central zones compared to those in the North where Agro-pastoral and riverine livelihoods found in South Central zone The regions that consistently exceeded 40% prevalence were Bay, Gedo, Bakool, Mudug, Lower and Upper Juba. All these regions are in the South-Central zone.
Kehinde *et al.*, 2021	Nutritional status of under five children in the Cameroonian Refugee Settlement in Ogoja, Cross River State, Nigeria	Nigeria	Descriptive cross-sectional study	211 children aged 6-59 months	• Prevalence of stunting was 41.7%, underweight 38.4%, and 26.8% wasting. • An overview of the risk of stunting based on the characteristics of the mother and household were as follows: 55.8% mothers' age was 25-34 years, 79.8% mothers age at delivery of first child was 14-17 years, 96.2% households had one child under five years old, 45.2% household often hungry, 70.2% households had 5 or more family members. • An overview of the risk of stunting based on characteristics and caring practices of under-five children were as follows: mean of ages was 30.33 months, 56.7% children were females, 83.2% of children under-five were exclusively breastfed for six months. Majority (55.8%) of children under-five were not fully immunised, and 29.8% had diarrhoea preceding two weeks.

Table 2. Summary of studies (continued)

Author	*Title*	*Country*	*Design study*	*Sample size*	*Result*
Sulaiman *et al.*, 2018	Prevalence and determinants of undernutrition among children under 5-year-old in rural areas: A cross-sectional survey in North Sudan	River Nile state (RNS) in North Sudan	Cross-sectional study	1477 children under 5 years	• Prevalence of stunting was 42.5%, underweight 32.7%, and 21% wasting. • Stunting was highest among 48–60 months age group. • Based on gender, boys had poorer indicators of undernutrition. • (+): age, gender, poorer household sanitation, and socio-economic class. The number of family members, less distance between families, and the baby being weaned suddenly are considered risk factors for malnutrition.
Adeniran *et al.*, 2017	Schistosomiasis, intestinal helminthiasis and nutritional status among preschool-aged children in sub-urban communities of Abeokuta, Southwest, Nigeria	Abeokuta, Southwest, Nigeria	Cross-sectional study	241 children aged 0-71 months	• Prevalence of stunting was 39.5%, underweight 22.8%, and 11.4% wasting. • There was no significant correlation between infected intestinal helminths, schistosomiasis, co-infection of schistosomiasis and intestinal helminths with stunting. • Mean z-scores were generally lower in infected than non-infected children, but not significantly different. • Children exposed to river were 61%. Bathing (20.2%) was the major activity predisposing to infection, and 63.6% bathed with water from the river at home. 13.2% households still depended solely on water from the river for domestic usage. • From sanitation aspect, 16.2% of the parent/caregiver engaged in open defaecation in surrounding bushes and 1.8% directly into the river. • From hygiene aspect, only 9% children had washed their hands with soap before eating. 63.55 had dirty fingers, and 92.3% had slippers/shoes.

Table 2. Summary of studies (continued)

Author	*Title*	*Country*	*Design study*	*Sample size*	*Result*
Mengesha *et al.*, 2021	The prevalence of stunting and associated factors among children under five years of age in Southern Ethiopia: Community based cross-sectional study	Southern Ethiopia	Cross-sectional study	660 children under five years	• Prevalence of stunting was 37.7% • (+): age (older), family size (more than five members), number of under-five children in the household (two or more), wealth status, source of drinking water (river, pond, or a spring), access to diversified diet, and household food security status.
Sanchez *et al.*, 2015	Needs, acceptability, and value of humanitarian medical assistance in remote Peruvian Amazon riverine communities	Peruvian Amazon Basin	Cross-sectional health assessment	457 children under five years	• Prevalence of stunting 20%, wasting 3%, mean of Hb 11.2g/dL, and prevalence of anaemia 37%. Prevalence of one or more intestinal parasites was 62%. • Immunisation and reported health status: only 71% of children over 1 year completed the full Peruvian immunisation schedule. The caretakers reported that 49% of their children had a cough, 34% had a fever, and 29% had diarrhoea 2 weeks before surveys. They also reported that 61% of their children had good physical well-being and 67% had good emotional well-being.
Kempton *et al.*, 2021	An assessment of health outcomes and methylmercury exposure in unduruku indigenous women of childbearing age and their children under 2 years old	Sawré Muybu Indigenous Land (IL) in the Tapajós River Basin, Brazil.	Cross-sectional study	16 infants under two years old	• Of 16 infants, four (25%) were found to be moderately to severely stunted and only one infant found to be underweight. Anaemia was found in 6 of 12 infants aged 6-24 months. • All infants had not received appropriate vaccination coverage for their age according to health booklets. • Of 16 infants, 3 (18.75%) had H-Hg levels over 6.0 µg/g.
Anticona & Miguel San, 2014	Anaemia and malnutrition in indigenous children and adolescents of the Peruvian Amazon in a context of lead exposure: a cross-sectional study	The Corrientesrive riverside located in the Northeastern Peruvian Amazon, in the Loreto region	Cross-sectional study	236 children and adolescents aged 0-17 years.	• Prevalence of stunting in children aged 0-4 years was 42.1%, underweight 31.6%, and wasting 6.5%. • (+): underweight, age group, and blood lead levels ≥5µ/dL

Table 2. Summary of studies (continued)

Author	*Title*	*Country*	*Design study*	*Sample size*	*Result*
Benefice *et al.*, 2006	Nutritional status of Amerindian children from the Beni River (lowland Bolivia) as related to environmental, maternal and dietary factors	Beni River (lowland Bolivia)	Cross-sectional study	354 children aged 0-10 years old (175 children aged less than 5 years old)	• Prevalence of stunting in children less than 5 years was 41%. • During survey, main alleged cause of children's illness was simple diarrhoea (20%). • This study found 75% children positive for at least one helminth. • After adjusting for age, risk factors for malnutrition (height-for-age) were ethnic group, clinical status, and food diversity.
Yori *et al.*, 2014	Santa Clara de Nanay: the MAL-ED cohort in Peru	Santa Clara de Nanay, Peru	Cohort Study. Data were analysed descriptively.	270 households with children under 5 years	• Prevalence of stunting was 46.3% and 0.2% wasting. • 20.3% of children in the cohort were stunted at 3 months of age; this rose to 38.2% by 12 months, 43.7% by 24 months, and 55.9% by 36 months of age. • Risk factors studied: Access to clean water, improved toilet/sanitation, maternal level of education electricity in household, cooking with fuel, charcoal or wood, wall material from wood, household without concrete or wood floor, roofing material made of thatch, iron, etc.
Roche, Creed-Kanashiro *et al.*, 2011	Infant and young child feeding in the Peruvian Amazon: the need to promote exclusive breastfeeding and nutrient-dense traditional complementary foods	Peru	Descriptive cross-sectional study	32 children aged 0-23 months	• Prevalence of stunting was 39.4%. • Only 12.6% of mothers had stopped breastfeeding before 1 year of age. • Adequate intake of energy and protein from complementary food in comparison with WHO recommendation. • Vitamin A, iron, zinc, and calcium were still inadequate in comparison with WHO recommendations.

Table 2. Summary of studies (continued)

Author	*Title*	*Country*	*Design study*	*Sample size*	*Result*
Muldiasman, *et al.*, 2008	Can early initiation to breastfeeding prevent stunting in 6-59 months old children?	Jambi Province, Indonesia	Cross-sectional study from National Nutrition Survey in Jambi province in 2015	2,502 children aged 6-59 months	• Prevalence of stunting: 27.5% (95% CI: 25.2-29.9) • (+): Early initiation of breastfeeding, birth weight, diarrhoea, house structure, water source, living in rural region, parent's ethnicity and age
Febry *et al.*, 2022	Identification of food diversity factors to overcome stunting in toddlers on the Musi River suburbs, Palembang South Sumatra, Indonesia	South Sumatera, Indonesia	Cross-sectional study	170 children aged 6-59 months	• Only 54.7% households had food diversity for children's diets. • Factors related to food diversity in household were mother's education and food availability. • Factors not related significantly with food diversity were number of family, family's income, livestock ownership, food crop ownership, mass media access, food decision-making, breastfeeding, father's education, and dietary habit.
Islam *et al.*, 2014	Nutritional status of under 5 children belonging to tribal population living in riverine (Char) areas of Dibrugarh district, Assam	Dibrugarh district, Assam, India	Cross-sectional study	500 children under five years	• Prevalence of stunting was 30.4%, underweight 29%, and 21.6% wasting. • Prevalence of stunting was highest in the age group 48-60 months (58.6%), followed by 57.3% in the age group 36-48 months. • (+): gender, socioeconomic class, literacy status of both parents, exclusive breastfeeding, colostrum, age of introducing complementary foods (-): number of family members and pre-lacteal feed given.

(+) Significant risk factor
(-) Insignificant risk factor

Table 3. Research variables related to stunting in children under five years

No	*Research variable*	*No*	*Research variable*	*No*	*Research variable*
1	Age	29	Birth order of child	57	Number of parent's children
2	Gender	30	Type of house floor	58	Feeding frequency
3	Diarrhoea	31	Parent's literacy	59	Mother's illness
4	Water source	32	Source of safe water (pipe/deep well/others)	60	Milk feeding frequency
5	Parent's education	33	Birth weight	61	Mother's income
6	Immunisation	34	House structure	62	Animal kept inside the house
7	Inappropriate complementary feeding practices	35	Living in rural region	63	Language group of head of household
8	Early initiation to breastfeeding	36	Length at birth	64	Hanging utensils after washing
9	Exclusive breastfeeding	37	Cold	65	Handwashing with running water
10	Sanitation	38	Shortness of breath in previous month	66	Use of dry utensils
11	Parent's occupation	39	Quality of water source	67	Water source sharing with animal
12	Age of mother	40	Number of household members	68	Interaction of human-animal.
13	Number of children under 5 years	41	Good physical well-being	69	Receiver of hygiene education
14	Number of people in the household	42	Good emotional well-being	70	Riverine livelihood
15	Parent's ethnicity	43	H-hg level	71	Prelacteal feed given
16	Hand washing with soap	44	Underweight	72	Maternal adiposity
17	Fever	45	Blood lead level	73	Maternal stature
18	Female household head	46	High exposure to oil activity	74	Mother's age at delivery of first child
19	Vitamin A	47	Anaemia	75	Household often hungry
20	Parasite infection	48	Acute respiratory infection	76	Family income
21	Food diversity	49	Household size	77	Type of cooking fuel
22	Socioeconomic class	50	High-carbohydrate foods	78	Type of wall
23	Visiting community base health service	51	High-protein foods	79	Dirty fingers
24	Cough	52	Fats	80	Had slippers/shoes
25	Boiling drinking water	53	Fruits and vegetable	81	Food security status
26	MUAC of mother	54	Suspected measles	82	Breastfeeding
27	Electricity	55	Number of father's children	83	Inadequate nutrition intake
28	Number of mother's children	56	Father lives with another family		

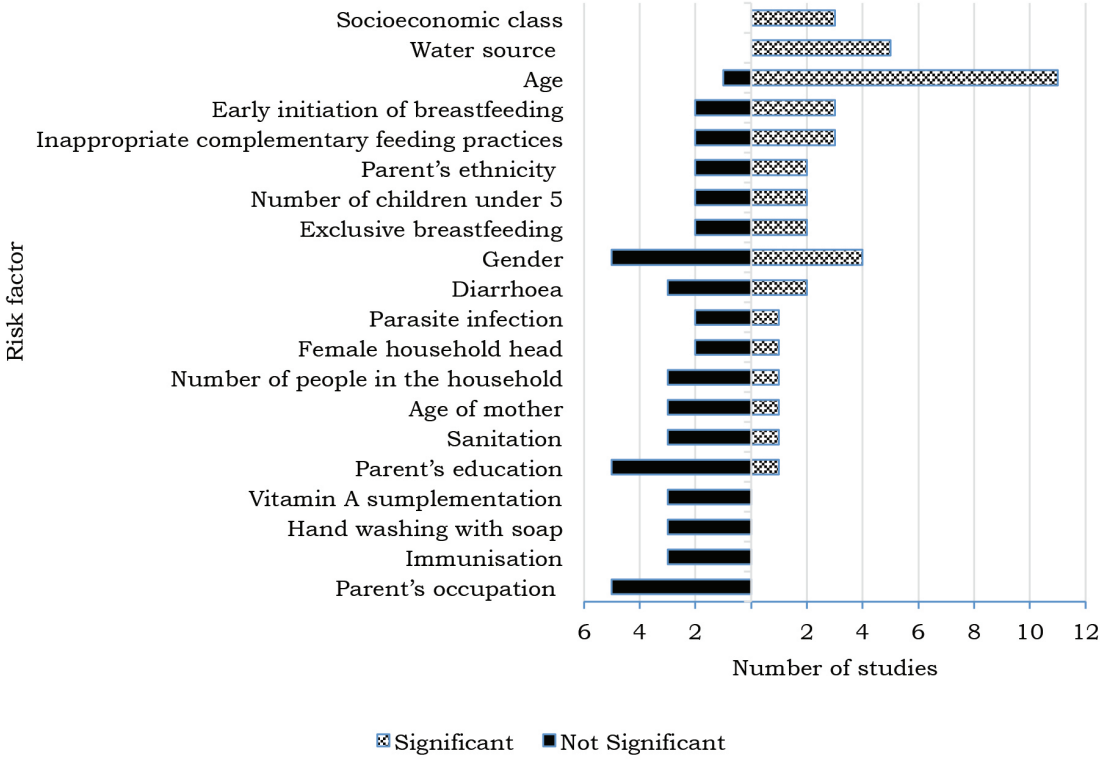

Figure 2. Comparison of risk factor significance

stunting. The majority of studies showed that the age of the child was related to stunting (11 of 12); the older the age of the child, the higher the prevalence of stunting. Parent's occupation, vitamin A supplementation, hand wash, and immunisation have also consistently been shown to be unrelated to stunting. However, apart from parental occupation, these three insignificant variables should be investigated further because they were found in three studies to be significantly related to stunting. Early breastfeeding initiation, inappropriate complementary feeding practices, parent's ethnicity, number of children under the age of five in the household, exclusive breastfeeding, gender, and diarrhoea had all been extensively studied (more than five studies), but the evidence had been inconclusive. The remaining variables were not included in Figure 2, because they were only found in two or fewer studies.

DISCUSSION

Socio-demography

Socio-demography is a study exploring the determinants and consequences of population size, distribution and composition of the demographic process (Murdock, 2019). Referring to the determinants of stunting by WHO, these social and demographic factors are the foundation of various factors that directly cause stunting (WHO, 2014). This study found that the most studied socio-economic-demographic factors related to stunting were age of children, gender, parental education and occupation, parental ethnicity, and socio-economic class. A study on children aged 0-36 months in 179 demographic and health

surveys from 64 low- and middle-income countries (1993-2015) showed a trend of decreasing average HAZ in children with increasing age (Roth *et al*, 2017). This finding is in line with the prevalence of stunting found in other studies in which children aged over 24 months have a greater risk of experiencing stunting than children aged under 24 months (Adhikari *et al.*, 2019; Atlas, 2020; Blankenship *et al.*, 2020; Khan *et al.* 2019; Mengesha *et al.*, 2021; Mutunga *et al.*, 2021; Rajpal *et al.*, 2020; Roba *et al.*, 2021). The risk of stunting between males and females in this study was still inconclusive. The results of the study found that females were more at risk of experiencing stunting in Asia (Khatun *et al*, 2004), and vice versa in research in the African region (Wamani *et al.*, 2007).

Economic vulnerability can occur in communities living in riverside areas. Research from Brazil found that the majority of riverside residents were involved in the agricultural sector, with 65.8% having a family income of up to 1 minimum wage (Rodrigues *et al.*, 2020). Furthermore, in the urban area, households that do not have adequate income will be forced to live near the river. This condition is very likely to form a slum area, which is identical with poor sanitary and drainage. This study found economic status to be consistently correlated with stunting. This finding is considered very important because they are related to the family's ability to meet the nutritional needs of children, prevent infectious diseases, and access a healthy home environment.

Maternal health

Factors of nutritional status and maternal health can lead to stunting in children. In this study, from the aspect of maternal health, the variables found were age of the mother, mid-upper arm circumference (MUAC) of the mother, number of mother's children, mother's illness, maternal adiposity, maternal stature, and mother's age at delivery of the first child. Maternal age was found to be significant in four studies, while the others were only present in one to two studies. Therefore, maternal health still cannot be concluded in this research. Malnutrition during preconception, pregnancy and breastfeeding, mother's short stature, intrauterine growth restriction (IUGR), premature birth, and teenage pregnancy have been shown to be associated with stunting (Beal *et al.*, 2018). Pregnant women require approximately 10-15% more energy than non-pregnant women. A deficiency of energy and protein causes pregnant women to be unable to meet the nutritional needs for foetal growth and development (Achadi *et al.*, 2020; Li *et al.*, 2020).

Short adult women represent a history of suboptimal growth in height, not only due to genetic factors, but also a long history of malnutrition and chronic recurrent infections. This affects organ development. Short pregnant women are at risk of having low birth weight (LBW), small-for-gestational age (SGA), or short babies (Achadi *et al.*, 2020). Several studies have found a strong correlation between mothers who have a short stature and stunting in children (Beal *et al.*, 2018; Li *et al.*, 2020; Oddo *et al.*, 2012; Rachmi *et al.*, 2016; Svefors *et al.*, 2019).

Household condition

Stunting can occur as a result of household conditions. This study found that the variables related to stunting were the number of children under 5 years of age, density of people in the house, electricity, type of house floor, wall, cooking fuel, house structure, high exposure to oil activity, animal kept inside the house, interaction between humans and animals, and hunger and food security status.

Food security refers to the ability of individuals or groups to accomplish access to good, safe and nutritious food. Family food security status is a crucial factor that can affect the nutritional status of family members, especially children under 5 years (Fadzila & Tertiyus, 2019; Helmyati *et al.*, 2019; Raharja *et al.*, 2019; Safitri & Nindya, 2017). The number of family members also plays a role in household food availability. A large number of children and family members will affect the food intake of children in the family (Helmyati *et al.*, 2019; Titaley *et al.*, 2019).

The quality of housing can affect people's health. The various characteristics of housing by the riverside depend on their socio-economic developmental level and cultures. The slum residential areas may be found in urban rivers with low socioeconomic levels. Otherwise, a high-quality living environment can be realised with the support of urban planning and development.

Poor housing is associated with a wide range of health conditions such as respiratory diseases including asthma, cardiovascular diseases, injuries, mental health, and infectious diseases including tuberculosis, influenza, and diarrhoea (WHO, 2018b). The improved housing criteria based on the United Nations are improved drinking water, improved sanitation, sufficient living area, and finished building materials (Tusting *et al.*, 2020).

Water sanitation and hygiene (WaSH)

WaSH can be a determining factor in the incidence of stunting (Cumming & Cairncross, 2016; Helmyati *et al.*, 2019). Inadequate WaSH can result in malnutrition (WHO, 2019). Mechanisms that play a role in the link between WaSH and malnutrition include recurrent diarrhoea, worm infections (soil-transmitted helminths, STH) such as *Ascaris lumbricoides, Trichuris trichiura, Aancylostoma duodenale*, and *Necactor americanus,* and subclinical conditions of the gastrointestinal tract. The exposure to enteric pathogens and symptomatic and asymptomatic infections mediates the impact of WaSH on undernutrition (Budge *et al.*, 2019; Helmyati *et al.*, 2019; WHO, 2019; Zavala, *et al.*, 2021).

Of these three factors, water sources from rivers are consistently associated with stunting. As a result of human activities, both chemical and biological river water pollution can occur. Human activities strongly influence the concentration of faecal coliform. The presence of faecal coliform almost always indicates water contamination by faeces, both human and animal (Bartram & Ballance, 1996). Several studies showed contamination of river water by faecal coliform (Pratama *et al.*, 2020), *Cryptosporidium spp, Giardia spp* (Tandukar *et al.*, 2018), and *Escherichia coli* (Verbyla *et al.*, 2021).

In addition to biological pollution, chemicals originating from industry or mining can pollute river water. We found one study on H-Hg levels in children, which exceeded maximum level (Kempton *et al.*, 2021) and one study on blood lead levels, which correlated with stunting (Anticona & Miguel San, 2014).

Breastfeeding

The most efficient method for ensuring that children receive adequate nutrition is through breastfeeding. Breast milk is believed to benefit infant growth because of its appropriate nutrient composition for children's nutritional requirements (Sirajuddin *et al.*, 2020). Inadequate breastfeeding in the WHO framework related to stunting includes non-exclusive breastfeeding, early initiation of breastfeeding, and weaning (Beal *et al.*, 2018). A study in Indonesia found that stunting occurred outside the age

period of breastfeeding (Sirajuddin *et al.*, 2020). In this study, two of four articles found non-exclusive breastfeeding as a risk factor, but this was still inconclusive.

Dietary intake

Food diversity and the amount of food a child eats per day are significant determinants of stunting and underweight status in children under 5 years of age (Hashmi *et al.*, 2021; Motbainor, Worku & Kumie, 2015). Adequacy of child nutrition includes adequacy of macronutrients and micronutrients; whereby carbohydrates, proteins, and fats fulfil the macronutrient needs (Sudargo, Aristasari & Afifah, 2018). Deficiency of some common micronutrients are vitamin A, zinc, ferum, and iodine (Black *et al.*, 2008), which can affect various aspects of physiology, including immune function and neurodevelopment (Prendergast & Humphrey, 2014).

Inappropriate complementary feeding practices have been extensively studied and showed their potential as a risk factor for stunting. The other variables related to dietary intake were food diversity, high carbohydrate, protein, and fat foods, fruits and vegetables consumption, feeding frequency, and inadequate nutrition intake. They were only found in 1-2 articles; therefore, no conclusion could be drawn from them.

Infection

According to WHO, infectious diseases in children that cause stunting include both clinical and subclinical, namely digestive infections (diarrhoea, environmental enteropathy, and worm parasitic infections), respiratory tract infections, and malaria (Beal *et al.*, 2018). Infectious diseases including diarrhoea, respiratory infections, and fever are associated with stunting in children aged 6–59 months living in urban and rural poor areas (Bardosono, Sastroamidjojo & Lukito, 2007). Additionally, Semba *et al.* (2007) reported that children aged 12-59 months who received complete, partial, or no immunisation had a stunting prevalence of 37%, 47%, and 54%, respectively (Semba *et al.*, 2007). Diarrhoea as a risk factor for stunting was found in five and three articles for parasite/helminth infection. However, both are still inconclusive evidence.

Infectious diseases are highly correlated with the environment. A study found that almost half of the children living by the riverside had experienced diarrhoea in the previous month, with sanitation and water sources as significant risk factors (Susanti, 2019). The other study found that upstream river pollution caused by bathing and sanitary practices explained as many as 7.5% of all diarrhoea-related deaths annually (Garg *et al.*, 2018). Therefore, poor environmental conditions can increase the risk of infectious diseases. Recurrent infections in children will eventually lead to malnutrition, including stunting.

CONCLUSION

The river is a source of life for the communities around it. The prevalence of stunting in children under five years living by the riverside ranged from 20% to 48.3%. Household water sources from rivers and economic status were consistently correlated with stunting. Water sources were an important risk factor for stunting in children living by the riverside. This was not only limited to the use of river water as a source of drinking water, but also for cooking, bathing, sanitary practices, and cleaning food equipment. Although the studied variables aligned with established stunting determinants, further investigation is required to explore the influence of environmental factors and the behaviours of individuals residing by the riverside.

Authors' contributions

Bambang W, Ririn A and Ridha R, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; Umar FA, Tri YM, Defriman D and Miko H, reviewed the manuscript.

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Conflict of interest

Authors declare no conflict of interest in this research.

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Characteristics of dietary intakes including NOVA foods among pre-adolescents living in urban Kuala Lumpur – Findings from the PREBONE-Kids study

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ABSTRACT

Introduction: Evidence showed considerable variability of health risk factors within different socioeconomic groups. This study aimed to characterise dietary intakes by total household income among a sample of Malaysian pre-adolescents in urban Kuala Lumpur. **Methods:** Baseline data of 243 healthy, pre-adolescent children between 9 and 11 years old including socio-demographic background (gender, ethnicity, and total household monthly income), anthropometry (body weight and height), and 7-day diet histories were collected. Secondary analysis was performed on dietary intakes to quantify food groups based on the Malaysian Dietary Guidelines and NOVA classification systems besides nutrients. Differences and associations between total monthly household income categories with anthropometry and dietary intakes were tested using independent *t*-test/Mann-Whitney U (depending on normality) and chi-square tests, respectively. **Results:** Most children in this study population had dietary intakes below the recommended serving sizes for five food groups, except meat/poultry (195.2±107.2%) and fish (110.1±106.3%) and consumed about 32% of energy from ultra-processed foods (NOVA food group 4). While there was no difference in dietary intake between the bottom 40% with the middle 40% and high 20% household income groups, the percentage of energy contributed by NOVA food group 4 (processed fats/oils, condiments, and sauces) was higher in the bottom 40% households (p=0.024). **Conclusion:** Most pre-adolescent children in this study, regardless of household income, did not meet dietary recommendations and ate diets comprised of less nutritious foods. Comprehensive approaches that aim to improve dietary patterns and reduce the risk of diet-related chronic diseases are warranted.

Keywords: children; dietary intake; income; Malaysian; NOVA group

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INTRODUCTION

The exponential increase in the prevalence of non-communicable diseases and associated risk factors in developing countries is of concern (WHO, 2014). The negative implications of globalisation, rapid urbanisation, sedentary lifestyles, and poor dietary habits are evident and constitute considerable challenges. Studies have reported that rates of health risk factors showed patterns of considerable variability within the developing countries and may be unequally experienced by different socioeconomic groups (Magnusson, 2010). Populations that are socioeconomically disadvantaged tend to fare worse with regard to non-communicable diseases risk factor prevalence (WHO, 2014), though important exception exists where overweight and obesity remain concentrated in higher socioeconomic groups.

While socioeconomic status is a complex construct, income is only one of the possible indicators. Inadequate income, a frequently used indicator of lower socioeconomic status, puts individuals at risk of developing unhealthy dietary patterns that could contribute to inadequate or excess intakes of energy and nutrients, thus poorer diet quality (Darmon & Drewnowski, 2008). Studies on Western populations provide strong evidence of children from low-income households being more likely to have inadequate intakes of macro- and micronutrients (Kumanyika & Krebs-Smith, 2001). In contrast, a local nutrition survey found that more than 80% of these children consumed two main meals (lunch and dinner) and snacked significantly more than those of higher household income (Chong *et al.*, 2016). Hence, understanding the extent to which differences in dietary intakes are of concern can be fostered by examining how the intakes of the subpopulation groups compare to dietary guidelines.

The low diet quality among children from low-income populations has been closely associated with the consumption of low nutrient-dense foods that are high in cholesterol, saturated fat, added sugar, and sodium, as well as low in fibre (Mayen *et al.*, 2014), which are largely contributed by ultra-processed foods (U.S. Department of Agriculture and U.S. Department of Health and Human Services, 2020; Monteiro *et al.*, 2019). Ultra-processed foods are characterised by foods or drinks formulated mostly or entirely from substances extracted from foods or derived from food constituents to make them highly convenient, attractive, and profitable. While such processes and ingredients can make ultra-processed foods nutritionally unbalanced and displace other types of foods (Monteiro *et al.*, 2019), there is emerging evidence that excluding ultra-processed foods may result in lowered intakes of key nutrients of particular concern for at-risk groups (Estell *et al.*, 2021). Ultra-processed foods are rapidly growing in middle-income countries (Baker & Friel, 2016) and contribute to almost half of the total dietary energy intake in some high-income country populations (Monteiro *et al.*, 2019).

Despite the risk of inadequate nutrition for low-income children, there is a paucity of literature on the extent of these nutritional issues, particularly in the Asian developing countries including Malaysia. Direct evidence on the dietary intake among Malaysian children from low-income families in urban communities is limited (Shariff *et al.*, 2015; Koo *et al.*, 2016; Yang *et al.*, 2017). Poor nutritional status increases health risk factors, which could be more prevalent amongst the lower-income group due to health and nutrition disparities. Hence, the purpose of this

paper was to characterise dietary intakes by household income among Malaysian children in Kuala Lumpur.

MATERIALS AND METHODS

This paper reports the secondary analysis of the baseline data from participants in the PREBONE-Kids Study, which was a 1-year randomised, double-blind, placebo-controlled trial of soluble corn fibre on bone indices in pre-pubertal children with ClinicalTrials.gov identifier: (NCT03864172). This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Research and Ethics Committee of the International Medical University (IMU R/2016). Written informed consent was obtained from all subjects, and assents were obtained from the children. The detailed protocol has been published elsewhere (Arasu *et al.*, 2021) and is briefly explained here.

A total of 243 school children aged 9 to 11 years (127 boys and 116 girls) were recruited from March 2017 through March 2018. The study included participants who were healthy as determined by a standard medical assessment, at Tanner Stage 1 or 2 based on breast development for girls and pubic hair in boys, pre-menarcheal for girls, and able to provide assent. Participants were excluded if they had a history of serious medical conditions and received therapy with medications known to interfere with bone metabolism (e.g., steroids, hormones, diuretics, cortisone, or anti-seizure medication).

Participants' socio-demographic background was collected by interviewing their parents on gender, ethnicity, and total monthly household income. Household income was obtained as a range and subsequently reclassified based on the three broad national classifications of total monthly household income for urban areas (Department of Statistics, 2020). These are Bottom 40% (B40), Middle 40% (M40), and Top 20% (T20). The thresholds of the monthly household for each group refer to 1) B40: less than Ringgit Malaysia (RM) 4850; 2) M40: between RM4850 and RM10960; and 3) T20: RM10960 and above. The B40 household covers both poor and low-income groups.

Body weight and height were measured according to standard protocols using a calibrated weighing scale (TANITA HD-314, Tanita Corporation, Tokyo, Japan) and a portable stadiometer (SECA 213, SECA Group, Hamburg, Germany), respectively. Z-scores for body mass index (BMI)-for-age (5 to 19 years), weight-for-age (5 to 10 years), and height-for-age (5 to 19 years) were determined using the World Health Organization (WHO) AnthroPlus software (version 1.0.4, 2009 WHO Int, Geneva, Switzerland) (WHO, 2006). The interpretations of BMI z-score and height z-score were based on the WHO cut-offs to classify the participants into four BMI categories: thin (z-score <-2.0), normal (z-score ≥ -2.0, ≤ 1.0), overweight (z-score > 1.0, ≤ 2.0), and obese (z-score > 2.0); and two height categories: stunting (z-score < -2.0) and normal (z-score ≥ -2.0) (WHO, 2006).

Dietary intake data were collected using interviewer-administered 7-day diet histories assisted by household tools and a food photographs album. Participants were asked to recall their usual food and beverage intakes for breakfast, lunch, dinner, and snacks consumed, including the frequency of intake for these foods for a week (five school days and two weekend days). Standard training was provided to all enumerators before research commencement. The collected dietary assessment forms were counter-checked to ensure completeness and validity of

reporting. The diet histories were further verified by randomly weighing the foods consumed at the school canteen and checking with participants' mothers whenever possible. The reported foods and beverages in the diet histories were converted into grams prior to analysis.

In determining serving sizes, foods and beverages obtained from the 7-day diet histories were classified into seven major food groups based on the Malaysian Dietary Guidelines (MDG)'s food pyramid (NCCFN, 2013): cereals; fruits; vegetables; meat/poultry; fish; legumes; milk and dairy products. The number of servings for these main food groups was calculated by aggregating the total amount for each food group and dividing it by the standard serving size from MDG. Subsequently, the number of daily servings consumed was compared to MDG recommendations for serving sizes (RSS). Using the similarities of the items' physical or preparation characteristics, nine sub-food groups were created: mixed dishes, sugar-sweetened beverages; western fast food; kuih/pastries/dessert; snacks; spread; sugar; oil and condiments. Mixed dishes were assigned a major food group based on primary ingredients, e.g., fried rice was assigned to 'cereals/tubers/grains'.

The individual mean daily nutrient intakes were analysed using Nutritionist Pro™ Diet Analysis software (Axxya Systems, Washington, United States of America), referencing against two national food composition databases from Malaysia (Tee *et al.*, 1997) and Singapore (Singapore HPB, 2003) due to similarities in food habits and cultures. Participants with implausible dietary intake were checked using a histogram plot on the distribution of energy intakes. Dietary outliers were defined as those with baseline energy intake >±2 standard deviations (*SD*) from mean energy intake of the population (Field *et al.*, 2004) and were removed from the final analysis. Total energy, macronutrients, and micronutrients intake values were compared with age-relevant nutrient recommendations; Estimated Average Requirement (EAR) by the National Academy of Medicine, USA (Food and Nutrition Board, Institute of Medicine, 2016) and Recommended Nutrient Intakes (RNI) 2017 for Malaysia (NCCFN, 2005).

Aside from the dietary adequacy perspective and given the lack of evidence on in-depth dietary patterns of Malaysian children, the Food and Agriculture Organization's NOVA classification system was subsequently used to characterise the participants' intakes. Foods and beverages were classified into four major groups: unprocessed or minimally processed foods (NOVA group 1), processed culinary ingredients (NOVA group 2), processed foods (NOVA group 3), and ultra-processed foods (NOVA group 4) (Monteiro *et al.*, 2019). The food description and ingredient list were obtained from the 7-day diet histories for assignment of a food or beverage into one of the four NOVA groups. The NOVA classification system was adapted to suit local variations in food preparation and food availability to improve accuracy (Table 1). For foods with a homemade recipe, the classification was applied to the underlying ingredients.

Data were tabulated using Microsoft Excel version 2016 (Microsoft Corporation, Redmond, Washington, USA). Analyses were carried out using IBM SPSS Statistics for Windows Version 27.0 (IBM Corp®, Armonk, New York, United States of America), and $p<0.05$ was considered statistically significant. Normality checking was done using the Shapiro-Wilk test, which results from skewness and kurtosis found the distributions to be normal for all variables, except for some micronutrients and food groups. Descriptive statistics were presented

Table 1. NOVA classification system adapted from the Food and Agriculture Organization

Food groups and subgroups	*Descriptors*
Unprocessed or minimally processed foods (NOVA group 1)	
Meats, poultry, fish, and eggs	-
Milk and plain yoghurt	-
Fruits	-
Grains	Dry, raw, or cooked whole grain and bran of rice, oat, corn, wheat; white rice; pasta; and grain flour
Vegetables	-
Nuts, seeds, or legumes	-
Other	Dried fruits without added sugars, dried vegetables, non-flavoured coffee and tea, coconut water, and homemade soup
Processed culinary ingredients (NOVA group 2)	
Plant oils	-
Animal fats	Butter and ghee
Sugar	Brown, granulated, or powdered sugar and honey added when cooking unprocessed or minimally processed foods at home/ restaurants
Processed foods (NOVA group 3)	
Cheese	-
Canned, smoked, or cured meats and fish	-
Canned fruits and vegetables	-
Rice-based dishes	Rice-based dishes such as oil rice, coconut milk rice, fried rice, fried noodles, and noodles soup
Meats, poultry, fish, and eggs-based dishes	Meats, poultry, fish, and eggs-based dishes such as sambal chicken, braised chicken with soya sauce, *ayam masak merah*, braised fish in tamarind sauce, and sambal cuttlefish/prawn
Other	Salted or sugared nuts and seeds
Ultra-processed foods (NOVA group 4)	
Industrial grain foods	Breads, rolls; breakfast cereals; biscuits, muffins, and quick breads; and pancakes, waffles, and French toast
Ready-to-heat and -eat mixed dishes	Pizza; sandwiches or hamburgers; and other mixed dishes that are ready-to-eat or ready-to-heat
Sweet snacks and sweets	Sweet bakery products (cakes, cookies, pies, and pastries); ice cream and desserts (ice pops, pudding, and fruit); candy
Savory snacks	Crackers, chips, and popcorn
Fast food or reconstituted meat, poultry, or fish	Meat patties, fried chicken, fish sticks, patties, or fillets; chicken nuggets, sausages, ham, and luncheon meats; and beef or other types of jerky
Sugar-sweetened beverages	Sugar-sweetened and diet soft drinks; fruit and other sweetened drinks (e.g., cordial)
Processed fats or oils, condiments, and sauces	Dressing, gravy, dips, spreads, mustard, margarine, and industrial fats
Flavoured dairy foods and substitutes	Flavoured milk, flavored yoghurt, milk shakes, and other dairy drinks
Fast food or prepared potato products	French fries, hash browns, potato puffs, and stuffed potatoes
Other	Soy products such as meatless patties; and distilled alcoholic drinks

Table 2. Characteristics of the children (*N*=230)

	Total (N=230)	*B40 (n=180)*	*Non-B40 (n=50)*	*p-value*
Age (years), mean±*SD*	10.1±1.0	10.0±1.0	10.1±0.9	0.614
Gender, *n* (%)				
Boys	120 (52.2)	96 (80.0)	24 (20.0)	0.504
Girls	110 (47.8)	84 (76.4)	26 (23.6)	
Ethnicity, *n* (%)				
Malay	209 (90.9)	162 (77.5)	47 (22.5)	0.579
Indian	21 (9.1)	18 (85.7)	3 (14.3)	
Monthly household income category, *n* (%)				
Bottom 40 (B40)	180 (78.3)	NA	NA	NA
Middle 40 (M40)	37 (16.1)	NA	NA	NA
Top 20 (T20)	13 (5.7)	NA	NA	NA
Anthropometry, mean±*SD*				
Weight (kg)	33.7±12.0	33.1±11.4	35.8±13.9	0.161
Height (cm)	135.6±9.1	135.1±9.0	137.5±9.6	0.094
BMI-for-age (kg/m^2)	17.9±4.4	17.8±4.3	18.5±4.8	0.339
BMI-for-age *z*-score	0.2±1.7	0.1±1.7	0.3±1.7	0.413
Weight-for-age *z*-score[a]	-0.3±1.6	-0.3±1.7	-0.3±1.4	0.898
Anthropometry, *n* (%)				
BMI *z*-score classification				
Thinness	19 (8.3)	14 (7.8)	5 (10.0)	0.862
Normal	136 (59.1)	109 (60.6)	27 (54.0)	
Overweight	37 (16.1)	28 (15.6)	9 (18.0)	
Obese	38 (16.5)	29 (16.1)	9 (18.0)	
Height *z*-score classification				
Normal	206 (89.6)	161 (89.4)	45 (90.0)	0.909
Stunting	24 (10.4)	19 (10.6)	5 (10.0)	
Percentage of intake compared to RSS (%), mean±*SD*[b]				
Cereals/tubers/grains	70.4±24.7	70.9±25.5	68.6±21.7	0.561
Fruits[d]	0.0±6.5	0.0±4.6	0.0±19.1	0.070
Vegetables[d]	53.7 (148.7)	44.8 (125.5)	108.5 (165.1)	0.014*
Meat/poultry	195.2±107.2	197.9±110.4	185.6±95.4	0.476
Fish	110.1±106.3	110.0±110.0	110.4±92.8	0.978
Legumes[d]	0.0±6.0	0.0±6.0	0.0±8.5	0.180
Milk & dairy products	8.6±15.5	7.8±14.6	11.6±18.2	0.174
Number of children below RSS, *n* (%)[c]				
Cereals/tubers/grains	196 (85.2)	152 (84.4)	44 (88.0)	0.531
Fruits	227 (98.7)	179 (99.4)	50 (100.0)	1.000
Vegetables	146 (63.5)	117 (65.0)	29 (58.0)	0.363
Meat/poultry	32 (13.9)	25 (13.9)	7 (14.0)	0.984
Fish	127 (55.2)	103 (57.2)	24 (48.0)	0.246
Legumes	224 (97.4)	177 (98.3)	47 (94.0)	0.119
Milk & dairy products	230 (100.0)	180 (100.0)	50 (100.0)	NA

NA: not applicable; SD: standard deviation; RSS: recommended serving size; B40: less than RM 4850; M40: between RM4850 and RM10960; T20: RM10960 and above

[a]*n*=109 as *z*-scores for 5 to 10 years

[b]Independent *t*-test

[c]Chi-Square/Fisher's exact test

[d]Median (inter-quartile range) tested with Mann-Whitney U test

*p<0.05

as frequencies and percentages for categorical variables, and mean and *SD*s, or median and interquartile range for continuous variables.

For comparisons by total monthly household income, the household income of M40 and T20 were re-categorised into non-B40 (effect size, d=0.45) (Kang, 2015). Associations between total monthly household income categories with anthropometry, food groups, and nutrients were tested using parametric tests. Comparisons with recommendations (RSS, EAR, and RNI) and NOVA classification by total monthly household income, body weight status, and gender categories were completed using independent *t*-tests/Mann-Whitney tests/ analysis of variance tests (continuous variables) and chi-squared tests (categorical variables).

RESULTS

Of the 243 children enrolled at baseline, individuals with implausible dietary intakes (*n*=4) and missing data for household income (*n*=9) were excluded from this analysis. This resulted in a sample of 230 children for secondary

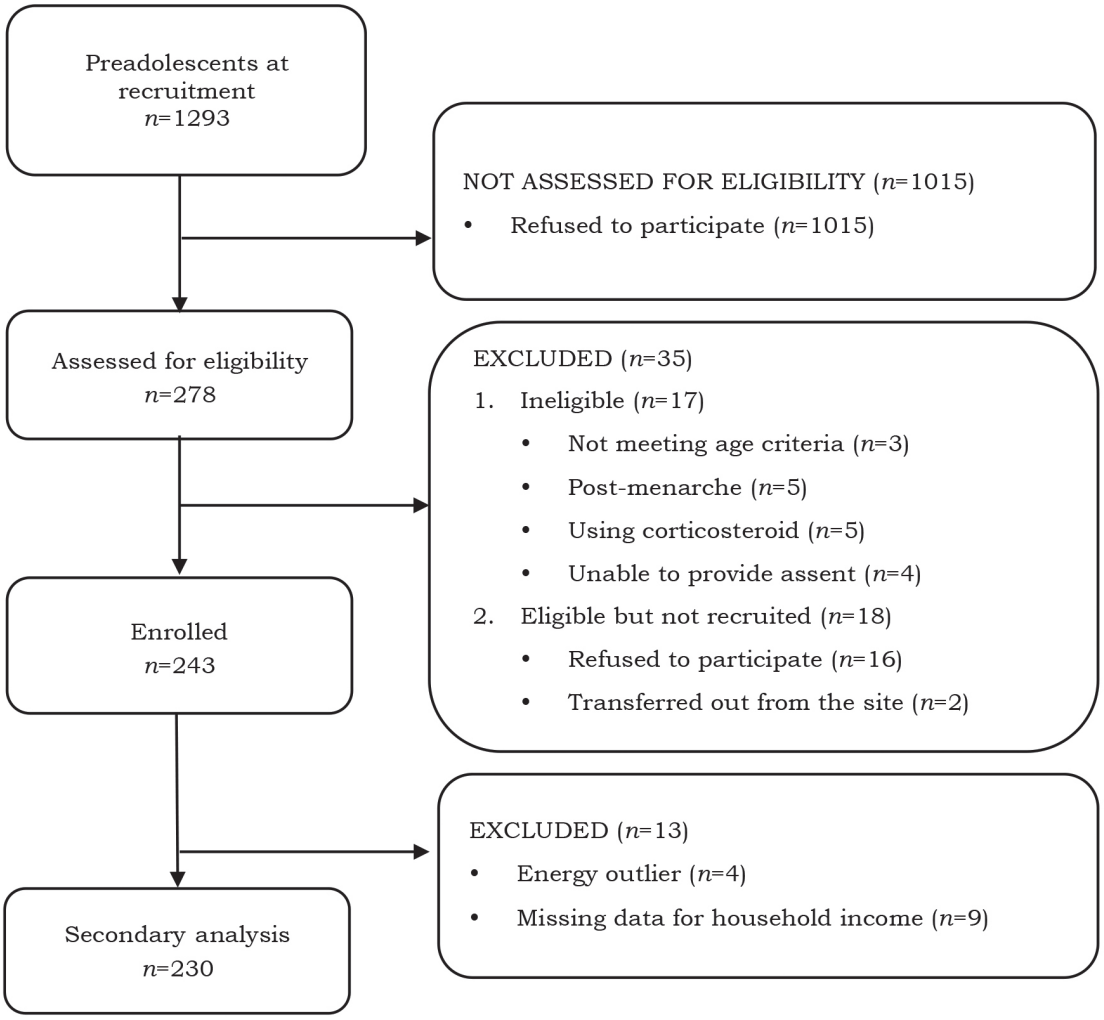

Figure 1. Flow chart of participants included in a secondary analysis

analysis (Figure 1). Table 2 describes the characteristics of all children including socio-demography, anthropometry, and dietary intakes. The children's mean age was 10.1±1.0 years, with equal gender distribution. Malay ethnicity was predominant (90.9%). Based on the national classification of total monthly household income, 78.3% of families reported a total household monthly income within the B40 household, and only 5.7% were in the T20 households.

Table 3 shows the estimated percentages of energy from NOVA food groups. The percentages of energy from the subgroup of processed fats/oils, condiments, and sauces in NOVA food group 4 were higher in B40 households (2.8±3.4% vs. 1.7±1.8%, p=0.024). In the NOVA food groups analyses, some subgroups showed a trend of non-B40 having higher intakes of processed food groups than B40 households. This included the unprocessed or minimally processed foods (NOVA food group 1), such as milk, plain yoghurt, and grains; and sugar (NOVA food group 2). Overall, all children from both households had high percentages of energy from ultra-processed foods (NOVA food group 4), with a total of 33.3±12.9% for non-B40 vs. 31.4±14.5% for B40 group; with more than half contributed by industrial grain foods (5.5%), ready-to-heat and ready-to-eat mixed dishes (6.8%), and sugar-sweetened beverages (5.7%). Additional analyses on ultra-processed foods (NOVA food group 4) by body weight status and gender did not show any statistical differences (p>0.05).

As shown in Table 4, dietary intakes were presented as nutrients, in which all children in this study had reported poor mean intakes for calcium, potassium, vitamin C, and vitamin D. There were no significant differences for all nutrients between children from B40 and non-B40 households. When comparing with nutrient recommendations (EAR and RNI), the percentages of intake achieving EAR were higher than RNI in this sample for both households. Those nutrients with percentages of intake that exceeded the EAR were carbohydrates, protein, iron, and vitamin A, while only protein and sodium intakes were above RNI. Children from B40 households had significantly higher percentage of intake meeting RNI for phosphorus (58.8±46.0% RNI vs. 55.3±24.9% RNI, p=0.049).

All or most children, regardless of their household income, had intakes below both nutrient recommendations (EAR and RNI) for calcium and vitamin D. In contrast, only four children were not able to meet their protein requirement based on RNI, mainly from non-B40 households (6.0% vs. 0.6%, p=0.033). Other nutrients including energy, vitamin C, and thiamine followed a similar trend of intake below recommendations amongst the non-B40, but these did not achieve statistical significance.

DISCUSSION

The secondary analysis of the PREBONE-Kids study aimed to characterise dietary intakes by total household income among a sample of Malaysian children in Kuala Lumpur. Results from the present study extend previous studies (Shariff *et al.*, 2015; Yang *et al.*, 2017) by describing dietary intakes in terms of food groups (including the NOVA classification system) and nutrients of children aged between 9 and 11 years, and comparing the differences between two levels of household income. The main findings accepted the null hypothesis that there was no difference in dietary quality between low-income and higher-income households. Irrespective of household income, most children had dietary intakes below the recommendations and consumed 32% of energy from ultra-processed foods, with a relatively high prevalence of stunting (10.4%) and more

Table 3. Estimated percentage of energy from NOVA classification system by household income

Food groups and subgroups	*Estimated % of energy from consumption of foods by household income, mean±SD*			*p-value*	*Mean difference (95% CI)*
	Total (N=230)	*B40 (n=180)*	*Non-B40 (n=50)*		
Unprocessed or minimally processed foods (NOVA group 1)					
Total	33.9±13.5	34.3±13.6	32.5±13.0	0.416	1.76 (-2.50 to 6.01)
Meats, poultry, fish, and eggs	14.5±8.5	14.7±8.5	13.6±8.6	0.406	1.13 (-1.55 to 3.81)
Milk and plain yoghurt	1.0±2.5	1.2±2.7	0.6±1.6	0.058	0.58 (-0.02 to 1.17)
Fruits	0.8±1.9	0.8±2.0	0.9±1.7	0.751	-0.10 (-0.71 to 0.51)
Grains	16.3±8.2	16.3±8.3	16.1±7.5	0.823	0.29 (-2.28 to 2.87)
Vegetables	1.0±1.6	1.0±1.6	1.0±1.4	0.885	-0.04 (-0.54 to 0.47)
Nuts, seeds or legumes	0.1±0.5	0.1±0.5	0.0±0.2	0.368	0.07 (-0.08 to 0.21)
Other	0.3±0.7	0.2±0.5	0.4±1.1	0.269	-0.18 (-0.50 to 0.14)
Processed culinary ingredients (NOVA group 2)					
Total	6.2±4.7	6.4±4.8	5.6±3.9	0.314	0.75 (-0.72 to 2.22)
Plant oils	3.8±3.4	3.8±3.4	3.9±3.2	0.873	-0.09 (-1.15 to 0.98)
Animal fats	0.2±0.6	0.2±0.6	0.1±0.4	0.691	0.04 (-0.14 to 0.21)
Sugar	2.2±3.5	2.4±3.8	1.6±2.3	0.158	0.80 (-0.31 to 1.91)
Processed foods (NOVA group 3)					
Total	28.1±12.8	27.9±12.5	28.5±13.9	0.775	-0.59 (-4.63 to 3.46)
Cheese	0.2±0.8	0.2±0.8	0.2±0.8	0.750	-0.04 (-0.29 to 0.21)
Canned, smoked, or cured meats and fish	0.4±1.1	0.4±1.1	0.4±0.8	0.660	0.07 (-0.25 to 0.41)
Canned fruits and vegetables	0.0±0.0	0.0±0.0	0.0±0.0	0.599	0.00 (-0.009 to 0.01)
Rice-based dishes	19.6±11.6	19.6±11.6	19.4±11.8	0.891	0.25 (-3.41 to 3.91)
Meats, poultry, fish, and eggs-based dishes	7.7±6.1	7.5±6.0	8.5±6.6	0.328	-0.96 (-2.88 to 0.97)
Other	0.3±1.0	0.3±1.1	0.2±0.8	0.627	0.09 (-0.24 to 0.40)
Ultra-processed foods (NOVA group 4)					
Total	31.8±14.2	31.4±14.5	33.3±12.9	0.397	-1.92 (-6.39 to 2.54)
Industrial grain foods	5.5±6.1	5.4±6.0	6.0±6.5	0.522	-0.63 (-2.54 to 1.30)
Ready-to-heat and -eat mixed dishes	6.8±7.4	6.8±7.7	6.8±6.4	0.992	-0.01 (-2.34 to 2.32)
Sweet snacks and sweets	2.9±5.4	2.7±5.0	4.0±6.5	0.129	-1.30 (-2.99 to 0.38)
Savoury snacks	2.6±5.4	2.3±4.8	3.8±7.2	0.174	-1.49 (-3.64 to 0.67)
Fast food or reconstituted meat, poultry, or fish	3.0±4.7	3.0±4.9	3.1±4.0	0.938	-0.06 (-1.56 to 1.44)
Sugar-sweetened beverages	5.7±4.6	5.8±4.8	5.3±3.8	0.456	0.55 (-0.90 to 2.00)
Processed fats or oils, condiments, and sauces	2.6±3.2	2.8±3.4	1.7±1.8	0.024*	1.15 (0.15 to 2.15)
Flavoured dairy foods and substitutes	0.7±2.0	0.7±2.1	0.5±1.2	0.438	0.24 (-0.37 to 0.86)
Fast food or prepared potato products	1.9±3.4	1.8±3.4	1.7±1.8	0.518	-0.35 (-1.43 to 0.73)
Other	0.1±0.5	0.1±0.4	0.1±0.6	0.751	-0.02 (-0.17 to 0.12)

SD: standard deviation; B40: less than Ringgit Malaysia (RM)4850; non B40: RM4850 and above
*$p<0.05$ tested with independent *t*-test

Table 4. Dietary intakes of children compared with the National Academy of Medicine's estimated average requirement (EAR) and Malaysian recommended nutrient intake (RNI) by household income

			Intakes, mean±SD		*% of USA NAM EAR, mean±SD*		*% of Malaysian RNI, mean±SD*		*No. Children < USA NAM, n (%)*		*No. Children < Malaysian RNI, n (%)*	
Nutrients	*USA NAM EAR*	*Malaysian RNI*	*B40 (n=180)*	*Non- B40 (n=50)*	*B40 (n=180)*	*Non- B40 (n=50)*	*B40 (n=180)*	*Non- B40 (n=50)*	*B40 (n=180)*	*Non- B40 (n=50)*	*B40 (n=180)*	*Non- B40 (n=50)*
Energy (kcal)	NA	1607	1421±376	1403±360	NA	NA	92.1±24.0	91.4±24.4	NA	NA	103 (57.2)	31 (62.0)
Carbohydrates (g)	100	NA	173.6±53.2	170.8±49.9	174.8±54.2	166.8±45.3	NA	NA	10 (5.5)	3 (6.0)	NA	NA
Protein (g)	0.76[b]	32.5	59.4±18.0	59.7±17.0	233.5±69.9	228.1±68.1	223.5±75.6	225.0±79.9	1 (0.6)	1 (2.0)	1 (0.6)*	3 (6.0)*
Fats (g)	NA	NA	54.8±16.6	53.3±15.7	NA	NA	NA	NA	NA	NA	NA	NA
Sodium (mg)	NA	1500	1999±709	1971±647	NA	NA	141.3±54.0	130.9±45.7	NA	NA	35 (19.4)	15 (30.0)
Potassium (g)	NA	4500	840.0±310.2	806.2±268.2	NA	NA	19.3±7.3	18.1±6.2	NA	NA	180 (100.0)	50 (100.0)
Phosphorus (mg)	1055	1250	644±329[a]	690±304[a]	61.4±30.8[a]	62.7±31.5[a]	58.8±46.0[a,*]	55.3±24.9[a,*]	164 (91.1)	48 (96.0)	173 (96.1)	48 (96.0)
Calcium (mg)	1100	1200	345.0±182.7	328.7±146.2	31.9±16.7	28.2±12.4	30.5±17.4	28.3±13.9	180 (100.0)	50 (100.0)	179 (99.4)	50 (100.0)
Iron (mg)	5.8	12.7	11.3±5.6	11.6±5.4	225.1±173.0	220.3±174.6	96.4±66.0	94.5±55.0	10 (5.5)	6 (12.0)	89 (49.4)	25 (50.0)
Vitamin C (mg)	39	55	17.7±28.0[a]	21.1±47.0[a]	45.3±71.8[a]	54.0±77.1[a]	35.9±56.9[a]	37.0±49.8[a]	140 (77.8)	38 (76.0)	142 (78.9)	44 (88.0)
Vitamin D (μg)	10	15	1.5±1.6	1.2±1.1	15.0±16.5	12.2±10.7	10.1±11.0	8.0±7.3	180 (100.0)	50 (100.0)	180 (100.0)	50 (100.0)
Vitamin A (μg)	433	568	459.7±262.9	487.6±261.9	106.6±59.8	111.0±63.7	83.4±48.1	89.2±51.6	99 (55.0)	27 (54.0)	129 (71.7)	34 (68.0)
Thiamine (mg)	0.7	1.1	0.6±0.3	0.6±0.2	85.9±41.5	81.2±32.5	58.7±30.0	55.3±22.3	134 (74.4)	40 (80.0)	157 (87.2)	47 (94.0)
Riboflavin (mg)	0.8	1.1	0.7±0.5[a]	0.8±0.5[a]	83.8±60.7[a]	95.9±76.7[a]	65.6±48.8[a]	74.6±45.5[a]	115 (63.9)	26 (52.0)	138 (76.7)	37 (74.0)
Niacin (mg)	9.0	14.7	6.0±4.4[a]	6.3±4.2[a]	66.4±48.4[a]	70.8±46.9[a]	41.7±36.6[a]	44.1±26.7[a]	139 (77.2)	38 (76.0)	170 (94.4)	49 (98.0)

USA NAM: United States of America's National Academy of Medicine; EAR: estimated average requirement; RNI: recommended nutrient intake; SD: standard deviation; NA: not available; B40: less than RM4850; Non-B40: RM4850 and above[j]

[a] expressed as median (inter-quartile range) tested with Mann-Whitney U test

[b] expressed as g/kg/day (calculated by mean weight; *p<0.05

than 30% of overweight and obesity in this population.

At present, evidence on the prevalence of stunting is mixed within studies from Malaysia depending on the location and age group of comparison (Chong *et al.*, 2016). Stunting is an indication of chronic malnutrition reflecting the cumulative effects of undernutrition and infections, and is associated with delayed mental development and reduced intellectual capacity (WHO, 2006). On the other hand, results on body weight categories were consistent with local studies (Chong *et al.*, 2016; Yang *et al.*, 2017) and similar to those reported from high-income countries globally (González-Álvarez *et al.*, 2020). Childhood obesity and stunting are major risk factors of poor health and early death due to chronic diseases (WHO, 2006). In the context of the co-existence of the triple burden of malnutrition and from the perspective of meeting nutrient needs, the available evidence suggests that the entire children population could benefit from positive changes in dietary intakes.

The percentages of energy from ultra-processed foods found in this study (about 32%) is consistent with the literature for children; much lower than the US and United Kingdom (>50%), but higher than Italy (about 10%) (Monteiro *et al.*, 2019; Estell *et al.*, 2021). Specifically, the dietary share of ultra-processed foods was associated with a deranged nutrient profile including excessive consumption of sodium and a lower intake of calcium, potassium, phosphorus, vitamins C and D, thiamine, riboflavin, and niacin. The observed effects between the intake of ultra-processed foods and dietary intakes are in agreement with the evidence available since the concept of ultra-processed foods was first introduced in 2009 (Monteiro *et al.*, 2019). A recent systematic review of worldwide consumption of ultra-processed foods concluded high variability in the intake, with young people, men, and overweight/obese generally having higher levels of consumption (Marino *et al.*, 2021). Taken together, the results of relatively high percentages of ultra-processed food intake and its dietary share within the children's dietary intake may suggest the potential of increased non-communicable disease risk development amongst children to be further explored in future studies (WHO, 2014; Magnusson, 2010).

In-depth analyses using the NOVA classification system highlighted the contribution of processed grains to the pre-adolescents' dietary intakes irrespective of income status in this study. A NOVA system states that ultra-processed foods contain additives that automatically include the majority of processed grains such as fortified ready-to-eat breakfast cereal or whole wheat bread (Monteiro *et al.*, 2019). Hence, healthful nutrients or other beneficial elements like whole grains and dietary fibre are not represented in a person's diet. A secondary analysis using dietary modelling on grain foods consumed in a national nutrition survey discovered the potential for altered nutrient intakes (thiamine, folate, iron, and iodine) while avoiding ultra-processed foods and replacing them with minimally processed foods (Estell *et al.*, 2021). This emerging evidence predicts similar outcomes in other countries with fortification programmes and suggests that the NOVA system should be used cautiously (Estell *et al.*, 2021).

The differences in consumption of processed fats or oils, condiments, and sauces within the ultra-processed food group among children from different incomes in this study reflected the global pattern in the nutrition transition stage. Countries in Latin America have demonstrated that those from the highest income group consumed more ultra-processed foods (Marrón-Ponce *et*

al., 2018), while in developed countries, such as the United Kingdom, France, and United States, the opposite trend was observed (Monteiro *et al.*, 2019; Estell *et al.*, 2021). Although ultra-processed foods are rapidly growing in middle-income countries (Baker & Friel, 2016), these foods are considerably more expensive than fresh or minimally processed foods, hence higher-income households with higher purchasing ability are able to afford these types of foods, whereas, this might not be the case in high-income countries (Moubarac *et al.*, 2013). At present, Malaysia is in the upper middle-income group, modelling similar situation as high-income countries that warrants further attention (Department of Statistics, 2020).

Most children in this study had fewer than the recommended five servings of fruits and vegetables, coupled with low vitamin C and potassium intakes. Findings from nutrition surveys in Malaysia provided little evidence to conclude that socioeconomic status was associated with children's fruits and vegetables consumption (Shariff *et al.*, 2015; Yang *et al.*, 2017). Despite not meeting the national recommendations for fruits and vegetables, the common sources of fruits were watermelon as it was sold in the school canteen, while non-green leafy vegetables were more popular than green leafy vegetables. The poor intake of fruits and vegetables among children might be a result of a combination of factors such as taste, habit, social influences, and availability. Research has shown differential sensitivity to prices of fruits and vegetables across income, suggesting that changing food prices may be a viable strategy for influencing dietary quality (Beydoun & Wang, 2008).

This is one of the few studies in Malaysia to characterise dietary intakes, including the NOVA classification system, among children living in urban Kuala Lumpur. The strength of this study is the comprehensive method of dietary data collection with verification by mothers and weighing of foods sold in school canteens to obtain a more accurate assessment of dietary intakes. Nevertheless, the 7-day diet history has its limitations on participants' bias including recording and recalling. The study, however, collected data from predominantly Malay children from low-income households, which could limit the generalisability of the results. Data on specific children receiving the supplementary food programme (*Rancangan Makanan Tambahan*) at school is lacking; hence, we were unable to differentiate the source of foods to understand the ability of the governmental programme in promoting diet quality amongst low-income households. If the differences in diet quality were a result of the foods served either at school or home, then specific strategies can be directed to increase nutrition awareness and improve the nutritional intake of children. The application of the NOVA classification system itself has limitations as it may not truly consider the nutritional values of commercially prepared foods.

CONCLUSION

Overall, children from the PREBONE-Kids study residing in urban Kuala Lumpur were experiencing a double burden of both malnutrition and overweight with diets comprised of less nutritious foods. Most children did not meet the national and international dietary recommendations, regardless of their household income, indicating the need for continued efforts to improve the dietary patterns of this population as a whole. Without a comprehensive approach, individual- and environmental-level initiatives that aim to improve dietary patterns

and reduce the risk of obesity or other micronutrient deficiencies diet-related chronic diseases, may not be effective but could potentially exacerbate disparities.

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Authors' contributions

Yang WY, conceptualised the manuscript, contributed to the investigation, conducted the formal analysis and data curation, and prepared the original draft of the manuscript; Wong SY, contributed to the investigation, conducted the formal analysis and data curation, and reviewed and edited the manuscript; Ong SH, Arasu K, and Chang, contributed to the investigation and data curation, reviewed and edited the manuscript; Chong MHZ, Meenal M, and Khoo EJ, contributed to the investigation and reviewed and edited the manuscript; Weaver CM, conceptualised, reviewed and edited the manuscript; Chee WSS, principal investigator, contributed to the methodology, supervision, project administration, funding acquisition, conceptualised the manuscript, and contributed to the review and editing of the manuscript. All authors read and approved the final version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest. The sponsoring body had no role in the study design, implementation, outcome, and publication of the study.

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Examining the relationship between fast-food and sit-down restaurant consumption frequency and dietary patterns among adults in Riyadh City, Saudi Arabia

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ABSTRACT

Introduction: This study assessed the association between dietary patterns and the frequency of eating at fast-food or sit-down restaurants. **Methods:** A cross-sectional study, with a sample of adults living in Riyadh, Saudi Arabia (SA), was conducted between January and March 2021. An online, self-administered survey was conducted using social media and WhatsApp. A total of 399 adults living in Riyadh were included; 40.4% were males, with a mean age of 35 and standard deviation (*SD*) of 12 years, and had no significant health problems. **Results:** Using principal component analysis to analyse 15 food groups based on the food items consumed, four dietary patterns were extracted: Western, unhealthy, plant-based, and healthy. After adjusting for potential covariates, a higher frequency of eating at fast-food restaurants was significantly associated with unhealthy dietary patterns [odds ratio (OR) = 4.85, 95.0% confidence interval (CI) = 1.80, 12.59]. In contrast, participants in the highest tercile of the frequency of eating at fast-food restaurants showed lower odds of adhering to a healthy dietary pattern (OR = 0.29, 95.0% CI = 0.11, 0.71). No significant association was found between participants in the highest tercile of the frequency of eating at sit-down restaurants and their dietary patterns. **Conclusion:** Frequently eating at fast-food restaurants was associated with increased odds of adherence to less healthy dietary patterns and negatively associated with adherence to a healthy pattern. No association was found between sit-down restaurants and any of the identified dietary patterns. The present findings can assist stakeholders and policymakers in developing strategic plans and policies for targeted interventions for this population.

Keywords: dietary pattern, fast-food restaurants, principal component analysis, Saudi Arabia, sit-down restaurants

INTRODUCTION

In Saudi Arabia (SA), the eating behaviours and lifestyle habits of the general population have dramatically shifted over the past few decades, mostly towards unhealthy food habits, contributing to a considerable increase in the eating of fast-food items

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(Naeem, 2012). Furthermore, fast-food manufacturers and marketers have aggressively extended their services nationwide (Benajiba, 2016). In 2014, there were 15,782 restaurants nationwide providing various food services (General Authority for Statistics, 2014), including sit-down and fast-food restaurants. However, the latter is widely known to produce unhealthy and quick, easy-to-make foods (Naeem, 2012).

Fast-food restaurants are characterised by inexpensive, energy-dense foods with high amounts of saturated fat (Benajiba, 2016). In contrast, sit-down restaurants provide food services within their establishments and have a variety of dishes (Bouznif *et al.,* 2018). Although these two types of restaurants show clear distinctions, neither consistently offers healthy food to their customers. Notwithstanding, research shows that sit-down restaurants typically have a higher proportion of healthy dishes to offer compared with fast-food restaurants, even if the availability and variety of these healthier options differ by restaurants (Saelens *et al.,* 2007).

The frequency of eating out is correlated with an increase in total calorie and saturated fat intakes in daily diet, unhealthy dietary practices, and decreased intake of micronutrients (Lachat *et al.,* 2012). Furthermore, research shows that eating out one meal contributes an additional 134 kcal to a subject's daily energy intake (Todd, Mancino & Lin 2010). Eating out is also associated with an increase of 0.6–0.8 kg/m^2 in body mass index (BMI) (Bhutani *et al.,* 2018). A study conducted at the national level revealed a high prevalence of obesity (BMI ≥ 30 kg/m^2) in SA, with rates ranging between 24.7% and 46.9%; whereby dietary patterns and eating habits were among the main factors causing obesity (Althumiri *et al.,* 2021).

Interestingly, investigating dietary patterns allows for an examination of the overall diet of a group, while considering the interactive and cumulative effects of dietary components, and acknowledging the complexity of the human diet. This is important because the nutrients and dietary components consumed by humans are intricately combined, correlated, and difficult to examine in isolation (Zhang, Tapera & Gou 2018). Currently, we have a plethora of scientific data backing up that lifestyle greatly influences dietary patterns (Beck *et al.,* 2018; Krieger *et al.,* 2018). However, the influence of frequency of eating out on dietary patterns has yet to be determined and remains in the field of speculation. Therefore, identifying factors contributing to specific dietary patterns seems crucial for enabling public health authorities to develop and implement well-informed nutrition strategies, as well as disease prevention and control policies.

Riyadh City is the capital of SA with a population density of ~ 5 million Saudis and a total number of 3,245 restaurants. Riyadh faces rapid development, with increased markets and cuisine types including fast-food and sit-down restaurants. The relationship between eating at fast-food restaurants, dietary patterns, and body weight is well-reported (Poti, 2014). However, information on how other restaurant types, such as sit-down restaurants, contribute to changes in dietary patterns and body weight is lacking.

Therefore, we aimed to examine the association between the frequency of eating at fast-food restaurants and dietary patterns, as well as between the frequency of eating at sit-down restaurants and dietary patterns in adults living in Riyadh, SA. The hypothesis was that frequent eating at fast-food restaurants is strongly associated with unhealthy eating patterns compared

with eating at sit-down restaurants. As healthy and unhealthy options are available at sit-down restaurants, consumers can choose healthier options from the menu.

MATERIALS AND METHODS

Study design

A cross-sectional study, with a sample of the adult population living in Riyadh, SA, was conducted between January and March 2021. The study protocol was approved by the Institutional Review Board of Princess Nourah bint Abdulrahman University (IRB no. H-01-R-059, dated 17/01/2021). All participants, before participating in the study, provided informed consent by clicking the 'I agree' button that was presented before starting the online questionnaire. It was explained, on the first page of the questionnaire, that the participants had the right to withdraw from the study at any time and that they had no obligations to participate in this study.

Participants

The inclusion criteria were male and female adults aged 18 years and above living in Riyadh, SA. The exclusion criterion was dietary restriction owing to any health conditions that may affect food choices (e.g., coeliac disease, food allergies, diabetes, heart diseases, and renal problems). Sample size was calculated based on the sample size equation using the total population of 5 million, , where Z for 95% confidence level=1.96, p= prevalence of factor under study, estimated to be 50%, q=1-p =50%, and d= margin of error=0.05% (Daniel, 1995). Therefore, we estimated a minimum study sample of 384 Saudi citizens. A total of 399 participants enrolled in this study. Convenience sampling was used for recruitment.

Study tools

Data were collected using an online self-administered survey. The research team posted the link on social media and sent it via WhatsApp to their contacts and encouraged people to disseminate it to others. The online survey included items regarding the following topics.

Sociodemographic information

These included age, sex, nationality, marital status, monthly household income, education level, and occupation.

Frequency of eating at fast-food and at sit-down restaurants

Fast-food restaurant examples were those that served Shawarma, bakery items, chicken nuggets, burgers, or chips; they could be found at local takeaway food places, canteens, cafeterias, or fast-food franchises, such as McDonald's, Hardee's, IHOP, Domino's Pizza, and others. Sit-down restaurants were defined as those that served full menus with various food choices, including breakfast menus, soups, salads, hot and cold appetisers, main courses, desserts, juices, and drinks (e.g., Lebanese, Italian, Chinese, and mixed international restaurants).

The response options for frequency of eating at each of these two types of restaurants were: never, 1–3 times per month, 1–2 times per week, 3–4 times per week, and five times or more per week. We used a continuous variable that represented the overall frequency of eating at fast-food and sit-down restaurants by adding breakfast, lunch, and dinner responses to assess participants' overall frequency of eating according to restaurant type. The overall frequency of eating at each restaurant type was categorised into terciles, with the lowest number of visits used as the reference.

The eating habits assessment tool

The tool used for eating habits over the past few months was adapted from a scale used by Paxton *et al.* (2011). Nonetheless, we added some questions to this tool to ensure that our tool covered all food groups based on the latest US Department of Agriculture and US Department of Health and Human Services dietary guidelines for Americans, 2020-2025.

Accordingly, the adapted eating habits tool included questions on the frequency of eating fruits, vegetables, dairy, whole and refined grains, poultry, chicken and eggs, red and processed meat, fish, beans, nuts, seeds, and soy products, fried and junk food, sweetened beverages, dessert and sweets, and butter and margarine. There were three different frequency options per day or week, depending on the food group and their relevant dietary recommendations. For example, the options for the frequency of consumption for fruit and vegetable groups were as follows: two or less per day, 3–4 per day, and five or more per day; whereas for meat groups, the options were as follows: five or more per week, 3–4 per week, and 0–2 per week.

The questionnaire was first developed in English. The authors then translated it into Arabic. The translated version was reviewed by three nutrition professional experts using a scoring sheet to determine the accuracy of the actual translation and its content. The experts were asked to score each question and add comments if necessary. All comments given by the experts were discussed and considered. The final translated questionnaire was tested for readability on 15 adults that were representative of the target study population. Our sampling process was conducted to ensure that these participants varied in their educational and socioeconomic characteristics. Participants were interviewed about the questionnaire layout, information, length, ease and time for completion, and any difficulties in understanding the questions. They were given a chance to ask any questions and raise queries. This step revealed the need to further clarify the definition of each restaurant type by giving examples and increasing the comprehensibility of the questionnaire by modifying the language.

Food component derivation

Principal component analysis (PCA) was used to identify dietary patterns that specifically reflected the food items consumed. Patterns were extracted based on the 15 food groups entered into the PCA. The Kaiser-Meyer-Olkin (KMO) measurement of adequacy and Bartlett's test of sphericity were performed. For Bartlett's test of sphericity, a $p<0.05$ showed statistical significance and a KMO value of >0.6 indicated that the data were suitable for factor analysis. An orthogonal rotation using the varimax option of the retained components was then obtained to interpret the dietary patterns following the extraction of the components. The number of principal components using Kaiser's criterion with eigenvalues >1, the Eigen plot (scree plot), and the interpretability of the principal components were identified.

In our data, the KMO and Bartlett's tests were 0.67 and $\chi^2=690.92$, $p<0.001$, respectively. This meant that the correlation among the variables was sufficiently strong, and homogeneity in the variance for the intake of different food groups was observed. When identifying the major dietary patterns and reducing bias induced by multiple testing, we extracted only the factor components with eigenvalues >1. Based on the principal component interpretability and scree plots (Figure 1), the established cut-off point showed an obvious break after the fourth factor, with an eigenvalue >1.

Food groups with factor loadings ≥0.3 were accepted as crucial contributors to the component. In contrast, those with factor loadings <0.3 were ignored because they corresponded only to small displacements in the direction of the variables they multiplied. Furthermore, the dietary patterns were named according to those with factor loadings ≥0.3.

Covariates

Self-reported demographic characteristics were sex (male or female), nationality (Saudi or non-Saudi), marital status (married or not married), education level (less than bachelor's degree or bachelor's degree or above), and monthly household income (Saudi Riyals [SR]) (≤ SR 5,000, SR 6,000–10,000, or ≥ SR 11,000).

Data analysis

All data were analysed using the IBM SPSS Statistics for Windows version 26.0 (IBM Corp., Armonk, New York, USA). We conducted a descriptive analysis of the participants' sociodemographic characteristics. Continuous and categorical data were presented as mean and standard deviation (*SD*), and numbers and percentages (%), respectively. The factor scores for each dietary pattern were divided into quartiles (the first quartile represented low adherence to the pattern and the fourth quartile represented high adherence to the pattern), and the distribution of sociodemographic characteristics across these quartiles was explored. Furthermore, to compare the differences in the participants' sociodemographic

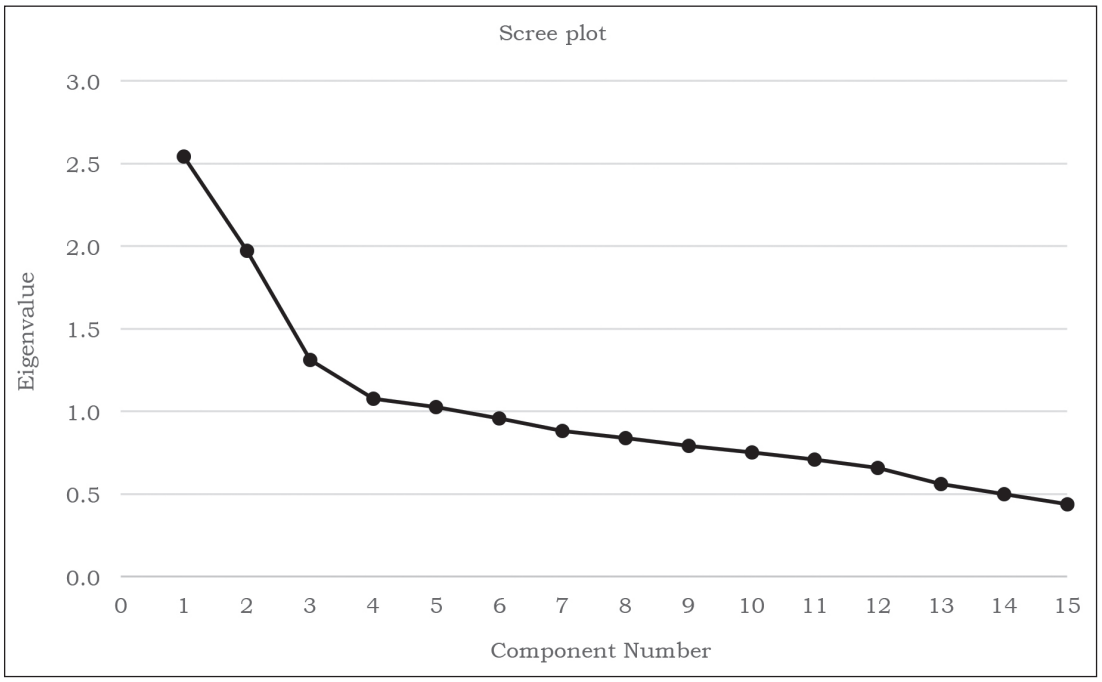

Figure 1. Scree plot for identification of dietary patterns (components) by principal component analysis. Food intakes were aggregated into 15 food groups and used as input variables. Factors considered appropriate for the pattern shown in Table 2 were the four factors with eigenvalues >1.00

Table 1. Demographic characteristics of the studied participants (*N*=399)

Characteristics	*mean±SD*	*n*	*%*
Age (years)	35±12		
Sex			
Female		238	59.6
Male		161	40.4
Marital status			
Married		218	54.6
Not married		181	45.4
Nationality			
Saudi		381	95.5
Non-Saudi		18	4.5
Education			
Less than bachelor's degree		85	21.3
Bachelor's degree or above		314	78.7
Occupation			
Governmental sector		112	28.1
Private sector		83	20.8
Business		9	2.3
Retired		24	6.0
Student		91	22.8
Housewife/Do not work		80	20.1
Household income (SR)†			
≤ 5000		169	42.4
6000 – 10,000		84	21.1
≥ 11,000		146	36.6
Fast-food restaurant frequency, by tercile			
Low tercile		189	47.4
Mid tercile		172	43.1
High tercile		38	9.5
Sit-down restaurant frequency, by tercile			
Low tercile		322	80.7
Mid tercile		71	17.8
High tercile		6	1.5

†SR: Saudi Riyal; 1 US dollar = SR 3.75 (as of May 28, 2023)
Frequency of restaurants by tercile: low tercile (less than 7), mid tercile (7–11), and high tercile (12–15) times per month

characteristics across these quartiles, a chi-square test for categorical data and a test of variance (ANOVA) for continuous data were performed.

Additionally, to evaluate the associations between the frequency of eating at restaurants (independent variable) and dietary patterns (dependent variable), the odds ratios and 95.0% confidence intervals (CIs) were computed using a multiple logistic regression test. Based on the scores for dietary patterns, we generated dichotomous dependent variables for each dietary pattern, representing either low or high adherence to that pattern. In Model 1, the relationships between dietary patterns and eating at fast-food restaurants, and between dietary patterns and eating at sit-down restaurants were examined.

Table 2. Factor loading matrices for the four dietary patterns among the studied participants

Food groups	*Dietary patterns*			
	Western pattern	*Unhealthy pattern*	*Plant-based pattern*	*Healthy pattern*
Fresh or dried fruits	-0.168	-0.026	**0.830**	-0.157
Fresh or cooked vegetables	-0.222	-0.048	**0.527**	**0.343**
Dairy products	-0.088	0.114	0.221	0.030
Refined grains	0.013	-0.286	0.076	-0.055
Whole grains	0.230	**0.307**	**0.470**	-0.179
Sweetened beverages	**0.621**	0.164	0.238	0.206
Poultry meat and chicken	**0.554**	0.101	-0.037	-0.088
Red meat	**0.642**	0.078	-0.118	-0.129
Processed meat	**0.683**	0.050	-0.201	-0.227
Fish meat	-0.168	-0.054	0.163	**0.756**
Beans	-0.109	0.026	-0.080	**0.687**
Nuts, seeds, and soy products	0.109	-0.149	**0.400**	**0.366**
Fried food and pre-packaged snacks (high in salt/fat)	0.221	**0.756**	0.057	-0.121
Desserts, sweet baked goods, and candy (not low-fat)	-0.071	**0.810**	-0.122	0.121
Butter and margarine	0.208	**0.518**	0.090	-0.085
% variance explained per pattern	12.2	11.7	10.2	10.1

Numbers in bold represent food groups strongly loaded in the factor component
Factor loadings ≥|0.30| were considered significant
The total variance for the four dietary patterns is 44.2%

In Model 2, the following covariates: age, sex, marital status, education, and income were adjusted. A *p*-value <0.05 was considered statistically significant.

RESULTS

Participants' sociodemographic and basic information

The demographic characteristics of the 399 participants are shown in Table 1. Mean age was 35±12 years, 59.6% were females, 54.6% were married, 95.5% were Saudis, 42.4% earned < SR 5,000 (~USD 1333), and just over three-quarters (78.7%) had a bachelor's degree or higher. Almost half worked in the government sector (28.1%) or the private sector (20.8%). On average, approximately 50.0% of the participants reported eating at fast-food restaurants 7–15 times per month, while only 19.3% ate at sit-down restaurants 7–15 times per month.

Identification of dietary patterns

Table 2 presents the factor loading matrices of the dietary patterns. PCA with the 15 food groups revealed four major dietary patterns, which explained 12.2%, 11.7%, 10.2%, and 10.1% of the variances in dietary intake, respectively. The four factors accounted for 44.2% of the total variance in dietary intake.

The four components were labelled based on the food items that showed high factor loadings. First, the Western pattern, which was characterised by high intakes of sweetened beverages,

Table 3. Distribution of sociodemographic characteristics across highest and lowest quartiles of dietary patterns scores

Variable	*Western pattern*			*Unhealthy pattern*			*Plant-based pattern*		*Healthy pattern*		
	Q 1	*Q 4*		*Q 1*	*Q 4*		*Q 1*	*Q 4*	*Q 1*	*Q 4*	
Age in years (mean±*SD*)	39±11	29±9	***	35±11	32±12	*	34±11	35±22	31±11	39±12	***
Sex, *n* (%)											
Female	70 (73.3)	41 (41.4)	***	67 (67.0)	42 (42.4)	***	69 (69.0)	50 (51.0)	59 (59.0)	54 (54.5)	
Male	25 (26.3)	58 (58.6)		33 (33.0)	57 (57.6)		31 (31.0)	48 (49.0)	41 (41.0)	45 (45.5)	
Marital status, *n* (%)											
Married	67 (70.5)	36 (36.4)	***	61 (61.0)	38 (38.4)	**	54 (54.0)	50 (50.0)	41 (41.0)	67 (67.7)	**
Not Married	28 (29.5)	63 (63.6)		39 (39.0)	61 (61.6)		46 (46.0)	48 (49.0)	59 (59.0)	32 (32.3)	
Nationality, *n* (%)											
Saudi	91 (95.8)	96 (96.0)		95 (95.0)	95 (96.0)		96 (96.0)	94 (95.9)	95 (95.0)	93 (93.9)	
Non-Saudi	4 (4.2)	3 (3.0)		5 (5.0)	4 (4.0)		4 (4.0)	4 (4.1)	5 (5.0)	6 (6.1)	
Education, *n* (%)											
Less than bachelor's degree	19 (20.0)	20 (20.2)		25 (25.0)	18 (18.2)		17 (17.0)	21 (21.4)	19 (19.0)	20 (20.0)	**
Bachelor's degree and above	76 (80.0)	79 (79.8)		75 (75.0)	81 (81.8)		83 (83.0)	74 (74.7)	81 (81.0)	79 (79.8)	
Income level (SR), *n* (%)											
≤ 5000	29 (30.5)	50 (50.5)	**	33 (33.0)	52 (52.4)		42 (42.0)	44 (44.9)	56 (56.0)	36 (36.4)	**
6000 – 10,000	22 (23.2)	22 (22.2)		24 (24.0)	16 (16.2)		20 (20.0)	21 (21.4)	22 (22.0)	19 (19.2)	
≥ 11,000	44 (30.3)	27 (27.3)		43 (43.0)	31 (31.3)		38 (38.0)	33 (33.7)	22 (22.0)	44 (44.4)	

SR: Saudi Riyal; 1 US dollar = SR 3.75 (as of May 28, 2023)
Categorical variables *n* (%), number and percentages, and continuous variables are represented as mean±*SD*; *p*-values for continuous variables (Analysis of variance) and categorical variables (chi-square test); *$p<0.05$, **$p<0.01$, ***$p<0.001$

Table 4. Associations between frequency of restaurant eating occasions and the major dietary pattern scores

	Western pattern		Unhealthy pattern		Plant-based pattern		Healthy pattern	
	Model 1 OR [CI]	*Model 2 OR [CI]*	*Model 1 OR [CI]*	*Model 2 OR [CI]*	*Model 1 OR [CI]*	*Model 2 OR [CI]*	*Model 1 OR [CI]*	*Model 2 OR [CI]*
Fast-food restaurant								
Low tercile	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Mid tercile	2.36***	1.21	1.99***	1.91**	0.68	0.65	0.46***	0.52**
	[1.54, 3.60]	[0.73, 2.00]	[1.30, 3.04]	[1.17, 3.13]	[0.45, 1.04]	[0.39, 1.05]	[0.30, 0.71]	[0.32, 0.86]
High tercile	6.37***	2.20	3.34**	4.85***	1.97	1.88	0.25***	0.29**
	[2.76, 14.67]	[0.84, 5.80]	[1.15, 7.04]	[1.80, 12.59]	[0.93, 4.13]	[0.78, 4.52]	[0.11, 0.54]	[0.11, 0.71]
Sit-down restaurant								
Low tercile	Ref	Ref	Ref	Ref	Ref	Ref	Ref	Ref
Mid tercile	1.47	0.53	1.08	0.80	1.09	2.00	0.67	0.65
	[0.87, 2.47]	[0.75, 3.83]	[0.65, 1.82]	[0.46, 1.40]	[0.21, 5.48]	[0.32, 12.23]	[0.40, 1.14]	[0.11, 3.83]
High tercile	2.15	0.62	0.00	0.00	0.65	1.08	0.92	0.51
	[0.38, 11.93]	[0.08, 4.54]	[0.00]	[0.00]	[0.12, 3.45]	[0.17, 6.68]	[0.18, 4.66]	[0.08, 3.05]
Age		0.95**		1.01		0.99		1.02
		[0.93, 0.98]		[0.98, 1.03]		[0.97, 1.02]		[0.99, 1.04]
Sex								
Female		0.35***		0.78		0.74		0.71
		[0.21, 0.58]		[0.48, 1.24]		[0.46, 1.17]		[0.44, 1.15]
Education		1.09		1.15		0.70		1.17
Bachelor's degree or above		[0.62, 1.90]		[0.68, 1.96]		[0.42, 1.17]		[0.69, 1.99]
Marital status								
Married		1.34		0.75		0.89		0.95
		[0.71, 2.49]		[0.41, 1.33]		[0.49, 1.59]		[0.52, 1.72]
Income level (SR)								
6000 – 10,000		0.76		0.58		1.18		0.69
		[0.42, 1.37]		[0.32, 1.03]		[0.67, 2.06]		[0.38, 1.23]
More than 10,000		0.53*		0.81		1.27		1.31
		[0.29, 0.97]		[0.46, 1.42]		[0.72, 2.21]		[0.74, 2.30]

OR: odds ratio; CI, 95.0%: confidence interval; Ref: reference; SR: Saudi Riyal; 1 US dollar = SR 3.75 (as of May 28, 2023)
Model 1 the association between fast-food and sit-down restaurant eating frequency with dietary patterns extracted by PCA; Model 2, estimation of models after adjustment for sociodemographic factors
*p<0.05, **p<0.01, ***p<0.001

as well as poultry, chicken, red, and processed meats. Second, the unhealthy pattern, characterised by high intakes of fried food, pre-packaged snacks (high in salt/fat), desserts, sweet baked goods, candy (not low in fat), butter, margarine, and whole grains. Third, the plant-based pattern, which was characterised by a high intake of fresh or dried fruits, fresh or cooked vegetables, whole grains, nuts, seeds, and soy products. Fourth was the healthy pattern, characterised by a high intake of fresh or cooked vegetables, fish meat, beans, nuts, seeds, and soy products.

Dietary patterns and sociodemographic characteristics

The distribution of the participants' general characteristics across quartiles (Q1–Q4) for the four dietary patterns is presented in Table 3. Regarding the Western pattern, compared with participants in the lowest quartile, those in the highest quartile were more likely to be younger, male, not married, and have a lower income level. Regarding the unhealthy pattern, those in the highest quartile were more likely to be younger, male, and not married. The healthy pattern was significantly associated with older participants who had a bachelor's degree or higher, had higher income levels, and were married. Regarding the plant-based pattern, we found no significant associations with any sociodemographic characteristics across the quartiles.

The association between dietary patterns and frequency of eating at either fast-food or sit-down restaurants

Results of the multiple logistic regression analysis for the two models are shown in Table 4. In Model 1, regarding the frequency of eating at fast-food restaurants, participants in the highest tercile were associated with higher odds of adherence to the Western pattern (*OR*=6.37, 95.0% *CI*=2.76, 14.67) and unhealthy pattern (*OR*=3.34, 95.0% *CI*=1.15, 7.04). Moreover, they were significantly associated with lower odds of adherence to the healthy pattern (*OR*=0.25, 95.0% *CI*=0.11, 0.54) compared with those in the lowest tercile. No statistically significant associations were found between participants in the highest tercile of eating at sit-down restaurants and dietary patterns.

In Model 2, after adjusting for potential covariates, the association between the frequency of eating at fast-food restaurants and the Western pattern was attenuated; however, this association remained significant for the unhealthy pattern (*OR*=4.85, 95.0% *CI*=1.80, 12.59) and healthy pattern (*OR*=0.29, 95.0% *CI*=0.11, 0.71).

DISCUSSION

The findings of this study suggested that a higher frequency of eating at fast-food restaurants was positively associated with unhealthy dietary patterns. Another study that discussed several factors associated with unhealthy eating habits among Saudis found that a lack of knowledge about healthy diets, lack of enjoyment, and lack of family support in healthy food preparation were the main contributing factors to unhealthy eating; while inaccessibility and inability to buy healthy foods were the most common barriers to healthy eating (AlQuaiz *et al.,* 2021).

Comparing the frequency of eating at the two types of restaurants, fast-food restaurants were higher than sit-down restaurants. Furthermore, four major dietary patterns were identified for our sample: Western, unhealthy, plant-based, and healthy patterns. The correlation coefficients between food groups of the extracted dietary patterns

justified the labelling of patterns. After adjusting for potential covariates, the findings indicated that those who frequently ate at fast-food restaurants were 4.8 times more likely to have an unhealthy dietary pattern. A previous study demonstrated that fast-food consumers with poor diets had dietary patterns characterised by a higher consumption of energy, fat, sodium, and sweetened beverages, and a lower consumption of fruits and vegetables (Powell & Nguyen, 2013).

After adjusting for potential covariates, no significant associations were observed between those who ate more frequently at sit-down restaurants and their dietary patterns. This could be explained by relatively fewer participants eating at sit-down restaurants than at fast-food restaurants. However, according to the Riyadh Chamber of Commerce and Industry, the proportion of fast-food restaurants in Riyadh is much higher (56%) than that of other restaurants (Bouznif *et al.,* 2018). Therefore, the availability of Western fast-food restaurant chains in Riyadh might explain the more frequent choice to visit fast-food restaurants compared with sit-down restaurants (Benajiba, 2016). Another explanation for the lack of a clear association between sit-down restaurants and the identified dietary patterns could be due to the nature of the various dishes served in sit-down restaurants, which include both healthy and unhealthy options.

We cannot rule out the possible interference of cravings for fast-food items either. Frequent intake of energy-dense foods significantly increases the cravings for high-fat foods and reduces the cravings for healthy options. In contrast, calorie-restricted diets reduce cravings for energy-dense foods, including sweets, fats, and starches, and increase cravings for fruits and vegetables (Anton *et al.,* 2012). Furthermore, research shows that hunger susceptibility and the dose-response effect of energy-dense foods could be possible mechanisms for the frequent intake of fast-food items compared to other foods (Gilhooly *et al.,* 2007).

Other research has examined the association between the frequency of eating out and dietary intake (Lachat *et al.,* 2012). The current study differed from previous research (Bhutani *et al.,* 2018) in that it identified different dietary patterns associated with eating at fast-food and sit-down restaurants. Research conducted on a healthy population showed that the high density of restaurants has contributed to poor quality diets. This, in turn, has been found to significantly affect metabolic functions, particularly blood pressure, in the studied population (Alsabieh *et al.,* 2019). Therefore, the results of the current study can be helpful in designing effective intervention programmes about nutrition and policies to promote healthier lifestyles. A community-based intervention review found that point-of-purchase advertising and increased availability of healthy choices were effective strategies for enhancing healthier dietary intake outside the home (Valdivia Espino *et al.,* 2015). Additionally, increasing the awareness of calorie labelling in menus could improve food choices in restaurants (Bawazeer *et al.,* 2021). Moreover, improving cooking skills has been reported to decrease eating out (Robson, Crosby & Stark, 2016), promote healthier eating behaviours (Tani, Fujiwara & Kondo, 2020), and improve diet quality and cooking frequency (Alpaugh *et al.,* 2020).

The current findings also highlighted the role of sociodemographic characteristics in dietary patterns and their association with frequent eating out. Specifically, a significant effect was found based on age, sex, education, marital status, and income level. This

supports the idea that dietary patterns vary according to sociodemographic factors (Beck *et al.,* 2018; Krieger *et al.,* 2018). Notably, in this study, sex was positively associated with less healthy eating habits; male adults had a higher tendency to have Western and unhealthy eating patterns, whereas female adults tended to adhere more to healthy eating patterns. A similar result has been observed at global levels (Muga *et al.,* 2017).

Differences in dietary patterns by age were observed, where younger individuals tended to have less healthy dietary patterns and older individuals were more adherent to healthier diets. The findings of the National Nutrition Survey *menuCH* corroborate age as a determinant of eating patterns (Krieger *et al.,* 2018). Furthermore, age is negatively correlated with the frequency of eating fast food, thereby, contributing to unhealthy food choices (Hidaka *et al.,* 2018). In our sample, we hypothesised that these age-related results could be partially attributed to the lower income levels of younger people. This hypothesis stemmed from one of our observations that the significant association between the Western pattern and a higher frequency of eating at fast-food restaurants (which have more accessible prices) was stronger among participants who had a monthly income of < USD 1333. Contrastingly, those with a monthly income of > USD 2933 showed greater adherence to healthy eating patterns. These patterns were inversely associated with a higher frequency of eating at fast-food restaurants in our sample.

In the current study, education may have mediated the relationship between income differences and dietary patterns. It has been shown that participants who had a bachelor's degree or higher were more conscious about health and had higher healthy pattern scores. Greater adherence to healthy dietary patterns can be attributed to the role of education in evoking higher health consciousness and its indirect impact on food purchase patterns. Specifically, education has often been associated with higher income (Beck *et al.,* 2018), thus affecting food purchase patterns. Research shows that lower income significantly contributes to people's decision to purchase less healthy foods, and vice versa (French *et al.,* 2019).

Strengths

This study was the first to investigate the association between dietary patterns and frequency of eating at either fast-food or sit-down restaurants in a sample of adults living in Riyadh city. Additionally, using data-driven approach was a strength of this study; the use of PCA in dietary pattern analysis has been proven to be consistently reproducible longitudinally and across different dietary assessment methods in different populations (Murakami *et al.,* 2019). Moreover, significant differences in sociodemographic characteristics regarding adherence to dietary patterns and the relationship between dietary patterns and frequency of eating at different types of restaurants were demonstrated. Furthermore, multiple advanced analyses were used to control for different potential covariates (i.e., age, sex, marital status, income, and education level).

Study limitations

Firstly, the cross-sectional design of this study and its data hindered our ability to establish longitudinal and causal inferences for the associations; thus, the results need to be verified in future prospective studies. Secondly, the data collected through an online self-report survey may be prone to recall or selection bias. However, online surveys are becoming a more popular research

method for assessing dietary patterns because they have the advantages of being low cost, easy to access, large population recruitment, and rapid data collection (Nayak, MSDP & Narayan, 2019). Thirdly, the convenience sampling technique used was vulnerable to selection bias; thus, the findings did not comprehensively reflect the target population. However, our population was clearly defined, and regular participation was guaranteed. Furthermore, the relatively small sample size may have affected the reliability of the results, thereby limiting their generalisability to different populations. Nevertheless, the sample size was calculated and the optimal number was achieved. Lastly, using PCA was subjected to researchers' decisions during the process. This involved subjective choices regarding appropriate rotation methods, factor loadings (correlation coefficients) to interpret the components, eigenvalues, quantity, and labelling of the identified patterns. These choices may lead to inconsistent results; however, PCA is a well-known method used in many nutritional epidemiological studies.

CONCLUSION

The results suggested that frequently eating fast food was strongly associated with increased adherence to less healthy dietary patterns and negatively associated with healthy dietary patterns. Furthermore, the frequency of eating at sit-down restaurants was not associated with any of the identified dietary patterns. Despite the relatively limited sample size, the current study offered valuable insights into the relationship between the frequency of eating out and dietary patterns in an understudied population (the Saudi population in Riyadh) experiencing rapid lifestyle changes. The study's findings can assist stakeholders and policymakers in developing strategic plans and policies for targeted interventions for this population.

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Authors' contributions
Enas A, performed data analysis and evaluation, and drafted the paper; Nahla MB, contributed towards project administration, designed research study and methodology, interpreted data, and reviewed the paper; Arwa A, Salma A, Shahad A, Haya A, and Hajer A, drafted the research proposal, conducted research, and data collection. All authors reviewed and approved the final manuscript.

Conflict of interest
The authors declare that there are no conflicts of interest.

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Sports nutrition knowledge, attitude, and practice among male gymnasium users in Jordan

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ABSTRACT

Introduction: Proper nutrition is important for the health and performance of athletes. However, a lack of knowledge might lead to negative attitude and poor dietary practices. This study aimed to determine the levels of nutrition knowledge, attitude, and practice (KAP) among male gymnasium users in Jordan. **Methods:** A total of 138 male gymnasium users (21.1±2.8 years) from Jordan were recruited. Their height, weight, body mass index, and age were recorded. The KAP-Sports nutrition questionnaire that contains 25 questions regarding knowledge on basic sports nutrition, 12 questions regarding usual food choices and practices, and 20 questions on attitude towards nutrition and sports-enhancing diet were administrated. Descriptive and Pearson correlation analyses were used to analyse the data. **Results:** Majority of the participants had moderate knowledge on sports nutrition (77.6%), neutral attitude (84.7%), and fair dietary practice (67.4%). Mean score for knowledge, attitude and practice were 18.1±2.6, 58.8±6.2, and 29.7±3.6, respectively. There was significant, weak correlation between knowledge and attitude (r=0.18, p=0.048), as well as between knowledge and practice (r=0.20, p=0.018). **Conclusion:** Knowledge is important to affect positive attitude and good dietary practices. However, gymnasium users in Jordan did not have a high level of knowledge that could lead to their positive attitude and good dietary practices. Thus, sports nutrition education is recommended to ensure that adequate and correct knowledge are disseminated to gymnasium users.

Keywords: exercise, gym goers, KAP, physically active

INTRODUCTION

Gymnasium exercises require an array of physical attributes, which include strength, power, endurance, and aesthetics. They have become popular nowadays not only for health and fitness purposes, but also for physical appearances. Besides following a proper exercise regime with correct techniques, a balanced nutrition is also essential (Farhud, 2015). The main dietary goal for gymnasium users is to have proper nutrition to ensure optimum health, fitness, performance, and also to promote healthy dietary practices in the long term (Iwasa-Madge & Sesbreno, 2022; Purcell, 2013). It has been reported that nutrient deficiency can negatively affect health and performance of an individual (Saunders & Smith, 2010). Nevertheless,

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as reported in a recent article, it is still one of the problems that physically active individuals and athletes face (Jordan, Albracht-Schulte & Robert-McComb, 2020).

Nutrition knowledge positively impacts the implementation of healthy eating habits (Bradette-Laplante *et al.*, 2017). A previous study reported that higher knowledge resulted in higher adherence to nutritional recommendations (Scalvedi *et al.,* 2021). A sufficient amount of energy must be consumed by physically active individuals and athletes. Their total energy intake requirement is usually higher than the general population because they burn more fuel to produce energy during exercise (France, 2011). At least 55-65% of total energy should come from carbohydrates, 10-35% from protein, and 20-35% from fat in order to meet the nutritional requirements of physically active individuals for health and sports efficiency (ACSM, 2009). Inadequate caloric intake may result in muscle loss, hormonal dysfunction, bone density loss or failure, increased weakness, injury, and illness risks, and a longer recovery time (Burke, Loucks & Broad, 2006). Low energy intake can also have an influence on the body's micronutrient levels. Vitamins and minerals are essential for metabolising energy substrates, aiding in tissue building, fluid balance, carrying oxygen etc. Furthermore, increased exercise-induced reactive oxidative stress among athletes can be reduced by certain vitamins and minerals (Beck *et al.*, 2021; Benardot, 2012).

A variety of factors influence an individual's eating habits and food choices. These factors include food supply and security, individual's diet, bad habits such as alcohol and drug dependency, low income, busy lifestyle, lack of social support (i.e., living alone), nutrition knowledge, as well as personal or family attitudes (Thurecht & Pelly, 2020). Currently, the level of nutrition knowledge has been a popular question among researchers. This is because a person with lack of knowledge is most likely to have negative attitude and poor dietary practices (Bakhtiar *et al.*, 2021). Furthermore, it was reported that malnutrition is generally due to a lack of nutrition information rather than a lack of food (James, Ralph & Bellizzi, 1997).

Several researches have been conducted to assess the knowledge, attitude, and practice (KAP) levels regarding sports nutrition among athletes, but reports among gymnasium users are lacking to date. Research has shown that many athletes possess limited nutrition knowledge (Riviere *et al.*, 2021; Spendlove *et al.*, 2012). Possibly, this may represent a misunderstanding of ineligible individuals providing nutrition tips for athletes, which consequently leads to principles of nutrition being misunderstood and/or improperly applied (Zinn, Schofield & Wall, 2006). It was previously reported that sports nutrition education increases sports nutrition knowledge, attitude, and dietary practice (KAP) among university student athletes (Kamarun Zaman, Muhamad & Che Jusoh, 2021). Thus, focus should be placed on educating physically active individuals and athletes about sports nutrition to increase their knowledge level and to ensure that they are getting correct information from valid and reliable sources.

To date, reports on sports nutrition KAP among gymnasium users is lacking. Hence, this study aimed to assess the sports nutrition knowledge, attitude, and practice of male gymnasium users in Jordan, as well as to look at the correlations between them. Findings from this research will add knowledge regarding KAP assessment. This could also create awareness among gymnasium users and their coaches to

improve knowledge and avoid myths and misinformation, which hopefully might lead to a positive attitude and good dietary practices in order to achieve optimum health, fitness, and performance.

MATERIALS AND METHODS

Research design

This study was a quantitative, cross-sectional study that included the assessment of sports nutrition knowledge, attitude, and practice (KAP) among male gymnasium users in Jordan. This study obtained ethical approval from the Human Research Ethics Committee of Universiti Sains Malaysia (JEPeM- USM, Approval code: 21030216). The study was conducted between July to December 2021.

Study population and recruitment process

A total of 138 participants (n=138) were recruited via convenient sampling method from seven gymnasiums located in different states of Jordan, which included Irbid, Jarash, Ajloun, Al-Mafraq, Al-Zarqa'a, Al-Salt, and Al-Karak. Male gymnasium users who exercised in the gymnasium for more than three times per week at least six months prior to the study, healthy, aged between 18–35 years, and understood the English language were recruited. All participants were given a detailed explanation regarding the objectives, procedures, benefits, risks, and possible discomforts that might occur during this study before signing the informed consent form.

Study procedures

Researchers visited several gymnasiums in Jordan to recruit the participants. Upon obtaining their written informed consent, anthropometry measurements were carried out, including standing height and body weight (Stadiometer, SECA 284, German), and body mass index (BMI) calculation. Participants were also asked about his age and education level, which were recorded in the sociodemographic form.

To assess KAP scores on sports nutrition, a validated questionnaire developed by Hornstrom *et al.* (2011) was used. Cronbach's alpha score for this questionnaire was 0.79. The questionnaire was in the English language and was self-administered by each participant. The researcher was on standby whenever assistance was needed. The questionnaire was divided into three sections:

i) The first section comprised of knowledge on basic sports nutrition, which consisted of 25 questions. Each correct answer was scored as one mark (1) and each incorrect answer was given a zero (0). Total score was calculated by summing up the marks obtained for all questions. The maximum score for knowledge was 25. Knowledge level was categorised into three levels: High (scores 20 - 25), Moderate (scores 15 - 19), and Low (scores 0 - 14).

ii) The second section comprised of nutrition choices and nutrition practices, which consisted of 12 questions. For questions 1 to 7, participants were required to choose one out of four answer options (1 = every day, 2 = most days, 3 = occasionally, and 4 = rarely). For questions 8 – 12, participants needed to choose one out of four answer options (4 = four times or more per day, 3 = two or three times per day, 2 = once per day, and 1 = less than once per day). Total score was calculated by adding the marks obtained for each of the 12 questions. The maximum score for practice was 48. The level of nutrition practice was categorised into three:

Good (scores 40 - 48), Fair (scores 29 - 39), and Low (scores 0 - 28).

iii) The third section comprised of questions on attitude towards nutrition and sports-enhancing diet, which consisted of 20 questions. This section required participants to choose one out of four options (1 = strongly agree, 2 = agree, 3 = disagree, and 4 = strongly disagree). Total score was calculated by summing the marks obtained from questions 1 to 20. The maximum score for attitude was 80. Attitude level was categorised into three levels: Positive (scores 65 - 80), Neutral (scores 48 - 64), and Negative (scores 0 - 47).

Statistical analysis

Data were analysed using the IBM SPSS Statistics for Windows version 26.0 (IBM Corp, Armonk, New York, USA). Descriptive statistics and Pearson correlation analysis were carried out and the results were presented as mean±standard deviation (*SD*). Statistical significance was accepted at $p<0.05$.

RESULTS

In this study, a total of 138 male gymnasium users were recruited and completed the study. Anthropometry data of the participants are presented in Table 1. Education level of all participants was Bachelor's degree. The average duration of participation in gymnasium exercise was 2.46±1.5 years, at least three times per week.

Table 1. Anthropometric data of the participants (*N*=138)

Characteristics	*Mean±SD*
Age (years)	21.1±2.8
Height (cm)	173.7±8.1
Weight (kg)	67.2±10.6
Body mass index (kg/m^2)	22.2±3.1

Table 2 shows the mean KAP score, as well as the frequency and percentage of each level of knowledge, attitude, and practice. The mean scores for knowledge, attitude, and practice were 18.1±2.6, 58.8±6.2, and 29.7±3.6, respectively. Majority of the participants had moderate level of knowledge on sports nutrition (*n*=107), making up 77.6%; while only 4.3% (*n*=6) and 18.1% (*n*=25) of the participants had low and high knowledge on sports nutrition, respectively. Out of 25 questions for knowledge, there were five questions that were wrongly answered by majority (more than 50%) of the participants. These questions asked about protein as the main energy source during exercise and fluid intake during exercise.

A majority (84.7%; *n*=117) of the participants had a neutral attitude, while another 5.1% (*n*=7) of participants had positive attitude and the remaining 10.2% (*n*=14) had negative attitude with regards to sports nutrition. It is interesting to highlight that majority of the participants (91.3%) cared about their coach's opinion in considering sports-enhancing diet practices and about 80% of the participants reported that their coach suggested that they should adopt a sports-enhancing diet. However, about 50% of them felt that their training schedule posed a problem to sports-enhancing diet practices.

For dietary practice, a total of 93 participants (67.4%) had a fair score and 45 participants (32.6%) had a poor score. However, none of the participants (0%) obtained a good score with regards to sports nutrition practice. From the data, it was noted that 50.7% of them frequently ate fast food like burgers, fried chicken, and sausages. In addition, majority had once a day or less of fruits (62%) and vegetables (71%).

Pearson correlation analysis revealed that there was a significant, positive

Table 2. KAP results of the participants (*N*=138)

KAP items	*Mean score±SD*	*n (%)*
Knowledge	18.1±2.6	
High (20-25 scores)		25 (18.1)
Moderate (15-19 scores)		107 (77.6)
Low (0-14 scores)		6 (4.3)
Attitude	58.8±6.2	
Positive (65-80 scores)		7 (5.1)
Neutral (48-64 scores)		117 (84.7)
Negative (0-47 scores)		14 (10.2)
Practice	29.7±3.6	
Good (40-48 scores)		0 (0.0)
Fair (29-39 scores)		93 (67.4)
Poor (0-28 scores)		45 (32.6)

weak correlation between knowledge and attitude (r=0.18, p=0.048) (Figure 1), as well as knowledge and practice (r=0.20, p=0.018) (Figure 2). However, attitude and practice were not significantly correlated (p=0.696).

DISCUSSION

In general, results from the KAP analysis showed that the mean scores for knowledge, attitude, and practice (18.1±2.6, 58.8±6.2, and 29.7±3.6, respectively) corresponded to moderate, neutral, and fair levels, respectively. In addition, it was found that knowledge and attitude, as well as knowledge and practice were significantly correlated, but there was no significant correlation between attitude and practice. These findings suggested that knowledge is a significant factor that affects attitude and practice.

As shown in Table 2, majority (77.6%) of the participants had a moderate knowledge regarding sports nutrition. As mentioned, studies among gym users

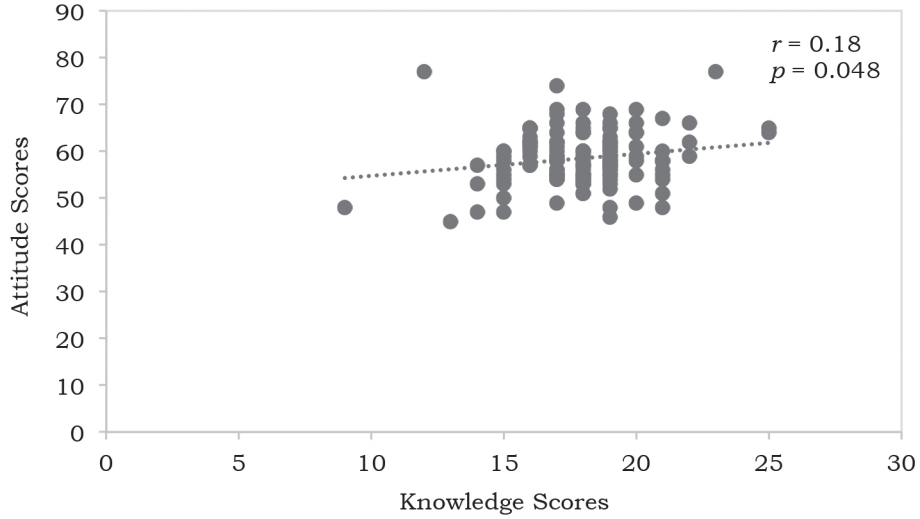

Figure 1. Correlation between knowledge scores and attitude scores

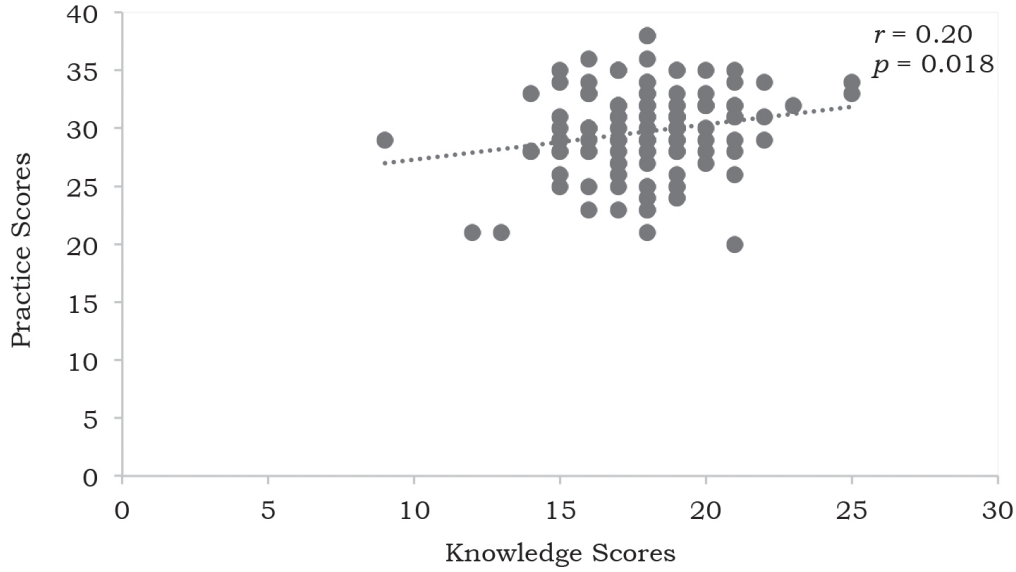

Figure 2. Correlation between knowledge scores and practice scores

are lacking to date, with less than five similar studies available in the literature. However, a previous study among university student athletes, which used a similar questionnaire to this present study, also reported that majority of the participants had a moderate knowledge level (Kamarun Zaman, Muhamad & Che Jusoh, 2021). In contrast to the present study findings, Wahlang & Baruah (2020) found that gym members in Guwahati, India had good knowledge, whereby males had better knowledge than females. The questionnaire used to assess nutrition knowledge level consisted of 19 sets of statements with three possible answers - "yes", "no", and "don't know". For evaluation, a correct answer was considered as one point, while an incorrect answer or a "don't know" was regarded as zero. Another previous study reported that majority of their amateur male bodybuilders had excellent knowledge, with only 8.6% having mediocre understanding (Wamiti, 2015). A five-question questionnaire was used to measure the participants' nutrition knowledge. The overall score was calculated after each response was graded, where the lowest score was 6 and the maximum score was ten. This study reported that the average score for knowledge was 9.2. Thus, it is speculated that the present study finding was not similar to these previous studies due to differences in the questionnaire used.

Although all participants in the present study had a bachelor's degree, less than 20% of them had a high knowledge level regarding sports nutrition. This could be attributed to lack of awareness, ignorance, or due to misunderstanding/misleading nutrition information received from people who are not qualified to provide such information. Besides that, it might also be due to their Bachelor's degree, which was not related to nutrition or sports nutrition. Sports nutrition education is essential to increase knowledge of individuals (Bakhtiar *et al.*, 2021, Siti Soraya *et al.*, 2018; Rossi

et al., 2017). As reported in a previous study, athletes' knowledge level was significantly increased after attending sports nutrition classes, with majority of them having a high level of knowledge (Kamarun Zaman *et al.*, 2021). Thus, it is important to provide correct and appropriate nutrition knowledge on a regular basis through proper educational training. It is important to choose a suitable content, study duration, and teaching techniques to ensure effective knowledge dissemination. Online classes or micro-credential courses can be considered as one of the feasible ways to get knowledge.

As mentioned, majority of the participants in the present study had a moderate level of sports nutrition knowledge. Hence, it was not surprising to observe that majority of them had a neutral attitude (84.7%) and fair dietary practice (67.4%), with only 5.1% of participants having a positive attitude and none of them had good dietary practice. Likewise, Kamarun Zaman *et al.* (2021) also found that majority of their participants did not have a positive attitude and good dietary practice. In fact, majority of them had a neutral attitude (57.1%) and poor dietary practice (71.4%). Wamiti (2015) also reported that majority (56.2%) of the bodybuilders recruited in the study had a negative attitude regarding nutrition despite excellent knowledge level obtained by the participants. This was quite surprising and it was speculated that this situation might be attributed to a lack of awareness despite having substantial knowledge.

The correlation analysis revealed that knowledge was significantly correlated with attitude and practice (Figures 1 and 2). The correlation was positive, which meant that as knowledge increased, attitude and practice also improved. This explains the neutral average score for attitude and fair average score for practice obtained in this study in response to the moderate knowledge level of the participants (Table 2). However, the correlation observed in the present study (r<0.4) was weak, according to LaMorte (2021), who described that an r value between 0.4 - 0.6 is considered as moderate, while $r \geq 0.6$ is considered as a strong correlation. This positive correlation also can be seen in a previous study, which found that increased knowledge after attending a nutrition class also increased the participants' attitude and dietary practice (Kamarun Zaman *et al.*, 2021). In this previous study, the percentage of participants having a positive attitude increased from 42.9% to 61.9%, while the percentage of participants having poor dietary practice reduced from 71.4% to 23.8%.

Similarly, researchers also noted that individuals who are familiar with nutrition guidelines will more likely incorporate them into their everyday eating patterns (Parr, Porter & Hodgson, 2016). In the study, a total of 348 coaches, 179 athletic trainers, and 2,977 athletes in high school and college settings answered a set of questionnaires about their nutrition knowledge and practices. Participants were quizzed on their knowledge, comprehension, and application of the basic four dietary groups (vegetables and fruits, grain foods, milk and milk products, and protein foods). As a result, 68% of the participants were aware of the basic four dietary groups and 71% of the participants consumed them accordingly.

CONCLUSION

In conclusion, knowledge is important to affect a positive attitude and good dietary practice. Male gymnasium users in Jordan did not have a high knowledge that could lead to their positive attitude and good dietary practices.

Sports nutrition education is therefore recommended to ensure that adequate and correct knowledge are disseminated to this population in order to improve their attitude and dietary practices. Future studies may look at actual dietary intake and its relation to knowledge level, as well as gender differences.

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Authors' contributions

Al-Quran M, principal investigator, led the data collection, data analysis and interpretation, and prepared the draft of the manuscript; Muhamad AS, conceptualised and designed the study, advised on data analysis and interpretation, reviewed the manuscript; Ahmad NS and Ooi FK, advised on data analysis and interpretation, reviewed the manuscript.

Conflict of interest

Authors declare no conflict of interest.

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Nutrient intake, oral symptoms, and oral health-related quality of life among antenatal mothers in Sarawak

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ABSTRACT

Introduction: This study aimed to determine the association between nutrient intake, oral symptoms, and oral health-related quality of life (OHRQoL) among antenatal mothers in the Sri Aman district, Sarawak. **Methods:** A total of 124 antenatal mothers in the second and third trimesters, through systematic random sampling, participated in this cross-sectional study. A validated self-administered questionnaire was used to collect their socioeconomic and obstetric profiles, perceived oral health status, and OHRQoL. Three days 24-hour diet recall assessment involving two weekdays and one weekend day was used for nutrient intake assessment. **Results:** Most mothers (75.0%) had at least one oral symptom: cavitated tooth (51.6%), bleeding gum (32.3%), halitosis (27.4%), gum pain (13.7%), toothache (12.1%), and swollen gum (6.5%). Most mothers also had inadequate intakes of energy, vitamin D, iodine, calcium, zinc, fluoride, and iron. The most affected OHRQoL domain was psychological discomfort, followed by functional limitation and physical pain. Nutrient intake was not significantly different between antenatal mothers with and without oral symptoms. Antenatal mothers with at least one oral symptom, presence of toothache, cavitated tooth, bleeding gum, and halitosis had significantly lower OHRQoL. **Conclusion:** Most antenatal mothers had inadequate nutrient intake, particularly vitamin D and calcium. Nutrient intake was not significantly associated with oral symptoms. The oral symptoms of dental caries and periodontal disease were prevalent; and the presence of toothache, cavitated tooth, bleeding gum, and halitosis were associated with poor OHRQoL.

Keywords: antenatal mothers, nutrient intake, oral health-related quality of life, oral symptoms

INTRODUCTION

The physiological hormonal changes during pregnancy are critical for positive pregnancy outcomes. However, it also causes detrimental effects on oral health among antenatal mothers. Recurrent vomiting enhances the acidic environment in the oral cavity (Shahid & Srivastava, 2019). Impaired oral hygiene due to nausea and vomiting, as well as food cravings for sugary food to offset it, further increases antenatal mother's risk of developing dental caries (Sari, Saddki & Yusoff, 2020). Besides that, oestrogen and progesterone enhance vascular permeability, and vascular proliferation

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could result in the recruitment of more inflammatory cells in the gingival (Costantinides *et al.*, 2020). As a result, the inflammatory response of periodontal tissues to bacteria in dental plaque is exaggerated even to a minimal amount of plaque accumulation (Rathee & Jain, 2022).

Therefore, antenatal mothers are susceptible to dental caries and periodontal disease. The presence of signs and symptoms of dental caries and periodontal disease could negatively affect the self-perception of oral health-related quality of life (OHRQoL) among antenatal mothers (Cordero *et al.*, 2019). This underlines the importance of maintaining good oral health during pregnancy.

A healthy diet is important for the preservation of oral health and positive pregnancy outcomes. However, a scoping review reported that nutrient intake for most antenatal mothers, particularly iron, folate, vitamin D, and calcium were inadequate in urban and suburban areas of Malaysia (Mohamed *et al.*, 2022). It is well established that nutrition and oral health has a bi-directional relationship in the general population (American Dental Association, 2021). However, there are very limited studies to establish the relationship between sufficient nutrient intake and oral symptoms suffered by antenatal mothers.

Thus, this study aimed to determine the nutrient intake, oral symptoms, and OHRQoL among antenatal mothers in Sri Aman district, Sarawak. The association between nutrient intake and oral symptoms, and the association between oral symptoms and OHRQoL were determined. The findings of this study can perhaps be further used to reinforce and strengthen the strategies outlined in the guidelines of "Oral Healthcare for Antenatal Mothers" by the Ministry of Health Malaysia (Oral Health Division, 2004).

MATERIALS AND METHODS

Population and sample

This study was a cross-sectional study of antenatal mothers who received antenatal care from the Maternal and Child Health (MCH) clinic of Sri Aman Health Clinic in Sri Aman district, Sarawak. There is a total of five Health Clinics in the Sri Aman district, Sarawak, and all Health Clinics offer maternal and child health care services at their respective MCH clinic. The MCH clinic at Sri Aman Health Clinic is the main MCH clinic in Sri Aman district, with the highest number of registered antenatal mothers. It is also the referral centre of antenatal care from other Health Clinics in the Sri Aman district.

The sample size for this study was determined based on research objectives, whereby the highest number of sample size was chosen. The final sample size selected was determined using the power and sample size calculations (PS) software version 3.1.6, based on comparison of mean nutrient (calcium) intake between subjects with oral symptoms and without oral symptoms, where the power of study was 80%, with 95% confidence. Standard deviation (*SD*) was determined at 240 mg (Adikari *et al.*, 2016). If the true mean difference between groups with and without oral symptoms was 120 mg, 64 subjects with oral symptoms and 64 control subjects were needed. The calculated sample size was 128. To accommodate for 10% non-response rate, a sample size of 141 was decided.

The inclusion criteria of this study were antenatal mothers in the second and third trimesters, aged 18 years and above, and able to read and write in the Malay language. Antenatal mothers who were non-citizen, and with mental disorders (which will affect memory) diagnosed by physicians were excluded by checking their antenatal health

record books. Potential participants were selected using a systematic random sampling method with sampling interval of three from patient registration books. Antenatal mothers who fulfilled the inclusion and exclusion criteria were invited to participate in this study. Written informed consent was obtained from antenatal mothers prior to data collection. Due to time constraint in this study, data collection was ceased when sample size achieved 130.

This study obtained ethical approval from the Human Research Ethics Committee of Universiti Sains Malaysia (Ref: USM/JEPeM/22010075) and Medical Research & Ethics Committee, Ministry of Health Malaysia (Ref: 22-00439-OKS (2).

Variables

Variables collected in this study were sociodemographic profile (age, ethnic group, highest education level, employment status, and monthly household income), obstetric profile (gestational age, gravida status, and parity status), perceived oral health status (perceived oral health status, oral symptoms, satisfaction with oral health status), nutrient intake (energy, carbohydrate, fat, protein, vitamin A, iron, folate, vitamin C, vitamin D, calcium, iodine, zinc, fluoride), and OHRQoL.

To assess the association between nutrient intake and oral symptoms, the independent variable was nutrient intake, while the dependent variable was oral symptoms. Nutrient intake was assumed to cause a change in oral symptoms. To assess the association between oral symptoms and OHRQoL, the independent variable was oral symptoms, while the dependent variable was OHRQoL. Oral symptoms were assumed to cause a change in OHRQoL.

Research tools

A structured self-administered questionnaire adapted from Sari *et al.* (2020), with original authors' permission, was used to obtain information on socio-demographic profile (including age, ethnic group, educational level, employment status, and monthly household income), obstetric profile (including gestational period, gravida status, and parity status), and perceived oral health status (including perceived oral health status, oral health problems, satisfaction with oral health status). The list of oral health problems was developed by a panel of clinical dental specialists (Sari *et al.*, 2020). Additionally, there was an open question through an "other" answer option with text entry on it for participants to specify other oral health problems they may have.

The Malay version of the short Oral Health Impact Profile questionnaire, designated as S-OHIP (M), validated by Saub, Locker & Allison (2005), was adopted to measure the OHRQoL of antenatal mothers. More specifically, the S-OHIP(M) measured perceptions of oral impacts on life experiences. Permission to use the questionnaire was obtained from original authors. There were two questions in each of the following seven domains in the questionnaire: functional limitation, physical pain, psychological discomfort, physical disability, psychological disability, social disability, and handicap. The frequency of oral impacts throughout the past 1 year was evaluated using a five-point Likert scale that were coded as '0' for 'never', '1' for 'hardly ever', '2' for 'occasionally', '3' for 'fairly often', and '4' for 'very often'. To measure the overall severity of the impacts, the total score of all 14 items (with a range of 0 to 56) was calculated. A higher S-OHIP(M) score implied a greater oral impact, and a worse OHRQoL. In

addition, the prevalence of impact for each item, which was the percentage of subjects who reported 'fairly often' or 'very often' was determined.

A 24-hour diet recall form by the Non-Communicable Disease (NCD) Section, Disease Prevention Division, Ministry of Health (MOH) Malaysia was used to measure the mothers' nutrient intakes. Information recorded in the standardised 24-hour diet recall form included the following: i) the recall day to indicate working day or weekend, ii) time of foods and beverages taken, iii) type and description of foods and beverages taken, and iv) household measurement of each serving.

Data collection

Before the study, the principal investigator was trained and calibrated by a qualified nutritionist on how to administer the 24-hour diet recall assessment. Problems encountered were discussed and improved before the study. An interview guideline on administering the 24-hour diet recall assessment was produced and endorsed by the nutritionist to be used in this study.

The antenatal mothers were approached while they were waiting to be seen by the medical officer in the waiting area. The inclusion and exclusion criteria were determined by interviewing the mothers and checking their antenatal health record books. Eligible mothers who were identified as potential participants using the systematic random sampling method were invited to participate in the study. Information regarding study rationale, objectives, procedures, risk, volunteering clause, and confidentiality were given; written informed consent was obtained from mothers who agreed to participate in this study.

The self-administered questionnaire was distributed to the participants. The participants took about 5-10 minutes to complete the questionnaire, which was collected back immediately after completion. Antenatal mothers were then informed of the 24-hour diet recall assessment that would be conducted over three non-consecutive days involving two weekdays and one weekend day. To increase the accuracy of diet assessment, a copy of samples of household measuring instruments was provided to them. An interviewer administered the 24-hour diet recall assessment. The first interview was conducted face-to- face right after they returned the self-administered questionnaire. The second and third 24-hour dietary recall assessments were conducted using phone interviews. Each diet recall assessment took about 15-20 minutes.

The three days 24-hour diet recall assessment data were analysed using the Nutritionist Pro™ Diet Analysis Software (Axxya System, Washington, USA) to obtain nutrient intake information. Malaysian Food Composition database was used to analyse diet data. The results of nutrient analyses were compared to the recommended nutrient intake (RNI) for Malaysian antenatal mothers (NCCFN, 2017). Nutrient intake of antenatal mothers was categorised as inadequate or adequate based on the given RNI values.

Statistical analysis

Data entry and analysis were performed using IBM SPSS Statistics for Windows version 26.0 (IBM Corp., Armonk, NY, USA). Data were first checked and cleaned. Descriptive analysis was first conducted. Categorical data were reported in frequency and percentage, and continuous data were reported as mean and *SD* or median and interquartile range (*IQR*), depending on the distribution of normality. Mean and *SD* were reported if the skewness was

within an acceptable range of -2 to +2, and kurtosis was within an acceptable range of -7 to +7 (Soh & Idris, 2017). In addition, the Kolmogorov-Smirnov test was also done, whereby significant findings indicated non-normal distribution.

To study the association between nutrient intake and oral symptoms, an independent *t*-test was used to compare the mean nutrient intake values between antenatal mothers with and without oral symptoms. The Mann-Whitney U Test was used if the observations were not normally distributed. An independent *t*-test was also used to study the association between oral symptoms and OHRQoL by comparing the mean S-OHIP (M) scores between antenatal mothers with and without oral symptoms. Similarly, the Mann-Whitney U Test was used if the observations were not normally distributed. Statistical significant level was set at p=0.05.

RESULTS

Sociodemographic and obstetric profiles of antenatal mothers

A total of 130 antenatal mothers participated in this study. However, only 124 of them completed the self-administered questionnaire and the three days 24-hour diet recall assessment, giving a response rate of 95.4%. Table 1 shows the sociodemographic and obstetric profiles of antenatal mothers. The age of mothers ranged from 18 to 42 years, with mean age of 29.1 (*SD* 5.55) years. Most of them were unemployed (61.3%) and the mean monthly household income was RM 2,284 (*SD* 1,846). Most antenatal mothers (71.0%) were in their second trimester. More than half of the antenatal mothers (66.1%) were pregnant for at least a second time.

Nutrient intake of antenatal mothers

Table 2 shows the nutrient intake of antenatal mothers. A total of 13 nutrients were extracted from the antenatal mothers' diet intakes. Most antenatal mothers in their second trimester (76.1%) and third trimester (88.9%) had inadequate energy intake. Nearly half (41.1%) had inadequate carbohydrate intake based on total energy intake (TEI). While most of the antenatal mothers had adequate folate (83.9%) and vitamin C (79.8%) intakes, the intakes for vitamin A, iron, vitamin D, calcium, iodine, fluoride, and zinc were mostly below the RNI values. All antenatal mothers had inadequate vitamin D and fluoride intakes, and almost all antenatal mothers (95.9%) had inadequate calcium intake.

Perceived oral health status and oral symptoms of antenatal mothers

Overall, half of the antenatal mothers (52.8%) were satisfied with their oral health status. Less than half of them perceived their oral health status as good (39.5%) and very good (7.5%), respectively. A quarter (25.0%) of antenatal mothers in this study reported not experiencing any oral symptoms, while most (75.0%) had at least one oral symptom. The most common oral symptom reported was cavitated tooth (51.6%), followed by bleeding gum (32.3%), halitosis (27.4%), gum pain (13.7%), toothache (12.1%), swollen gum (6.5%), and others (1.6%). Other oral symptoms included discomfort from wisdom tooth (0.8%) and yellowish teeth (0.8%).

Oral health-related quality of life (OHRQoL)

Table 3 shows the prevalence and severity of oral impact among antenatal mothers. The results were obtained using S-OHIP(M) instrument. The most affected oral health domain was psychological discomfort, with a mean score of 1.95 (*SD* 1.89). Meanwhile, the

Table 1. Sociodemographic and obstetric profiles of antenatal mothers (*N*=124)

Characteristics	*Mean±SD*	*n (%)*
Age (years)	29.1±5.6	
Age group (years)		
18 – 19		3 (2.4)
20 – 29		66 (53.2)
30 – 39		49 (39.5)
≥ 40		6 (4.8)
Ethnic group		
Malay		36 (29.0)
Chinese		13 (10.5)
Indian		-
Iban		63 (50.8)
Melanau		1 (0.8)
Bidayuh		7 (5.6)
Others		4 (3.2)
Highest education level		
No formal education		-
Primary school		8 (6.5)
Secondary school		67 (54.0)
Post-secondary or diploma		34 (27.4)
Tertiary		15 (12.1)
Employment status		
Employed		48 (38.7)
Unemployed		76 (61.3)
Mean household income (MYR)	2,284±1,847	
Household income (MYR)		
Below 999		16 (12.9)
1,000-2,999		70 (56.5)
3,000-4,999		20 (16.1)
5,000 and above		18 (14.5)
Trimester		
Second trimester		88 (71.0)
Third trimester		36 (29.0)
Gravida status		
Primigravida		42 (33.9)
Multigravida		82 (66.1)
Parity status		
Nulliparous		47 (37.9)
Primiparous		40 (32.3)
Multiparous		37 (29.8)

item of discomfort due to food getting stuck between teeth or dentures had the highest mean score of 1.15 (*SD* 1.07) among all 14 items, followed by feeling shy (mean 0.81, *SD* 1.09), and problems causing bad breath (*mean* 0.79, *SD* 0.95). The highest prevalence of impact experienced was discomfort due to food getting stuck between teeth or dentures (11.3%), followed by feeling shy (10.5%), problems causing bad breath (5.6%), and avoiding smiling (5.6%).

Table 2. Nutrient intake of antenatal mothers (*N*=124)

Nutrients	*RNI*	*Mean±SD*	*Frequency (%)*	
			Inadequate	*Adequate*
Energy (kcal)	1,890[b]	1,598±354	67 (76.1)	21 (23.9)
	2,080[c]	1,674±351	32 (88.9)	4 (11.1)
Carbohydrate (%)	50-65 [†a]	51.3±6.2	51 (41.1)	73 (58.9)
Fat (%)	25–30 [†a]	29.3±4.8	16 (12.9)	108 (87.1)
Protein (%)	10-20 [†a]	19.4±3.5	-	124 (60.5)
Vitamin A (µg)	800[a]	749.9±382.4	72 (58.1)	52 (41.9)
Iron (mg)	100[a]	78.3±39.7	88 (71.0)	36 (29.0)
Folate (µg)	600[a]	2,124±1,966	20 (16.1)	104 (83.9)
Vitamin C (mg)	80[a]	176.4±144.7	25 (20.2)	99 (79.8)
Vitamin D (µg)	15[a]	2.7±2.2	124 (100.0)	-
Calcium (mg)	1,300[§]	465.5 (379.3, 981.4)[‡]	3 (100.0)	-
	1,000[¶]	562.5±229.4	116 (95.9)	5 (4.1)
Iodine (µg)	200[a]	74.2±46.7	122 (98.4)	2 (1.6)
Zinc (mg)	7[b]	10.5±8.0	44 (50.0)	44 (50.0)
	10[c]	8.8±6.7	28 (77.8)	8 (22.2)
Fluoride (mg)	3[a]	0.0 (0.0, 0.0)[‡]	124 (100.0)	-

[†]% based on total energy intake (TEI)
[‡]Median (25th, 75th), Kolmogorov-Smirnov <0.05
[§]RNI calcium for age group 18-19 years
[¶]RNI calcium for age group 20 years and above
[a]Similar RNI for all trimesters; [b]RNI for second trimester; [c]RNI for third trimester

Association between nutrient intake and oral symptoms

Table 4 shows the differences in mean nutrient intake between antenatal mothers with at least one oral symptom and without oral symptoms. It was found that all 13 selected nutrients intake were not significantly different among antenatal mothers with and without oral symptoms.

Association between oral symptoms and OHRQoL

Table 5 shows the differences in mean S-OHIP(M) score by oral symptoms. It was found that the median S-OHIP(M) score among antenatal mothers with at least one oral symptom was significantly higher compared to those without oral symptoms. Antenatal mothers with at least one oral symptom had lower OHRQoL compared to those without oral symptoms. Antenatal mothers in this study with toothache, cavitated tooth, bleeding gum, and halitosis had significantly lower OHRQoL compared to those without symptoms.

DISCUSSION

Antenatal mothers in this study had a high prevalence of oral symptoms associated with dental caries. The self-perceived cavitated tooth in this study was slightly lower compared with the finding of 62% reported by Sari *et al.* (2020). The high prevalence of caries could be attributed to impaired oral hygiene due to nausea and vomiting, as well as frequent intake of sugary foods and drinks to alleviate pregnancy cravings (Sari *et al.*, 2020). In addition,

Table 3. Prevalence and severity of oral impact among antenatal mothers (*N*=124)

S-OHIP(M) domain and item	*Prevalence of impact (%)*	*S-OHIP Mean±SD*
Functional limitation		1.29±1.48
Difficulty chewing any foods	2.4	0.50±0.84
Problems caused bad breath	5.6	0.79±0.95
Physical pain		1.19±1.29
Discomfort eating any food	2.4	0.54±0.84
Ulcers in mouth	2.4	0.65±0.84
Psychological discomfort		1.95±1.89
Discomfort due to food getting stuck	11.3	1.15±1.07
Felt shy	10.5	0.81±1.09
Physical disability		1.02±1.54
Avoided eating certain foods	4.0	0.58±0.88
Avoided smiling	5.6	0.00 (0.00, 0.75)†
Psychological disability		0.00 (0.00, 1.00)†
Sleep been disturbed	4.0	0.00 (0.00, 0.00)†
Concentration been disturbed	4.0	0.00 (0.00, 0.00)†
Social disability		0.39±1.07
Avoided going out	0.8	0.00 (0.00, 0.00)†
Problems in carrying out daily activities	2.4	0.00 (0.00, 0.00)†
Handicap		0.94±1.31
Had to spend a lot of money	2.4	0.00 (0.00, 0.00)†
Felt less confident	4.8	0.65±0.90

†Median (25th, 75th), Kolmogorov-Smirnov (p<0.05)

untreated caries before pregnancy may worsen and progress to advanced stage, which may lead to dental pain.

Among the common symptoms of periodontal disease reported by antenatal mothers in this study were bleeding gum, followed by gum pain and gum swelling. The finding of self-perceived bleeding gum in this study was slightly higher than the finding of 28% reported by Sari *et al.* (2020). Pregnancy gingivitis was included in the new classification of periodontal and peri-implant diseases and conditions in 2017 as a dental plaque induced gingival condition modified by systemic condition of sex hormones. With the high levels of oestrogen and progesterone, gingival oedema and gingivitis are prevalent in most antenatal mothers, even to a minimal amount of plaque accumulation (Rathee & Jain, 2022). Untreated gingivitis may further progress to periodontitis, characterised by irreversible bone loss and tooth mobility.

In this study, the intakes of vitamin D and calcium were found to be inadequate. The insufficient vitamin D intake in this study was much higher compared to a study by Basha *et al.* (2021) in Malaysia, where the prevalence of vitamin D deficiency in late pregnancy was about 92 percent. This can be attributed to the relatively low awareness on vitamin D intake among antenatal mothers, which is related to lower educational background (Bukhary *et al.*, 2016). In addition, the sun avoidant lifestyles and also Muslims who cover themselves (especially ladies) during outdoor activities have been shown to be related to vitamin D insufficiency (Mohamed *et al.*, 2022). Meanwhile, majority of antenatal mothers in this

Table 4. Differences in mean nutrient intake by oral symptoms in antenatal mothers (*n*=124)

Nutrients	*With at least one oral symptom*	*No oral symptoms*	*t statistic (df)*	*p-value*
	Mean±SD	*Mean±SD*		
Energy (kcal)	1,629±351	1,592±364	-0.50 (122)	0.618
Carbohydrate (%)	50.8±6.1	52.9±6.2	1.58 (122)	0.116
Fat (%)	29.7±4.6	27.9±4.8	-1.75 (122)	0.082
Protein (%)	19.4±3.6	19.1±3.1	-0.41 (122)	0.683
Vitamin A (µg)	778.2±394.0	665.1±337.1	-1.43 (122)	0.155
Iron (mg)	75.5±39.6	86.6±39.3	1.35 (122)	0.178
Folate (µg)	1,923±2,008	1,727±1,884	-0.35 (122)	0.729
Vitamin C (mg)	176.9±148.9	175.1±133.5	-0.06 (122)	0.970
Vitamin D (µg)	2.1 (1.1, 3.7)†	1.7 (0.9, 3.3)†	1,257.5‡	0.288
Calcium (mg)	568.4±222.4	549.3±256.3	-0.39 (122)	0.691
Iodine (µg)	75.8±48.1	69.4±42.8	-0.66 (122)	0.508
Zinc (mg)	9.3±7.1	12.3±8.9	1.69 (43.45)	0.098
Fluoride (mg)	0.0 (0.0, 0.0)†	0.0 (0.0, 0.0)†	1,309.5‡	0.311

†Median (25th, 75th), Kolmogorov-Smirnov (p<0.05)
‡U statistics by Mann-Whitney U test

study had inadequate dietary calcium intake. The result is comparable with the findings from Hamid *et al.* (2019), where only 2.6% of antenatal mothers in suburban MCH clinics in Selangor, Malaysia achieved the RNI for calcium. It was reported that most Asians were not aware of the importance of calcium for their health and thus, authors suggested that their negative attitude must be corrected (Chan *et al.*, 2018).

Both vitamin D and calcium complement each other and are important in maintaining healthy bones and teeth, which is crucial for healthy periodontium during pregnancy. Besides that, adequate intakes of vitamin D and calcium are essential in the development, formation, and mineralisation of primary teeth starting at the 13th week of pregnancy (Suárez-Calleja *et al.*, 2021). It was found that maternal vitamin D insufficiency was associated with greater caries experience in primary dentition (Beckett *et al.*, 2022). Therefore, antenatal mothers should be educated on the importance of adequate intakes of vitamin D and calcium through diet.

According to the American Dental Association (2021), oral health and nutrition have a bi-directional relationship. However, in this study, there was no significant difference in all the selected nutrient intakes between antenatal mothers with and without oral symptoms. The insignificant results in this study may be attributed to the changes in dietary intake associated with food cravings, and the multivitamin and mineral supplements taken during pregnancy, which was irrelevant with pre-existing oral symptoms before pregnancy. Furthermore, food intake pattern, which is affected by food availability and affordability, culture and belief, and food taboos were not investigated in this study.

This study revealed that the OHRQoL was lower among antenatal mothers with at least one oral symptom compared to those without oral symptoms. The result of this study is in line with Fakheran *et al.* (2020) that antenatal mothers' perceptions of their OHRQoL were

Table 5. Differences in mean S-OHIP (M) score by oral symptoms in antenatal mothers (*N*=124)

Variable	*S-OHIP score Mean±SD*	*t statistic (df)*	*p-value*
Presence of oral symptom			
With at least one oral symptom	7.00 (2.50, 14.00)†	757.00‡	<0.001
No oral symptom	2.00 (0.00, 5.00)†		
Toothache			
Yes	12.27±9.79	-2.57 (122)	0.011
No	6.82±7.37		
Cavitated tooth			
Yes	7.00 (3.00, 14.00)†	1326.00‡	0.003
No	3.00 (1.00, 8.00)†		
Gum pain			
Yes	11.88±10.04	-2.01 (18.76)	0.058
No	6.78±7.27		
Bleeding gum			
Yes	7.50 (3.25, 15.75)†	1132.50‡	0.003
No	4.00 (1.00, 8.75)†		
Swollen gum			
Yes	11.50±11.69	-1.02 (7.40)	0.337
No	7.19±7.52		
Halitosis			
Yes	9.00 (4.75, 14.50)†	911.50‡	0.001
No	4.00 (1.00, 8.00)†		

†Median (25th, 75th), Kolmogorov-Smirnov (p<0.05)
‡U statistics by Mann-Whitney U test

significantly impacted by the presence of oral symptoms resulting from dental caries and periodontal disease. Dental caries in the advanced stage usually causes dental pain, food stuck in cavitated tooth, and difficulty in eating and chewing. To a greater extent, it may disturb sleep and affect daily living activities like absence from class or work (WHO, 2017).

The high impact of dental caries among antenatal mothers might be due to the practice of deferring dental extraction and restorative treatment to protect the foetus and keeping it safe and healthy (Fakheran *et al.*, 2020). Furthermore, antenatal mothers may become anxious when their gingiva bleeds during routine toothbrushing and it gets worse when oral hygiene practices are impaired due to nausea and vomiting (Fakheran *et al.*, 2020). Besides that, halitosis can significantly lower their self-esteem and result in impaired social interactions with other people. Most of the time, halitosis could be a consequence of periodontal disease, deep dental caries, dry mouth, and impaired oral hygiene practices causing dental plaque accumulation and food debris in cavitated tooth (Izidoro *et al.*, 2022).

Diet and nutrition should be seen as one of the common risk factors affecting oral health and pregnancy outcomes. More oral health and nutritional promotion programmes should be conducted at the community level to raise the awareness on the importance of nutrients towards positive pregnancy outcomes and oral health. Besides that, the dental referral pathway by antenatal

healthcare providers and monitoring mechanism should be strengthened. Clinical interventions like basic diet intervention and prompt dental treatments including preventive dental treatment, such as topical fluoride therapy, should be prioritised for all antenatal mothers. Tailored oral health education by emphasising effective dental plaque removal and use of fluoride toothpaste should be emphasised to all antenatal mothers.

The family wellness concept should be utilised in promoting oral health by using mothers as the agent of change in the family. Interventions could enhance positive preventive behaviours of mothers, who can then further good eating habits and oral health practices within the family unit and in turn the community at large. Through this, community wellness can be achieved (Oral Health Programme, 2019).

This study had several limitations. The study design was cross-sectional in nature and thus, had limitation in establishing the causal relationship between variables. The information from 24-hour diet recall assessment had limitations where antenatal mothers may unintentionally under-report their actual diet intake, including the dietary supplements taken. Using self-reported oral symptoms had limitations of biases. Often a discrepancy exists between a person's perceived oral health status and a professional's assessment found in clinical examination (Sari *et al.,* 2020). Future studies that measure oral health status using clinical examination is therefore recommended.

CONCLUSION

In this study, most antenatal mothers had inadequate nutrient intakes, particularly vitamin D and calcium. Majority of them had at least one oral symptom. The most prevalent symptom was cavitated tooth, followed by bleeding gum and halitosis. The most affected OHRQoL domain was psychological discomfort, followed by functional limitation and physical pain. Nutrient intake was not significantly associated with oral symptoms. The presence of at least one oral symptom was significantly associated with a lower OHRQoL among antenatal mothers. The presence of toothache, tooth cavitation, bleeding gum, and halitosis were found to be significantly associated with lower OHRQoL. This study highlighted the importance of reinforcing dental referral by antenatal healthcare providers and preventive dental care including use of topical fluoride and fluoride toothpaste, as well as incorporating diet and nutrition in oral health education and promotion activities for antenatal mothers. Through family wellness concept, mothers could play a role as the agent of change for better oral health, diet and nutrition in the family.

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Authors' contributions

Tiong IT, principal investigator, conceptualised and designed the study, conducted data collection, data management and analyses, data interpretation, and prepared the draft of the manuscript; Ruhaya H, conceptualised and designed the study, conducted data management and analyses, data interpretation, reviewed and edited the manuscript; Norkhafizah S, conceptualised and designed the study, conducted data management and analyses, data interpretation, reviewed and edited the manuscript; Tham H, conducted data collection. All authors have read and agreed to the manuscript.

Conflicts of interest

This research was self-funded. The authors declare no conflict of interest.

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The effect of photoperiodism on nutritional potency of *Euglena* sp. Indonesian strains

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ABSTRACT

Introduction: Biomass microalgae functional materials, such as drugs and food supplements, have recently received much attention. *Euglena* sp. is a particularly appealing microalgae because of its simplicity of culture and capacity to produce a wide range of bioactive compounds. Moreover, it is one of the few microorganisms that produces carbohydrate, lipid, protein, ß-1,3-glucans, antioxidants, phytotoxins, wax esters, and polyunsaturated fatty acids that can be used to make nutraceuticals, pharmaceuticals, and cosmeceuticals. However, the potential utilisation of *Euglena* sp. for production of food supplements has been exploited only on a limited basis. **Methods:** This study was modified by adding protocatechuic acid and photoperiodism for 12:12; 14:10; 16:8; and full dark to affect the metabolite content of *Euglena* sp. **Results:** Results showed that the photoperiod had significant effect on lipid, chlorophyll-a, and carotenoid levels in the control treatment, with the highest levels as follows: 0.52±0.03 g/L, 1.20±0.01 $x10^{-2}$ g/L, 0.30±0.02 $x10^{-2}$ g/L; while the others were not significantly affected by the treatment, with the highest protein content at full dark 3.10±0.2 $x10^{-2}$ g/L; chlorophyll-b at photoperiod 14:10 0.70±0.03 $x10^{-2}$ g/L; paramylon at photoperiod 12:12 1.90±0.02 $x10^{-1}$ g/L. The highest carbohydrates were found in control, with a level of 1.20±0.02 g/L. **Conclusion:** Photoperiodism is recommended to enhance productivity of protein, paramylon, and chlorophyll-b, while full light is recommended to enhance carbohydrate, lipid, chlorophyll-a, and carotenoid production in *Euglena* sp. to improve the quality of food nutrition.

Keywords: *Euglena* sp., photoperiod, protocatechuic acid

INTRODUCTION

In recent years, it has been known that scientists are interested in developing the use of microalgae biomass as a source of component for producing bioactive metabolites that are environmentally friendly and renewable. In addition, recent advances in biological engineering and multi-omics have revealed numerous potentials for microalgae bioproducts in the nutrition, media, pharmaceutical, and commodity industries (Bajhaiya,

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Moreira & Pittman, 2017; Stengel & Connan, 2015). *Euglena* (protist) is a flagellated microfactory organism living in autotrophic, heterotrophic, or mixotrophic environments. It can grow heterotrophically with various carbon sources and pH levels in wastewater (Mahapatra, Chanakya & Ramachandra, 2013). *Euglena* sp. is not like bacteria or protozoa, so it does not have a dangerous or pathogenic risk to other living things, especially humans, and their numbers can be increased easily.

Euglena is high in carbohydrate-active enzymes, with an exceptional ability to synthesise complex carbohydrates for a unicellular organism. Furthermore, *Euglena* cells contain many of nutrients like fatty acids, such as eicosapentaenoic acid, docosahexaenoic acid, and vitamins, implying the genus is a valuable and potential resource for food supplement. *Euglena* sp. also contains many nutrients, such as carbohydrates, lipids, proteins (primary metabolites), ß-1, 3-glucans (paramylon), carotenoids, tocopherol, essential amino acids, minerals, phycobiliproteins (PBPs), phytohormones, phytosterols, phenolic compounds, and mycosporine-like amino acids (secondary metabolites), that have been shown to possess nutritional, antioxidant, neuroprotective, anti-inflammatory, antimicrobial, anti-angiogenic, and anti-cancer properties (Haque *et al.,* 2014; Rico *et al.,* 2017; Singh *et al.,* 2017; Nakazawa, 2017). Therefore, the use of *Euglena* sp. as a healthy supplementary food is promising.

In the cultivation of *Euglena* sp., dark:light cycle influences its spectral composition, oscillation pattern, and photoperiod, all of which contribute significantly to the microalgae metabolic process. Optimal irradiance, oscillation pattern, and dark length all influence phytoplankton metabolic activity (Oostlander *et al.*, 2020). Changes in photoperiod can affect the production of total pigment, fatty acid, and protein content in *Chlorella vulgaris*, lipid formation and growth in *Porphyridium cruentum* (Khoyi *et al.*, 2009; Oh *et al.*, 2009), as well as nutrient utilisation and biomass in *Chaetoceros muelleri* production (Minggat, Roseli & Tanaka, 2020). The duration of photoperiod significantly impacts diatom cell development (dark: light cycle) (Palanisamy *et al.*, 2022). Light intensity greater than 150 E $m^{-2}s^{-1}$ is thought to inhibit cell development; it may oversaturate cell growth under more extended irradiation, as indicated by the difference between the dark:light cycles of 8:16 and 16:8 (Li, Talmy & Campbell, 2017). Therefore, to confirm the potential of *Euglena* sp. as a source of various bioactive for human nutrition, various cultivation techniques can be carried out, one of which is by using different photoperiods.

MATERIALS AND METHODS

Isolation & cultivation

Water samples for microalgae isolation were collected from the Dieng Plateau, Wonosobo, Middle Java, Indonesia and isolated using capillarity pipette methods in aseptic laminary air flow. Cramer–Myers (CM) medium was used as a growth medium, with the composition ($g.L^{-1}$): 1 gram NH_4SO_4, 1 gram KH_2PO_4, 0.2 gram $MgSO_4.7H_2O$, 0.02 gram $CaCl_2.2H_2O$, 100 µL trace metal mix, 100µL $Na_2MoO_4.2H_2O$ solution, 20 µL vitamin B1 solution, 25 µL vitamin B12 solution, with a pH of 5.5 and an additional carbon source of 0.8 g protocatechuic acid (PCA). The CM medium was previously sterilised using an autoclave at 121°C for 20 minutes. After making the media, the starter was left for two weeks to equalise the age of *Euglena* sp. The starter was then transferred to a 50 mL culture bottle and

the volume of CM was 450 mL; thus, the total in one culture bottle was 500 mL.

Identification and screening strain

Samples from various strains that were successfully isolated were observed under the Olympus CX22LED1000x magnification light microscope, with added immersion oil and connected with Optilab Advance Observer (MTN034). Strain screening was done by cultivating isolates that have successfully grown without contamination, namely seven strains out of a total of 35 strains. The seven lines were IDN 23, IDN 29, IDN Mix, IDN 33 A Aerobic, IDN 33 A Anaerobic, IDN 33 B Aerobic, and IDN 33 B Anaerobic. Then, their growth rates were compared, and the highest growth rate was selected as the best candidate line. Microalgal growth was measured every day. In addition, the concentration of microalgae cells was determined by measuring the culture's optical density (OD) with the Thermo Scientific Evolution 201 UV-Vis spectrophotometer at an absorbance of 680 nm in three replicates. Calculation of cell-specific growth rate was done using the following formulas (1,2):

$$\mu = \frac{Ln\,(Nt - N0)}{t1 - t0} \quad (1)$$

$$Dt = \frac{Ln2}{24x\mu} \quad (2)$$

μ=Specific growth rate; Dt=generation time (hours); Nt=cell population on the t-day exponential phase (cells mL^{-1}); N0= cell population on day 0 of the exponential phase (cells mL^{-1}); *t*1-*t*0 = time interval in the exponential phase (days).

Growth kinetics modelling

The Logistic and Gompertz models were used to model the growth kinetics of *Euglena* sp. First, the Logistics model was calculated using the following formulas (equations 3 and 4), where X is cell density, X0 is initial cell density, Xmax is maximum cell density, and max is maximum specific growth rate (Phukoetphim *et al.,* 2017).

$$\frac{dx}{dt} = \mu_{max}\left(1 - \frac{x}{\mu_{max}}\right)x \quad (3)$$

$$x = \left(\frac{xo.\exp(\mu_{max}t)}{1 - [(\frac{xo}{x_{max}})\,(1 - \exp(\mu_{max}t))]}\right) \quad (4)$$

The parameters in the Gompertz model were maximum cell production (rm) and lag time (tL). The model was determined using the following formulas (equations 5 and 6), where SSR is the sum square residual and SST is the sum square total (Phukoetphim *et al.,* 2017).

$$x = Xo + [X_{max}.\exp[-\exp((\frac{r_m.\exp(1)}{x_{max}})\,(t_l - t) + 1] \quad (5)$$

$$R^2 - (1 - \frac{SSR}{SST}) \quad (6)$$

Determination of cell growth

Cell growth in different photoperiods was compared by counting cells every 24 hours using a light microscope and Haemocytometer Neubauer 1 mm. After shaking the sample to homogenise it, 100 μL was pipetted into a 2 mL microtube using a micropipette. The model was then transferred to a haemacytometer and the cells were counted using a light microscope linked to a computer running optilab software. The number of cells in each of the four corners was calculated and the total number of cells was computed.

Biomass calculation of *Euglena* sp.

Biomass production was calculated every three days using the dry weight of cells. Two mL of sample culture was transferred into a 2 mL microtube. For

10 minutes, the sample was centrifuged at 4000 rpm. The supernatant was removed and washed with distilled water. Cell suspensions at the bottom of microtubes were dried in an incubator oven at 37°C until they had a constant weight. The final biomass was calculated by subtracting the sample's final weight from its initial weight and then dividing it by its initial volume.

Carbohydrate estimation of *Euglena* sp.

Dubois's method quantified the carbohydrate content in microalgae biomass using phenol-sulfuric acid. After creating a standard curve of carbohydrate concentrations, the sample's absorbance was measured using a spectrophotometer at 490 nm to determine carbohydrate concentration.

Lipid estimation of *Euglena* sp.

The Bligh and Dyer method was used to quantify the lipid content of *Euglena* sp. Extraction method that included a 1:2 ratio of chloroform and methanol, followed by a 1:1 ratio of chloroform and aquades was used. The solution was then centrifuged until three layers were formed, and the bottom layer was removed and incubated in an oven at 30°C for 24 hours.

Protein estimation of *Euglena* sp.

The Bradford method was used to determine the protein content of *Euglena* sp. This method was carried out by centrifuging the supernatant from the separation process and adding in a SDS solution. Following a 5-minute incubation at 95°C, a 5-minute incubation at 4°C was performed. Bradford's solution was then added to the incubation samples. Absorbance was then measured at 595 nm using an eLISA Reader Biotech. Protein content was calculated using standard linear curve regression equations from standard Bovine Serum Albumin (BSA) protein solutions from Abbkine kit at 20, 50, 75, and 100 µg/ml concentrations.

Percentage and productivity determination of *Euglena* sp. primary metabolites

Productivity measurements of primary and secondary metabolites were calculated based on the following equations (8,9) (Chen *et al.*, 2020).

$$\% \text{ cell compound} = \frac{\text{Total cell compound}}{\text{Biomass}} \times 100\% \quad (8)$$

$$\text{Productivity} = \text{Biomass productivity} \times \% \text{ cell compound} \quad (9)$$

Pigmentation analysis

A spectrophotometric method was used to determine pigment content. Two ml culture was centrifuged at 4000 rpm for 5 minutes. Then, the supernatant was removed and the pellet was extracted overnight in the dark at 4°C with 1.5 mL methanol (99.9%). The use of waves on the spectrophotometer ranged from 400-750 nm. The concentrations of chlorophyll-a (Chl-a), chlorophyll-b (Chl-b), and photoprotective carotenoid (PPC) were calculated using the following equations:

$$\text{Chl a } (\mu g/mL) = -8.0962 \times \lambda_{652} + 16.5169 \lambda_{665};$$

$$\text{Chl b } (\mu g/mL) = 27.4405 \times \lambda_{652} - 12.1688 \times \lambda_{665};$$

$$\text{Total Carotenoids } (\mu g/mL) = 4 \times \lambda_{480}$$

Paramylon extraction and analysis

A culture of 10 mL *Euglena* sp. was taken and centrifuged at 4000 rpm for 10 minutes. The supernatant was discarded to obtain pellets. The pellets were dissolved in 1% (w/v) SDS and 5% (w/v) Na2EDTA, then incubated in a water bath at 37°C for 30 minutes. The treatment was repeated without incubation in SDS-Na2EDTA solution

and then the paramylon was washed twice with distilled water. Paramylon pellets were dissolved in 2 mL NaOH. The phenol sulfuric acid method was used to determine paramylon concentration. The extraction solution was mixed in a test tube with 5% phenol and H_2SO_4 (sulfuric acid). The test tube was allowed to stand for 10 minutes in a standing position. Next, the solution was vortexed for 30 seconds before being allowed to stand at room temperature for another 20 minutes. Then, a 490 nm spectrophotometer solution was used. The paramylon standard was used to create standard curves. Paramylon productivity ($g\ L^{-1}\ day^{-1}$) = last exponential phase-first exponential phase (Zhu & Wakisaka, 2018).

Statistical analysis

All experiments were done in triplicates and data were shown as mean values of the three replicates. The various experiments' mean values and standard deviations were evaluated using Microsoft Excel 2007. Using IBM SPSS Statistics for Windows version 26.0 (IBM Corp., New York, United States), the results of each analysis were analysed using one-way analysis of variance (ANOVA) by rank at a 95% confidence level.

RESULTS

There were many species of *Euglena* in the Dieng plateau. Erfianti *et al.* (2023) stated that *Euglena* sp. is a member of the *Euglena* genus that has been successfully isolated under extreme conditions. The pH level of Dieng Peatland is 2.0-3.5, suitable for the growth of *Euglena*. The special characteristics of *Euglena* are elongated oblong or spherical shaped cells, green in colour because they contain the pigment chlorophyll-a and -b, and has a pellicle structure that allows its cells to make changes called euglenoid movement, as well as having eye spots (stigmas) containing carotenoids that control the intensity of light (Erfianti *et al.*, 2023). *Euglena* also has a cell size ranging from 31-68 µm, flagella for swimming, and a reservoir (Al-Ashra, Abiad & Allahem, 2014). Therefore, *Euglena* sp. was isolated and identified using a light microscope from all strains (Figure 1). The solitary *Euglena* sp. measured ±50 µm in length. Microscopical analysis revealed that *Euglena* cells were solitary and free to swim. They typically lacked a cell wall and were elongated and spindle-shaped with tapering ends. Seven different strains (IDN 23, IDN 29, IDN Mix, IDN 33 A Aerob, IDN 33 A Anaerob, IDN 33 B Aerob, and IDN 33 B Anaerob) were obtained based on the isolation process, which were then screened by growing the isolation results in laboratory-scale CM medium to get the strain type with the best growth rate.

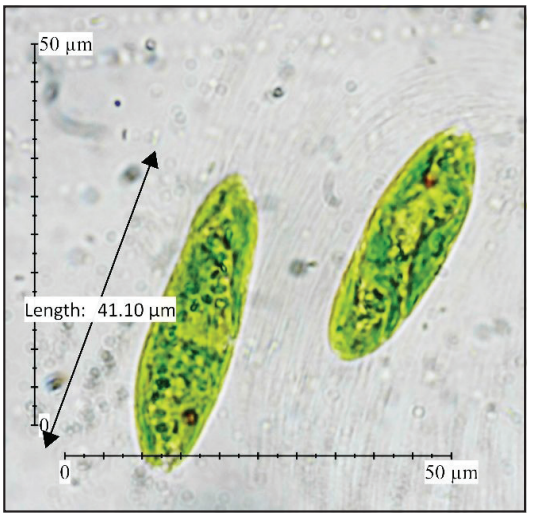

Figure 1. Cell of *Euglena* sp isolated from Dieng Plateau. Magnification 100 x

Table 1 and Figure 2 showed that the highest biomass productivity was found in strain IDN 33 A Aerobic, with a value of 0.76 g/L/day. The same results

Table 1. Screening productivity of biomass *Euglena* sp.

Strain	*SGR (μ)*	*DT (day^{-1})*	*Productivity biomass (g/L/day)*
IDN 23	0.149	0.193	0.614
IDN 29	0.153	0.189	0.668
IDN MIX	0.125	0.230	0.459
IDN 33 A Aerobic	0.168	0.172	0.764
IDN 33 A Anaerobic	0.122	0.238	0.450
IDN 33 B Aerobic	0.158	0.183	0.636
IDN 33 B Anaerobic	0.155	0.187	0.591

were obtained in measuring cell density using the spectrophotometric method. The results showed that strain IDN 33 A Aerobic had a growth phase close to the previously optimised IDN 29 strain. It is important to note that variation in microalgae can be seen between distinct genera, various species, and even strains of the same genus (Taleb *et al.,* 2016). From the two measurement processes, IDN 33 A Aerobic was then declared to be the selected strain that was to be used for the cultivation stage, with the highest specific growth rate value of 0.168 μ and the lowest doubling time of 0.172 day^{-1}.

The optical density of *Euglena* sp. cultivated in CM medium and the addition of PCA combined with specific photoperiod modes is presented in Figure 2. Optical density (OD) formed a different pattern between the control group without adding PCA and using the full light treatment with the addition of PCA and photoperiod. On day 2, the treatment group experienced a relatively rapid increase in OD compared to the control group. The treatment group entered the log phase faster than the control on day 2; the control group entered the log phase on day 6. Then, the treatment and control groups entered the stationary phase simultaneously on day 9. This meant that the photoperiod treatment group had a longer log phase, around seven days. The initial death phase in the treatment group began on day 13, while the control group started on day 15.

Based on the one-way ANOVA test, the photoperiod treatment of the control *Euglena* sp. had no statistically significant effect on the growth rate of *Euglena* sp. (p=0.440). In this experiment, higher carbohydrate content was obtained using a medium with dark condition and control treatment on day 9 at 1.10±0.02 g/L and 1.20±0.02 g/L, respectively (Figure 3a), with carbohydrate productivity of 0.64x10^{-1} g/L/day and 1.11x10^{-1} g/L/day, respectively. Moving to the following graph (Figure 3b), higher lipid content was obtained using a medium with dark condition on day 12 and control treatment on day 15 at 0.49±0.05 g/L and 0.52±0.03 g/L, respectively. Total lipid content of *Euglena* sp. in full dark treatment increased from 14.47% to 62.98%, while lipid content of *Euglena* sp. control increased from 10.70% to 43.94%, with lipid productivity of 0.32 x10^{-1} g/L/day and 0.26x10^{-1} g/L/day, respectively. A higher protein content was obtained using a medium with dark condition on day 15 and photoperiod 16:8 treatment on day 3 at 3.1±0.2 x 10^{-2} g/L and 2.6±0.1x10^{-2} g/L, respectively (Figure 3c). *Euglena* sp. in full dark treatment increased total protein content from 0.24% to 0.52%, and *Euglena* sp. in photoperiod 16:8 treatment increased protein content from 3.25% to 4.60%, with protein

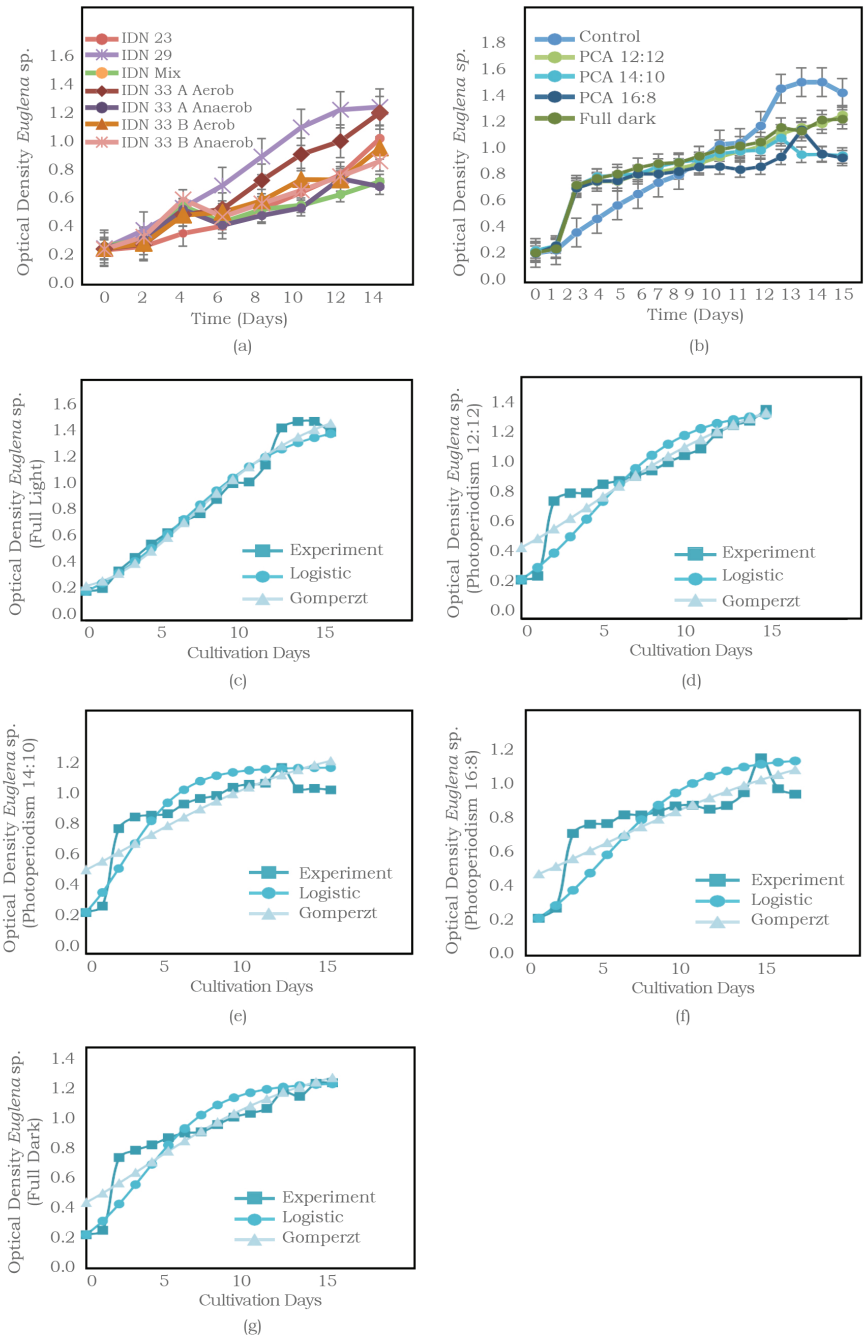

Figure 2. Growth of *Euglena* sp. (a) Screening potential strain of *Euglena* sp.; (b) Growth of *Euglena* sp. in photoperiod treatment and PCA addition; (c) Growth modelling of *Euglena* sp. (Full light); (d) Growth modelling of *Euglena* sp. (Photoperiodism 12:12); (e) Growth modelling of *Euglena* sp. (Photoperiodism 14:10); (f) Growth modelling of *Euglena* sp. (Photoperiodism 16:8); (g) Growth modelling of *Euglena* sp. (Full dark)

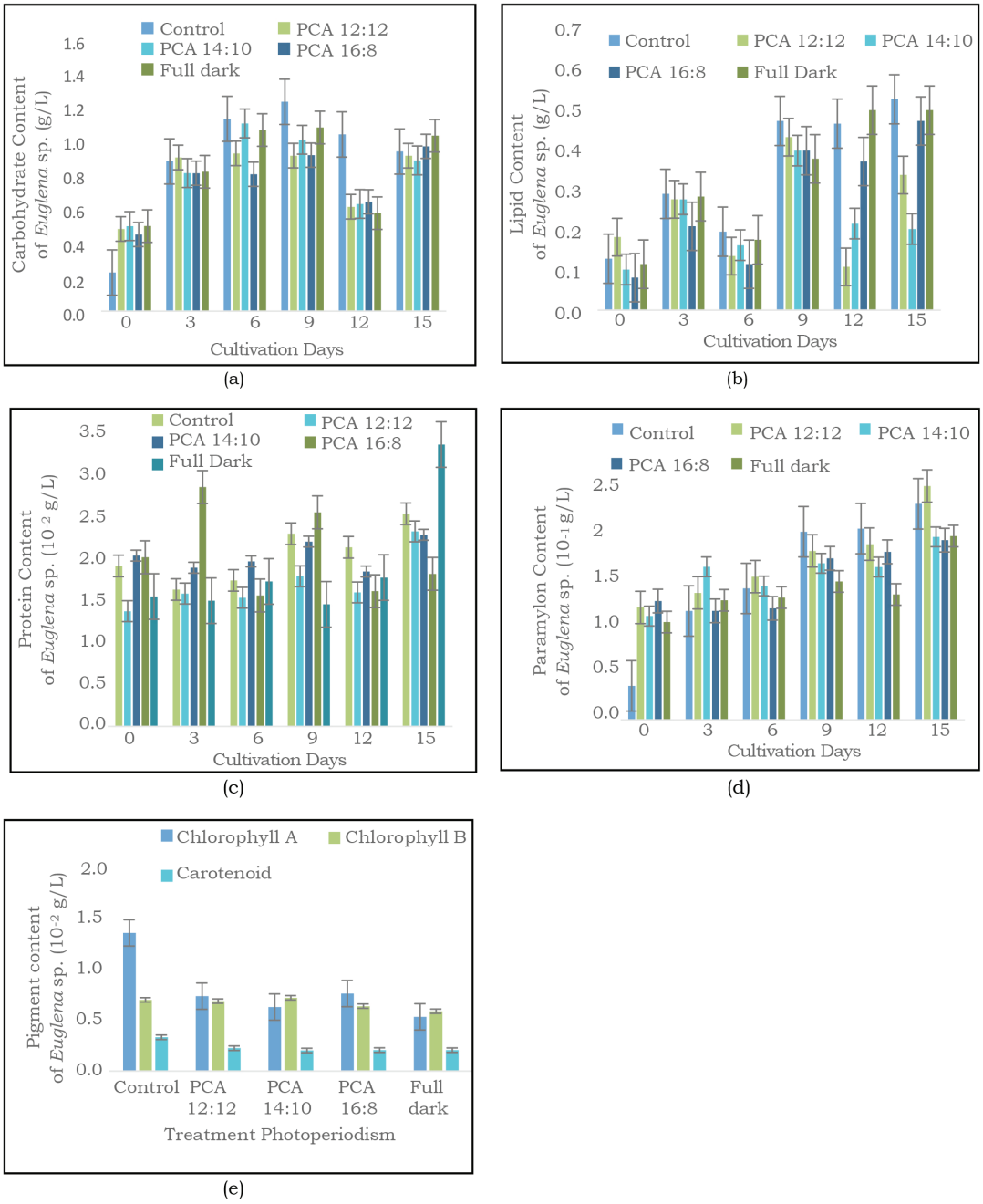

Figure 3. Metabolite content of *Euglena* sp (a) carbohydrate content; (b) lipid content; (c) protein content; (d) paramylon content; (e) pigment content.

productivity of 1.1 $x10^{-3}$ g/L/day and 2.5 $x10^{-3}$ g/L/day, respectively. Based on one-way ANOVA test, the photoperiod treatment of control *Euglena* sp. had no statistically significant effect on carbohydrate content (p=1.310) and protein content (p=0.060). In contrast, the result had a significantly different effect on lipid content (p<0.05); and from the Duncan post-hoc test, there was a significant difference between treatment and control groups.

In this experiment, higher pigment content was obtained. The overall type of pigment chlorophyll-a and carotenoid (Figure 3e) produced were highest in the control treatment, as extensive as 1.2±00.01 $x10^{-2}$ ±g/L and 0.30±0.02 $x10^{-2}$ g/L, respectively. The other most increased production was chlorophyll-b on the photoperiod treatment 14:10, as large as 0.70±0.03$x10^{-2}$ g/L. Different from pigment, higher paramylon content was obtained using a medium with photoperiod 12:12 treatment on day 15 at 1.90±0.02 x10-1 g/L (Figure 3d). Total paramylon content of *Euglena* sp. in photoperiod 12:12 treatment increased from 45.87% to 95.47%, with a productivity of 6.613 µg/ml/day. Based on the one-way ANOVA test, the photoperiod treatment of control *Euglena* sp. had no statistically significant effect on paramylon (p=0.457) and chlorophyll-b (p=0.192) content. However, the result had a significant effect on chlorophyll a content (p<0.05) and carotenoid content (p<0.05); and from the Duncan post-hoc test, there was a significant difference between treatment and control groups in chlorophyll-a and carotenoid content (Table 2).

DISCUSSION

In the initial phase of microalgae growth, cells adjust to the new media so that *Euglena*, with the addition of PCA and photoperiod treatment, requires a lag phase, namely the first and second days, while the control *Euglena* requires a longer time, namely the sixth day. Rapid exponential development in treatment culture begins on the second day of cultivation, reaches a stationary phase on day 9, and then begins to diminish. More extended periods of light exposure create photoinhibition in the cells and a lack of nutrients in the culture, causing growth to slow down. As a result, cells could not complete photosynthesis to multiply (Palanisamy *et al.,* 2022). In the control treatment, the death phase began on day 15. Due to decreased photosynthesis, continuous light generates the lowest cell multiplication number (373±104) and biomass productivity (0.371±21 g/L) on day 15 of cultivation (Palanisamy *et al.,* 2022). Based on ANOVA test, it was found that the photoperiod treatment did not significantly affect the growth rate of *Euglena*. This was possibly because some needed light, so *Euglena* became more adaptive. Microalgae requires light to produce adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH2), as well as critical chemicals for growth (Xie, Lin & Luo, 2021). Besides that, light is the source of energy that drives this process, and it must be considered in terms of intensity, spectrum quality, and photoperiod (Park & Craggs, 2011) so that a certain level of intensity or quality of light may have an impact on the growth rate when combined with a photoperiod.

Logistic and Gompertz were two non-linear models that were suitable for the rapid population growth of organisms such as microalgae (Lam *et al.,* 2017). Not limited by substrate type and consumption, the Logistic and Gompertz models (Figure 2c-g) were the simplest models and could be used for general microalgae growth rate. This research showed that the best pattern based on the Logistic and Gompertz kinetics models was on control cultivation. The resulting

regression treatment was below 0.9, which deviated from the model presented. Based on Logistic modelling, the maximum specific growth rate (μmax) of *Euglena* sp. was 0.2985/day. For the Gompertz modelling, the maximum cell production rate (rm) of *Euglena* sp. was 0.108 x106 cells/mL. The lag time (tL) of *Euglena* sp. was 1.428/day. Each of the *R* square error value for the Logistic and Gompertz models were 0.966 and 0.974, respectively. Therefore, based on the *R* square error values, the Gompertz model indicated a better-fit model compared to the Logistic model.

The proportions of chemical components (for example, carbohydrates, lipids, proteins, and pigments) in algal cells are closely related to cultivation conditions such as photoperiod and light intensity (Juneja, Ceballos & Murthy, 2013). Proteins are the most abundant component of dry microalgae biomass, accounting for 6-52% of the total; Zhu (2015) and Deng *et al.* (2018) found that protein content was similar (44.7-50.7%) when *C. kessleri* was grown in a mixotrophic condition. The highest carbohydrate production was on day 9, while the most increased lipid production was on day 12. As seen in Figure 3, productivity of carbohydrates on days 6, 12, and 15 were inversely proportional to the productivity of lipids. Carbohydrate biosynthesis is a competitive process in algal cells, requiring less ATP and NADPH per carbon than lipid synthesis (Subramanian *et al.,* 2013). Furthermore, microalgae can adjust their carbon partitioning programmatically in response to changes in culture conditions and environment (Wang *et al.,* 2013).

The effect of photoperiod on the chemical compositions of *C. sorokiniana* grown in CCW in a bubble-column bioreactor were investigated. Protein content increased with increasing illumination time, reaching a peak of

Table 2. Effects of photoperiod and addition of PCA on metabolite productivity of *Euglena* sp.

Treatment	*Biomass (g/L)*	*Productivity (g/L/day)*	*Percent protein (%)*	*Percent para-mylon (%)*	*Percent chlorophyll a (%)*	*Percent chlorophyll b (%)*	*Percent carotenoid (%)*
Control (Full light)	1.18	0.08	1.96±0.02	75.62±0.00	1.47±0.01[a]	0.76±0.00	0.60±0.01[b]
PCA-12:12	1.12	0.07	1.90±0.00	95.47±0.00	0.60±0.00[ab]	0.70±0.00	0.26±0.00[a]
PCA 14:10	1.03	0.07	2.02±0.00	56.04±0.01	0.97±0.00[b]	0.58±0.01	0.30±0.00[a]
PCA 16:8	3.27	0.22	4.60±0.00	67.74±0.00	0.21±0.00[b]	0.64±0.00	0.30±0.00[a]
PCA-Full dark	5.90	0.40	0.52±0.00	50.03±0.00	0.06±0.01[c]	0.68±0.03	0.03±0.00[a]

Note: Numbers followed by a different letter indicated significant differences between treatments and were calculated by one-way ANOVA followed by Duncan multiple range test (DMRT) ($p<0.05$)

54.92% at a photoperiod of 20L:4D (light:dark) (Gao *et al.,* 2022). George *et al.* (2014) also conducted similar experiments on *Ankistrodesmus falcatus* and discovered that protein production increased with increased illumination duration, light intensity, and prolonged exposure time. As for lipid content, it increased first, then decreased, reaching a maximum of 24.56% at 16:8. At the same time, carbohydrate content decreased first and then increased, reaching a minimum of 19.03% at 20:4. As photoperiods increased from 8:16 to 24:0, pigment content increased from 1.06 to 2.77% (Gao *et al.,* 2022). As previously stated, lipids and carbohydrates have two competing pathways for storing production in microalgae.

Short light exposure in microalgae can form stress signals, thereby encouraging cells to convert excess glucose into lipids and reduce cell division (Mitra, Leeuwen & Lamsal, 2012). This stress adaptation event is analogous to the modifications caused by hyperostosis or nitrogen limitation, which causes a rise in lipid synthesis (Hirai *et al.,* 2016). As a result, the principal chemical compositions of algae change as light conditions change, demonstrating that photoperiod is a significant factor in influencing the levels of chemical compositions in algal cells.

Cell wall resistance, solvent type, and extraction procedures all impact pigment extraction. To extract chlorophyll and fucoxanthin, methanol is used (Palanisamy *et al.,* 2022). Because chlorophyll accessory antenna transfers photosynthetic chemical energy from 10 NADPH2 to lipid, protein, and nucleic acid synthesis, its content must be quantified (Palanisamy *et al.,* 2022). Several studies have discovered that the type of solvent, photoperiod, wavelength, nitrogen starvation, and other physico-chemical properties significantly impact pigment content (Bhattacharjya *et al.*, 2020). In this research, the chlorophyll content was calculated and showed that the control of cultivation produced the highest quantities of chlorophyll and carotene pigments compared to treatment. This indicates that continuous light conditions are the optimum conditions for producing chlorophyll and carotenoids. However, in conditions of excess light, microalgae cells reduce the production and accumulation of chlorophyll to avoid excessive energy requirements, thereby reducing photodamage or photoinhibition due to too much light (Li *et al.,* 2017).

Paramylon production can be increased during the exponential growth phase (Mahapatra *et al.*, 2013), as well as by co-culture of *Pseudoalteromonas* sp. or marine microbes during the logarithmic phase. Another element that influences the existence of paramylon is light circumstances which affect the accumulation time of paramylon when grown in the dark versus grown in the light (Zeng *et al.,* 2016). In this research, the highest concentration of paramylon obtained at treatment photoperiod 12:12 was 95.47% for 15 days (Table 2). As a result, *Euglena* sp. produced high amounts of paramylon with a combination of light and darkness. As stated in a previous study, dark cultivation is an excellent strategy to accumulate paramylon until 80% of total biomass (Kim *et al.,* 2021). Besides that, higher irradiation levels, on the other hand, contribute to carbohydrate build up when grown in the light (Matsuda, Hayashi & Kondo, 2011). So, for the formation of paramylon, which is a carbohydrate derivative, it requires a combination of light and darkness.

Paramylon is an insoluble, linear, high-molecular-weight 1,3-glucan that occurs naturally in crystalline form. The euglenoids accumulate paramylon in granules that may be widely dispersed throughout the cytoplasm, form caps over

the pyrenoids or be packed. The various types of paramylon are classified into six morphological categories, all of which are present in various euglenids. Uridine diphosphate glucose (UDP-Glc) donates the glucose moiety that is subsequently linked to form the expanding polysaccharide (Skodová-Sveráková *et al.,* 2020). The sugar nucleotide formation depends on the enzymes UTP (D-glucose uridylyltransferase) or UDP-Glc pyrophosphorylase (Muchut *et al.,* 2018). In euglenoids, the appearance of paramylon requires the activity of paramylon synthase, a membrane-bound enzyme complex of approximately 670 kDa belonging to the eukaryotic glycosyltransferase 48 (GT48) family (Skodová-Sveráková *et al.,* 2020). In *Euglena gracilis*, the glucan synthase-like genes EgGSL1 and EgGSL2 have been identified. They encode 304 kDa and 258 kDa proteins with 15 and 19 transmembrane domains, respectively (Tanaka *et al.,* 2017). Tanaka *et al.* (2017) reported that paramylon synthesis depends on the activity of EgGSL2, which forms complexes with 37 and 54 kDa UDP-binding proteins. The membrane fraction surrounding the paramylon granules is associated with the activity of paramylon synthase (Skodová- Sveráková *et al.,* 2020).

CONCLUSION

This study found that photoperiod treatment had a significant effect on lipid, chlorophyll-a, and carotenoid levels of *Euglena* sp. in the control treatment (full light), with the highest total levels as follows: 0.52±0.03 g/L, 1.2±0.01 x 10^{-2} g/L, 0.3±0.02 x10^{-2} g/L, respectively; while the others were not significantly affected by the treatment. Photoperiod was able to increase the levels of metabolites when compared to control conditions, namely the highest protein content at full dark was 3.1±0.2x 10^{-2} g/L; chlorophyll-b at photoperiod 14:10 was 0.7±0.03 x10^{-2} g/L; and paramylon at photoperiod 12:12 was 1.9±0.02x10^{-1} g/L. The highest carbohydrate content was found in control conditions, with levels of 1.2±0.02 g/L. *Euglena* is a protist with high adaptability because it can live as an autotroph, heterotroph, or mixotroph, and the presence of a photoperiod does not significantly influence the accumulation of *Euglena* metabolites. Therefore, it is necessary to carry out further studies related to *Euglena*'s limitations in adapting to the environment so that it triggers stress and increases the targeted metabolisms.

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Authors' contributions

Khusnul QM, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; Tia E, advised on data analysis and interpretation, and reviewed the manuscript; Istini N, led the data collection and reviewed the manuscript; Ria A, reviewed the manuscript; Dedy K, reviewed the manuscript; Brilian RS, led the data analysis and reviewed the manuscript; Revata M, conducted the study; Bambang RA, conducted data analysis and interpretation; Arief B, assisted in drafting of the manuscript, Eko AS reviewed the manuscript.

Conflict of interest

Authors declare no conflict of interest. Authors have received a research grant from the Indonesian Ministry of Research, Technology and Higher Education (Ristekdikti).

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Demographic characteristics are associated with children's nutritional status: Findings from a pilot study on food insecure households in Simunjan district, Sarawak

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ABSTRACT

Introduction: Food insecurity is a public health concern that may lead to malnutrition in children. The purpose of this study was to determine the relationship between demographic characteristics and food security level with nutritional status among children from food-insecure households in Simunjan District, Sarawak. **Methods:** This study involved a total of 171 mother-and-child pairs from two Maternal and Child Health Clinics (Simunjan and Gedong) using non-probability convenience sampling technique. Food insecurity status was determined using Radimer/Cornell Hunger Food Insecurity Instrument, while child's weight and height were measured following standard procedures. Chi-squared test of independence and binary logistic regression were used during data analysis. **Results:** The prevalences for household food insecurity, individual food insecurity, and child hunger were 70.8%, 15.2%, and 14.0%, respectively. The main nutritional problems for children aged 24–59 months were underweight (17.9%) and stunting (17.9%), while for children aged 60–144 months were overweight and obesity (27.5%). Children of mothers over 34 years old (*AOR*=2.367; 95% *CI*: 1.085, 5.164), and those aged 60–144 months (*AOR*=3.619; 95% *CI*: 1.521, 8.613) had increased odds of being overweight or obese. Meanwhile, children of working mothers (*AOR*=6.526; 95% *CI*: 1.108, 38.449) were more likely to have a thinness problem than children of unemployed mothers. However, no association was found between the severity of food insecurity with children's nutritional status. **Conclusion:** Malnutrition in children remains a public health concern in Simunjan District, and it is linked to mother's age and employment status. An intervention programme is required to ameliorate the situation.

Keywords: children, nutritional status, demographic characteristics, food insecurity

INTRODUCTION

Food is a vital component of life, and getting enough food is a basic human right. Therefore, food security is an important agenda in a country. The National Plan of Action for Nutrition of Malaysia (NPANM) III, 2016–2025, is the master plan to achieve optimal nutritional well-being for Malaysians. Out of 46 NPANM III indicators, nine are related to food and nutrition security (NCCFN, 2016). Food security

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is described as a scenario in which all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life (FAO, 2002).

In 2021, an estimated 29.3% of the world's population, or approximately 2.3 billion people, were moderately or severely food insecure. In the Asia Region, the subregion that recorded the highest level of food insecurity was Southern Asia, where 40.6% of the population experienced moderate or severe food insecurity in 2021, followed by Western Asia (33.7%), South-eastern Asia (20.7%), and Central Asia (20.2%); Eastern Asia recorded the lowest level of food insecurity, which was 6.2% (FAO, IFAD, UNICEF, WFP & WHO, 2022). In Malaysia, according to the National Health and Morbidity Survey (NHMS) in 2014, food insecurity in terms of inadequate food quantity of purchased foods was 24.9%. By comparison between regions, East Malaysia was found to have a much higher prevalence of food insecurity (39.2%) than West Malaysia (21.1%) (IPH, 2014).

Food insecurity is caused by various comprehensive factors, including population growth, natural disasters, international commerce, demographic characteristics, economic factors, environmental factors, institutional factors, and socio-cultural factors (Sewnet & Sewnet, 2015). Family structure, number of children, household size, education level of mothers, household income, and poverty are factors under economy and demography that influence the level of food security (Hannum, Liu & Frongillo, 2014). Food insecurity is a public health issue that has various effects and may ultimately result in malnutrition (Ali Naser *et al.*, 2014). Children from food-insecure households were reportedly more likely to be underweight and stunted than children from food-secure households (Betebo *et al.*, 2017). With that, it is important to focus on addressing the problem of food insecurity because it will directly improve the nutritional status of children.

Since food insecurity is still an important issue and has a significant impact on health status, various efforts and intervention programmes have been planned and implemented by relevant agencies, such as the Ministry of Agriculture, Ministry of Higher Education, and Economy Planning Unit to improve Malaysia's situation (NCCFN, 2016). Based on studies conducted in Malaysia over the past two decades, research areas have mainly focused on the prevalence of food insecurity, severity of food insecurity, factors associated with food insecurity, and the association between food insecurity and health status among indigenous people, low-income households, university students, elderly population, and migrant workers. There is a lack of exploration on food insecurity issues, particularly among Sarawakians.

In this respect, this pilot study aimed to determine the relationship between demographic characteristics and food security level with nutritional status among children from food-insecure households in Simunjan District, Sarawak.

MATERIALS AND METHODS

Study design and samples

A cross-sectional study was conducted for two months, from March to April 2022 in Simunjan District, which is one of the 40 districts under the administration of the Sarawak State Government. It is approximately 2,217.7km^2, located about 176km from Kuching, the capital city of Sarawak. Simunjan District reportedly has the highest incidence of poverty among the administrative

districts in the southern region of Sarawak (DOSM, 2020). Given the close association between poverty and food insecurity, as indicated by Hannum *et al.* (2014), and the specific focus of this study on households experiencing food insecurity, the rationale for selecting this district as the research setting is thus justified.

As this pilot study was conducted among children from households experiencing food insecurity to determine the relationship between demographic characteristics and food security level with their nutritional status, a non-probability convenience sampling method was used. After discussions were held with the Samarahan Division Health Office, taking into consideration logistics feasibility and financial factors, study respondents were recruited from the Maternal and Child Health Clinics (MCHC) in Simunjan and Gedong, two clinics in the Simunjan District that serve populations with similar demographic characteristics. The study inclusion criteria were children aged 2–12 years old with mothers aged 15–49 years old from food insecure households, while the exclusion criteria were children suffering from delays in mental development and with physical disability. All children attending the clinics during the data collection period (March to April 2022) who met the inclusion and exclusion criteria were screened for food security status using the Radimer/Cornell Hunger and Food Insecurity Instrument (Radimer, Olson & Campbell, 1990). Only mother-and-child pairs from food insecure households were invited to participate in this study.

Study protocol

Ethical clearance was obtained from the Medical Research Ethics Committee, Universiti Malaysia Sarawak (Ethics Approval code: FME/21/13). Approval from the Medical Research Ethics Committee (MREC), Ministry of Health Malaysia was subsequently obtained, registered in the National Medical Research Register (NMRR) system [Approval code: NMRR ID-21-02001-HDV (IIR)]. All invited participants were given the respondent's information sheet and consent form. Only participants who provided consent were recruited in the study.

Data collection

Demographic characteristics

A questionnaire in the Malay language was used for data collection. All pertinent research information were collected through individual face-to-face interviews with the mothers. The demographic characteristics of the respondents consisted of age, gender, mother's age, ethnicity, religion, marital status, number of children, household size, parent's highest education level, parent's occupation, and household income.

Household food insecurity measurement

The Radimer/Cornell Hunger and Food Insecurity Instrument was used to assess the degree of household food insecurity (Radimer *et al.*, 1990). There were ten items and each item had three options: "not true," "sometimes true," or "often true." The ten items were then translated into the Malay language version by Sharif & Ang (2001) with good internal consistency (Cronbach's alpha ranged from 0.8-0.9). Ten items reflected four levels of food insecurity with increasing severity as shown in Table 1.

Anthropometric measurements

Anthropometric measurements were conducted by two trained research assistants in accordance with established standard protocols (IPH, 2020). Each measurement was taken twice, then the mean value of the two measurements was recorded and used for further analyses.

Table 1. Classification of food security according to the Radimer/Cornell Scale

Food security status	*Indicator*
Food security	Negative answers to all hunger and food insecurity items†
Individual food insecurity	Positive answer to at least one of items 1 to 4‡
Household food insecurity	Positive answer to at least one of items 5 to 8‡
Child hunger	Positive answer to items 9 and 10‡

†Negative answer is referred to as "not true".
‡Positive answer is referred to as "sometimes true" or "often true".
(Radimer *et al.*, 1996)

Body weight of children was measured using a calibrated weighing scale (Tanita HD-662, Japan), with minimum clothing, barefooted, and in a straight posture. The reading was recorded to the nearest 0.1 kg. Height of the children was measured using a portable stadiometer (SECA 213, Germany), with head, shoulders, buttocks, knees, and heels touching the board, and without shoes. The reading was recorded to the nearest 0.1 cm. The age of the children were calculated in months from their date of birth.

The World Health Organization (WHO) Anthro 3.2 software and WHO AnthroPlus software were used to obtain z-scores for four nutritional indicators, namely weight-for-age (WAZ), height-for-age (HAZ), weight-for-height (WHZ), and BMI-for-age (BAZ). The status of malnutrition for children aged 24–60 months was categorised according to the WHO Child Growth Standards (WHO, 2006), based on the six conditions, namely underweight [WAZ<-2 standard deviation (*SD*)], stunting (HAZ<-2*SD*), wasting (BAZ<-2*SD*), possible risk of overweight (BAZ>+1*SD* to ≤+2*SD*), overweight (BAZ>+2*SD* to ≤+3*SD*), and obese (BAZ>+3*SD*). Meanwhile, for children aged 61 to 144 months, the status of malnutrition was categorised according to the Growth Reference Data for 5-19 Years (WHO, 2007), based on the five conditions, namely underweight (WAZ<-2*SD* for children aged 61 to 120 months), stunting (HAZ<-2*SD*), thinness (BAZ<-2*SD*), overweight (BAZ>+1*SD* to ≤+2*SD*), and obese (BAZ >+2*SD*) (WHO, 2006; WHO, 2007).

Data analysis

Data were analysed using the IBM SPSS Statistics for Windows version 26.0 (IBM Corporation, Armonk, New York, USA). The frequency, mean, percentage, and standard deviation for demographic characteristics, food insecurity status, and nutritional status of children were determined using descriptive analysis. Chi-squared test, Fisher's Exact test or Likelihood Ratio were used to determine the associations between demographic characteristics and food insecurity status with nutritional status of children. Then, determinant factors (variables of demographic characteristics and food insecurity status with $p<0.05$ in the Chi-squared test) for nutritional status of children were subsequently determined by performing a simple logistic regression analysis.

RESULTS

The descriptive statistics for demographic characteristics, level of food insecurity, and children's nutritional status are illustrated in Table 2. A total of 171 children participated in this study. The age of children ranged 24–140 months, with a mean age of 65.9±30.6 months. The proportions of children aged 24–59 months and 60–144 months were equally distributed, at 50.3% and 49.7%, respectively. The maternal age ranged

Table 2. Characteristics of mothers, children and households (*N*=171)

Characteristics	*n*	*%*	*Mean±SD*
Age of children (months)			65.9±30.6
24-59	84	49.1	
60-144	87	50.9	
Sex of children			
Male	75	43.9	
Female	96	56.1	
Maternal age			32.6±6.1
<25 years	13	7.6	
25-34 years	96	56.1	
35-44 years	54	31.6	
≥45years	8	4.7	
Ethnicity			
Malay	145	84.8	
Iban	19	11.1	
Others	7	4.1	
Religion			
Islam	147	86.0	
Christianity	24	14.0	
Marital status			
Married	165	96.5	
Divorced/Separated	6	3.5	
Number of children in the household			3±1
<3	76	44.4	
≥3	95	55.6	
Household size			6±2
<7	115	67.3	
≥7	56	32.7	
Maternal highest education			
No formal/Primary education	18	10.5	
Secondary education	120	70.2	
Tertiary education	33	19.3	
Paternal highest education (*N*=165)†			
No formal/Primary education	28	17.0	
Secondary education	120	72.7	
Tertiary education	17	10.3	
Maternal occupation			
Housewife	123	71.9	
Self-employed	20	11.7	
Private	16	9.4	
Government	12	7.0	
Paternal occupation (*N*=165)†			
Self-employed	75	45.5	
Private	64	38.8	
Government	20	12.1	
Unemployed	6	3.6	

Table 2. Characteristics of mothers, children and households (*N*=171) *[continued]*

Characteristics	*n*	*%*	*Mean±SD*
Household income‡			1,935.64±1,506.00
<RM1096 (hard core poor)	51	29.8	
RM1096 – <RM2130 (poor)	76	44.4	
≥RM2131	44	25.7	
Food insecurity status			
Household food insecure	121	70.8	
Individual food insecure	26	15.2	
Child hunger	24	14.0	
Nutritional status of children			
24-59 months (*n*=84)			
Underweight	15	17.9	
Stunting	15	17.9	
Wasting	12	14.3	
Possible risk of overweight	0	0.0	
Overweight	4	4.8	
Obese	4	4.8	
60-144 months (*n*=87)			
Underweight (*n*=73)	10	13.7	
Stunting	14	16.1	
Thinness	6	6.9	
Overweight	7	8.0	
Obese	17	19.5	

†6 respondents divorced
‡Department of Statistics, Malaysia Sarawak, 2020

from 21 to 47 years old, with a mean age of 32.6±6.1 years old and majority were Malays (84.8%). Three-quarters (74.2%) of the respondents were living in a poor household. Of the 127 respondents from poor households, 51 of them (40.2%) were in the range of hard-core poor. With regards to the level of food insecurity, of the 171 respondents from food-insecure households who agreed to participate in this study, the majority were experiencing household food insecurity (70.8%), followed by 15.2% adult food insecurity, and 14.0% child hunger. In terms of children's nutritional status, the descriptive analysis revealed that children in this study had both undernutrition and overnutrition problems. For children aged 24–59 months, it was found that the main nutritional problems were underweight (17.9%), stunting (17.9%) and wasting (14.3%); while for children aged 60–144 months, the main nutritional problems were overweight/obesity (27.5%) and stunting (16.1%).

Association between demographic characteristics, level of food insecurity, and nutritional status of children

The associations between demographic characteristics, level of food insecurity, and nutritional status of children are presented in Table 3. Out of twelve variables, three variables were found to be significantly associated with a child's nutritional status, namely age of mother [$\chi^2(1, N=171) = 4.846$, $p=0.028$], occupation of mother ($p=0.040$), and

Table 3. Associations between demographic characteristics, level of food insecurity, and nutritional status of children aged 2–12 years old

Characteristics	*Weight-for-age* [a] *(WAZ)*			*Height-for-age* [b] *(HAZ)*			*Weight-for-height* [c] *(WHZ)*			*BMI-for-age* [d] *(BAZ)*			*BMI-for-age* [e] *(BAZ)*		
	Non-underweight (n=132) (%)	*Underweight (n=25) (%)*	χ^2 *(p-value)*	*Non-stunting (n=142) (%)*	*Stunting (n=29) (%)*	χ^2 *(p-value)*	*Non-wasting (n=72) (%)*	*Wasting (n=12) (%)*	χ^2 *(p-value)*	*Non-thinness (n=81) (%)*	*Thinness (n=6) (%)*	χ^2 *(p-value)*	*Non-overweight/ Obese (n=129) (%)*	*Overweight/ Obese (n=32) (%)*	χ^2 *(p-value)*
Age of children															
24–59 months	69 (82.1)	15 (17.9)	0.505 (0.478)	69 (82.1)	15 (17.9)	0.095 (0.758)	Nil	Nil	Nil	Nil	Nil	Nil	76 (90.5)	8 (9.5)	9.166 (0.002)
60–144months	63 (86.3)	10 (13.7)		73 (83.9)	14 (16.1)								63 (72.4)	24 (27.6)	
Sex of children															
Male	58 (81.7)	13 (18.3)	0.551 (0.458)	63 (84.0)	12 (16.0)	0.087 (0.768)	28 (82.4)	6 (17.6)	(0.534)†	37 (90.2)	4 (9.8)	(0.415)†	61 (81.3)	14 (18.7)	0.000 (0.989)
Female	74 (86.0)	12 (14.0)		79 (82.3)	17 (17.7)		44 (88.0)	6 (12.0)		44 (95.7)	2 (4.3)		78 (81.3)	18 (18.8)	
Maternal age															
≤ 34 years old	88 (84.6)	16 (15.4)	0.067 (0.796)	94 (86.2)	15 (13.8)	2.183 (0.140)	52 (82.5)	11 (17.5)	(0.279)†	43 (93.5)	3 (6.5)	(1.000)†	94 (86.2)	15 (13.8)	4.846 (0.028)
≥ 35 years old	44 (83.0)	9 (17.0)		48 (77.4)	14 (22.6)		20 (95.2)	1 (4.8)		38 (92.7)	3 (7.3)		45 (72.6)	17 (27.4)	
Marital status															
Married	126 (83.4)	55 (16.6)	(0.590)†	136 (82.4)	29 (17.6)	(0.591)†	69 (86.3)	11 (13.8)	(0.467)†	79 (92.9)	6 (7.1)	(1.000)†	134 (81.2)	31 (18.8)	(1.000)†
Divorced/ Separated	6 (100.0)	0 (0.0)		6 (100.0)	0 (0.0)		3 (75.0)	1 (25.0)		2 (100.0)	0 (0.0)		5 (83.3)	1 (16.7)	
Number of children															
< 3	64 (85.3)	11 (14.7)	0.169 (0.681)	66 (86.8)	10 (13.2)	1.404 (0.236)	41 (83.7)	8 (16.3)	0.400 (0.527)	27 (100.0)	0 (0.0)	(0.171)†	64 (84.2)	12 (15.8)	0.769 (0.381)
≥ 3	68 (82.9)	14 (17.1)		76 (80.0)	19 (20.0)		31 (88.6)	4 (11.4)		54 (90.0)	6 (10.0)		75 (78.9)	20 (21.1)	
Household size															
< 7	87 (82.9)	18 (17.1)	0.352 (0.553)	97 (84.3)	18 (15.7)	0.426 (0.514)	48 (87.3)	7 (12.7)	(0.744)†	55 (91.7)	5 (8.3)	(0.661)†	92 (80.0)	23 (20.0)	0.382 (0.536)
≥ 7	45 (86.5)	7 (13.5)		45 (80.4)	11 (19.6)		24 (82.8)	5 (17.2)		26 (96.3)	1 (3.7)		47 (83.9)	9 (16.1)	
Maternal highest education															
Primary/No formal education	12 (80.0)	3 (20.0)	(0.710)†	15 (83.3)	3 (16.7)	(1.000)†	7 (87.5)	1 (12.5)	(1.000)†	9 (90.0)	1 (10.0)	(0.530)†	17 (94.4)	1 (5.6)	(0.201)†
Secondary/ Tertiary education	120 (84.5)	22 (15.5)		127 (83.0)	26 (17.0)		65 (85.5)	11 (14.5)		72 (93.5)	5 (6.5)		122 (79.7)	31 (20.3)	

Table 3. Associations between demographic characteristics, level of food insecurity, and nutritional status of children aged 2–12 years old [continued]

Characteristics	*Weight-for-age* [a] *(WAZ)*			*Height-for-age* [b] *(HAZ)*			*Weight-for-height* [c] *(WHZ)*			*BMI-for-age* [d] *(BAZ)*			*BMI-for-age* [e] *(BAZ)*		
	Non-underweight (n=132) (%)	*Underweight (n=25) (%)*	χ^2 *(p-value)*	*Non-stunting (n=142) (%)*	*Stunting (n=29) (%)*	χ^2 *(p-value)*	*Non-wasting (n=72) (%)*	*Wasting (n=12) (%)*	χ^2 *(p-value)*	*Non-thinness (n=81) (%)*	*Thinness (n=6) (%)*	χ^2 *(p-value)*	*Non-overweight/ Obese (n=129) (%)*	*Overweight/ Obese (n=32) (%)*	χ^2 *(p-value)*
Paternal highest education [f]															
Primary/No formal education	18 (78.3)	5 (21.7)	(0.542)†	22 (78.6)	6 (21.4)	(0.588)†	9 (90.0)	1 (10.0)	(1.000)†	15 (83.3)	3 (16.7)	(0.106)†	23 (82.1)	5 (17.9)	0.019 (0.890)
Secondary/ Tertiary education	108 (84.4)	20 (15.6)		114 (83.2)	23 (16.8)		60 (85.7)	10 (14.3)		64 (95.5)	3 (4.5)		111 (81.0)	26 (19.0)	
Maternal occupation															
Unemployed	95 (82.6)	20 (17.4)	0.692 (0.406)	104 (84.6)	19 (15.4)	0.711 (0.399)	50 (84.7)	9 (15.3)	0.711 (1.000)	62 (96.9)	2 (3.1)	(0.040)†	102 (82.9)	21 (17.1)	0.775 (0.379)
Employed	37 (88.1)	5 (11.9)		38 (79.2)	10 (20.8)		22 (88.0)	3 (12.0)		19 (82.6)	4 (17.4)		37 (77.1)	11 (22.9)	
Paternal occupation [g]															
Unemployed	2 (50.0)	2 (50.0)	(0.128)†	3 (50.0)	3 (50.0)	(0.068)†	2 (100.0)	0 (0.0)	(1.000)†	3 (75.0)	1 (25.0)	(0.258)†	6 (100.0)	0 (0.0)	(0.595)†
Employed	124 (84.4)	23 (15.6)		133 (83.6)	26 (16.4)		67 (85.9)	11 (14.1)		76 (93.8)	5 (6.2)		128 (80.5)	31 (19.5)	
Household income															
≤ RM2,130	99 (84.6)	18 (15.4)	0.100 (0.752)	108 (85.0)	19 (15.0)	1.400 (0.237)	52 (85.2)	9 (14.8)	(1.000)†	63 (95.5)	3 (4.5)	(0.149)†	104 (81.9)	23 (18.1)	0.118 (0.731)
≥ RM2,131	33 (82.5)	7 (17.5)		34 (77.3)	10 (22.7)		20 (87.0)	3 (13.0)		18 (85.7)	3 (14.3)		35 (79.5)	9 (20.5)	
Level of food insecurity															
Household food insecurity	96 (84.2)	18 (15.8)	‡	102 (84.3)	19 (15.7)	‡	57 (89.1)	7 (10.9)	‡	53 (93.0)	4 (7.0)	‡	95 (78.5)	26 (21.5)	‡
Individual food insecurity	21 (84.0)	4 (16.0)	0.009 (0.995)	21 (80.0)	5 (19.2)	0.472 (0.790)	7 (70.0)	3 (30.0)	2.488 (0.288)	15 (93.8)	1 (6.3)	0.013 (0.993)	21 (80.8)	5 (19.2)	5.164 (0.138)
Child hunger	15 (83.3)	3 (16.7)		19 (79.2)	5 (20.8)		8 (80.0)	2 (20.0)		13 (92.9)	1 (7.1)		23 (95.8)	1 (4.2)	

Chi-squared test; †Fisher's Exact Test; ‡Likelihood Ratio

(α) Non-underweight: z-score ≥ -2*SD*; Underweight: z-score < -2SD; Total subjects 157, 6 subjects >120 months were excluded

(α) Non-stunting: z-score ≥ -2*SD*; Stunting: z-score < -2*SD*

(α) Non-wasting: z-score ≥ -2*SD*; Wasting: z-score < -2*SD*; Total subjects 84, 87 subjects ≥ 60 months were excluded

(α) Non-thinness: z-score ≥ -2*SD*; Thinness: z-score < -2*SD*; Total subjects 87, 84 subjects <60 months were excluded

(α) Non-overweight/Obese: z-score ≤ +1SD (for children aged ≥ 60 months) and ≤ +2*SD* (for children aged < 60 months); Overweight/ Obese: z-score > +1*SD* (for children aged ≥ 60 months) and > +2*SD* (for children aged < 60 months)

(f) &(g) Total subjects different with other variables due to marital status of 6 subjects being divorced/separated

Table 4. Children's nutritional status with associated demographic characteristics by simple logistic regression

Nutritional status	*Demographic characteristics*		*N*	*Adjusted OR*	*95% CI*	*p-value*
Overweight/ Obese	Age of children	24–59 months	84	(Ref)	(Ref)	(Ref)
		60–144 months	87	3.619	(1.521, 8.613)	0.004
	Maternal age	≤ 34 years old	109	(Ref)	(Ref)	(Ref)
		≥ 35 years old	62	2.367	(1.085, 5.164)	0.03
Thinness	Occupation of mothers	Unemployed	64	(Ref)	(Ref)	(Ref)
		Employed	23	6.526	(1.108, 38.449)	0.038

OR: Odds Ratio
CI: Confidence Interval
Omnibus Test of Model Coefficient; Total subjects 171.

age of child [$\chi^2(1, N=171) = 9.166$, $p=0.002$]. Variables with a significant *p*-value were further analysed with simple logistic regression (Table 4). The analysis indicated that children with mothers aged at or more than 35 years old were 2.367 times more likely to be overweight or obese compared to children of mothers less than or equal to 34 years old (*AOR* = 2.367; 95%*CI*: 1.085, 5.164). Children aged 60–144 months possessed increased odds of being overweight or obese, almost 3.5 folds compared to children aged 24–59 months (*AOR*=3.619; 95%*CI*: 1.521, 8.613). This study showed that children of working mothers had a higher percentage (17.4%) or were 6.526 times more likely to have a thinness problem than children of unemployed mothers (*AOR*=6.526; 95%*CI*: 1.108, 38.449). However, no statistically significant association was found between the level of food insecurity and children's malnutrition status ($p>0.05$).

DISCUSSION

This study showed that among the three categories of food insecurity, the highest proportion was household food insecurity, while the proportions for individual food insecurity and child hunger were approximately the same. There are slight differences in the results of this study with previous studies in terms of the proportion of child hunger (Hamid *et al.,* 2021; Mamat, Norhasmah & Mesbah, 2019). Hamid *et al.* (2021) conducted a study on 114 women aged 18–45 years old in Tuba Island, Kedah and found that of the 74 respondents who experienced food insecurity, the highest proportion was household food insecurity (64.9%), and the proportion of child hunger was 23.0%. Another study conducted by Mamat *et al.* (2019) in Mentakab, Pahang on 139 mothers aged 20–59 years old showed that the proportion of child hunger among food-insecure households was 29.3%. Both studies showed higher proportions of child hunger than this study, which was 14.0%. Although the age range of respondents for both studies were almost the same as this study, differences might be due to other demographic characteristics, such as lower percentage of married respondents, lower education attainment (Hamid *et al.*, 2021), different proportion of ethnicity, and bigger household size (Mamat *et al.*, 2019), compared with this study.

The current study findings indicated that more children aged 24–59 months were undernourished than overnourished. This finding is consistent

with NHMS (2019), where the prevalence of undernutrition (underweight 14.1%; stunting 21.8%; wasting 9.7%) was higher than overnutrition (overweight 5.6%; obese 5.6%) (IPH, 2020). In Sarawak, a study conducted by Rahman, Kiyu & Seling (2021) reported that the proportion of wasting was lower than in the present study, which was 6.9%. The difference in proportion may be due to different demographic characteristics, as the previous study was conducted among the Dayak community and in all divisions of Sarawak. In contrast, the current study was only conducted in the district of Simunjan only. Besides that, it might be due to the difference in the proportion of poor households between these two studies. Poor household is one of the factors related to wasting in children (Rahman *et al.*, 2021). Undoubtedly, the proportion of poor households in this study (74.2%) was much higher than the study conducted by Rahman *et al.* (2021), which was 27.3%. According to the Nutrition Landscape Information System's proposed prevalence thresholds, which are set to map countries based on severity levels, the prevalences of underweight and wasting in this study were categorised as medium and serious public health significance, respectively (de Onis *et al.*, 2019).

For children aged 60–144 months in this study, it was found that the prevalence of underweight was similar to the national prevalence (15.4%) and the same trend was also observed in the proportion of overweight or obesity (29.8%). Conversely, the prevalence of thinness in the present study (6.9%) was lower compared to the national prevalence (10.0%) (IPH 2020). The prevalence of overweight and obesity is inconsistent with the result of Cheah (2019), which reported a much higher rate (36.9%) than this study. It might be due to the differences in study areas. Both studies were conducted in Sarawak with the majority of Malay ethnicity. Still, the difference was the study area, which was conducted in a rural area, while the study of Cheah (2019) was conducted in an urban area. According to Agbozo *et al.* (2016), children who live in urban areas will have more problems with being overweight than underweight.

In the present study, the analysis indicated that older children (60–144 months) have a higher prevalence and significantly increased odds of being obese (almost fourfold) than younger children (24–59 months). The finding is consistent with previous research, where the proportion of overweight subjects increased with age (Abdelkarim *et al.*, 2020). In the United States, being overweight is more prevalent among girls aged 2–5 years old. Although there was no gender difference of being overweight among 2–5 years old in this study (data not shown), it did show the same trend in the increasing percentage of obese children with the advancement of age during childhood. This situation is due to changes in eating patterns and physical activity. Unhealthy eating patterns (excessive calorie intake) and too little physical activity as age increases during childhood contribute to this phenomenon (New York State Department of Health, 2012).

This study documented a significant association between the mother's age and the child's overweight and obese status. Children of mothers over 34 years old were more likely to be overweight or obese compared to children of mothers less than or equal to 34 years old. This finding is similar to a previous study (Barclay & Myrskyla, 2016), which found that children of older mothers were more likely to be overweight or obese than children of younger mothers. However, this contrasts with a study conducted by Farajian *et al.* (2014), in which children

whose mothers were older had reduced risk of being overweight or obese, with a possible explanation that family health awareness was better in older mothers. The difference in study population might also explain the disparity between these findings. While the study by Farajian *et al.* (2014) was based on children aged 10–12 years old in Greece, the present study was conducted among 2–12 years old children in Simunjan.

In this study, it was found that children of working mothers have higher risk of thinness than those of non-working mothers. Contrary to a previous study conducted among children aged 5–10 years in Sri Lanka by Shinsugi *et al.* (2019), thinness was highly prevalent among children of mothers who were housewives or not working. Therefore, the impact of maternal employment on child malnutrition is debatable. According to Ghosh (2020), children with working mothers will be well-nourished because involvement in work will mean more empowerment for the mothers. This will allow them to make better decisions in nutrition and child care. However, with employment, mothers with long working hours will have limited time spent with their children, and this could lead to malnutrition among children. Nonetheless, a theory stated that employment status is related to maternal education level and knowledge. In the study conducted by Fernández-Cornejo *et al.* (2015) among 2383 university students, it was found that female students were more likely to accept some sacrifice in their career progression for family reasons. This could be because they believed that their caregiving responsibilities require more time and effort than their career responsibilities. A woman who attains a higher level of education and possesses significant knowledge, but chooses to be a housewife or to not engage in formal employment tends to have a child with better health status. Therefore, the findings in this study, where working mothers have higher risk of having thin children than non-working mothers, may be due to the mother's education and knowledge; whereby only one out of five mothers in this study attained tertiary education.

Household food insecurity has been identified as a possible underlying determinant of childhood malnutrition (Drammeh, Hamid & Rohana, 2019). From previous studies, it was indicated that food security status increases the likelihood of children being malnourished – both undernutrition and overnutrition (Ali Naser *et al.*, 2014; Shahraki *et al.*, 2016; Papas *et al.*, 2016). However, there was no statistically significant association found between severity of food insecurity and children's malnutrition status in this study. There is overwhelming consensus in the research literature supporting the association between food insecurity and malnutrition in children as most studies conducted globally consistently demonstrate a strong link between food insecurity and adverse nutritional outcomes in children. However, the respondents in this study were mother-and-child pairs from food-insecure households, focusing on the relationship between the severity of food insecurity (household food insecurity, individual food insecurity, and child hunger) and child's malnutrition status, not between food security (food-secure household and food-insecure household) and child's malnutrition status. Furthermore, due to the small sample size of this study, causing the analyses to be inadequately powered, we were eventually unable to demonstrate the association between the severity of food insecurity and a child's malnutrition status.

Strengths and limitations

This study attempted to assess the severity of food insecurity among food-insecure households in the district that reportedly had the highest incidence of poverty in the southern region of Sarawak, which provided some insights into factors that contribute to the conditions of malnutrition in children. Besides providing information for designing, developing, and implementing intervention programmes at a later phase, this study would also provide the local authorities or related agencies with ideas on how resources can be allocated and the steps necessary to improve the food insecurity situation, which would eventually help improve the nutritional status of children. Additionally, trained researchers measured and collected all of the data and information in this study, which reduced potential bias compared to a study based on self-reported data. Furthermore, the study equipment and tools used were non-invasive, less time-consuming, and cheaper.

As this was only a cross-sectional study, it could only describe the relationship between demographic characteristics, the severity of food insecurity, and a child's malnutrition status, but not explain its causal effect. In addition, the sampling technique used was non-probability convenience sampling, hence, the present study findings would not apply to a broader population in general. Furthermore, there is a possibility that the statement regarding food insecurity given by the respondent was untrue due to embarrassment, which may cause bias (Shahraki *et al.*, 2016). Lastly, because of the small sample size and the fact that it only included households with children aged 2–12 years old, the findings may have overlooked the impact of other factors on the severity of food insecurity and children's malnutrition status.

CONCLUSION

Among the three food insecurity indicators, household food insecurity was more prevalent than individual food insecurity and child hunger among the household in Simunjan, Sarawak. At the same time, childhood underweight, stunting, overweight and obesity remained the main malnutrition problems in this district. The present study illustrated that the age of mother, employment status, and child's age are associated with malnutrition in children. However, no association was found between the severity of food insecurity and children's malnutrition status. As the prevalence of underweight and wasting in this study were categorised as medium and serious public health significance, respectively, therefore a programme focusing on improving household food insecurity and children's nutritional status should be conducted so that the situation will not worsen. Programmes, such as home gardening or small-scale farming, can be conducted to promote diverse and nutritious food production that can positively impact food availability and accessibility. Besides that, nutrition education campaigns can be undertaken to promote proper feeding practices and encourage healthy food consumption. These programmes focus on food security and nutrition aspects, which will benefit children's nutritional status. Further studies need to be carried out to determine children's dietary intake and quality, which may also contribute to malnutrition.

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Author's contributions

Tan BC, conceptualised and designed the study, conducted the study, conducted data collection, data analysis and interpretation, and prepared the draft of the manuscript; Cheah WL, principal investigator, conceptualised and designed the study, reviewed data analysis, interpretation, and the manuscript; Law LS, the grant owner, conceptualised and designed the study, reviewed data analysis, interpretation, and the manuscript.

Conflict of interest

The authors declare no conflicts of interest in undertaking this study.

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Rice bran oil reduces organ-specific fat deposition, attenuates hyperlipidaemia and abnormal liver function in Long Evans rats with high fat intake

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ABSTRACT

Introduction: High-calorie diets, particularly the quality of dietary fats, are regarded as an independent risk factor for developing obesity, hyperlipidaemia, and liver diseases. The present study examined the impact of rice bran oil (RBO) on organ-specific fat deposition, lipid profile, and liver function enzymes in Long Evans rats. **Methods:** Long Evans rats (n=24) were fed for six weeks with a controlled high-fat diet (HFD) to induce hyperlipidaemia and abnormal liver function. Rats were then divided into two groups: one group continued feeding on HFD, and the other group was fed with a RBO diet, replacing the fat source. After six weeks of feeding, six rats from each group were sacrificed and required analytical tests were performed. The remaining obese rats (n=12) were divided into continued HFD and RBO diet, and after sacrificing, essential analytical tests were done. **Results:** RBO feeding to hyperlipidaemic rats for six weeks significantly reduced brown adipose tissue, abdominal adipose tissue, epididymal adipose tissue, and liver fat compared to continuing HFD group (p<0.05). Similarly, serum levels of total cholesterol, triacylglycerides, and low-density lipoprotein cholesterol were all decreased, whereas high-density lipoprotein cholesterol increased in response to RBO compared to HFD (p<0.05). Additionally, rats fed with RBO showed reduced alanine aminotransferase, aspartate aminotransferase, and gamma-glutamyl transferase levels when compared with continuing HFD-fed rats (p<0.05). **Conclusion:** These findings suggest that RBO supports the reduction of fat storage from major fat depots, controls lipid profile, and restores healthy liver functions in rats.

Keywords: fat depots, high fat diet, hyperlipidaemia, liver enzymes, rice bran oil

INTRODUCTION

Overweight and obesity is a major risk factor for type 2 diabetes, cardiovascular diseases (CVD), some cancers, and overall mortality (Gray *et al.,* 2015). Dietary management has been recognised as a critical aspect of therapy for obesity since it can potentially improve adiposity and its related co-morbidities. Reducing saturated fat and cholesterol intakes through dietary interventions is an effective strategy in treating CVD

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and obesity-related disorders. Although extra energy consumed in any form of macronutrients can be converted and stored in the body as fat (Galgani & Ravussin, 2008), the amount and type of fat (saturated or unsaturated) consumed in the diet are significant contributors to the development of adiposity and hyperlipidaemia (Beulen *et al.,* 2018). Polyunsaturated fatty acids (PUFAs) are widely accepted as part of a healthy diet because of their beneficial effects on metabolism (Zarate *et al.,* 2017). Observational studies have suggested that consumption of PUFAs (omega-3 and omega-6 fatty acids) may reduce abdominal fat by decreasing fat cell size and contribute towards improving body composition by increasing metabolism and fat burning potential (Albar, 2022). Therefore, replacing saturated fat intake with PUFAs through dietary means can be a realistic approach to managing obesity and reducing hyperlipidaemia.

The coexistence of hyperlipidaemia and abnormal liver function are well-established risk factors for developing metabolic disorders, including CVD and diabetes (Chithra *et al.,* 2015). As a crucial metabolic organ, the liver plays a crucial role in lipid and lipoprotein metabolism, including the biosynthesis of cholesterol, fatty acids, apolipoproteins, and proteins involved in lipoprotein homeostasis. Proper liver function is essential for the regulation of these metabolic processes. Gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) are commonly used as markers of hepatic abnormality and are associated with numerous disease conditions, including non-alcoholic fatty liver disease (NAFLD) (Juo & Livingston, 2019). Recent studies have shown that a higher accumulation of adipose tissues in different fat depots can alter the endocrine and paracrine functions of these active organs. These changes can lead to insulin resistance, dysregulation of glucose and lipid metabolisms, coagulation, and inflammation, ultimately contributing to the progression of cardiometabolic diseases (Lim & Meigs, 2014).

Cooking oil is one of the vital contributors of fat to the diet. Rice bran oil (RBO) is a major source of PUFA, containing approximately 30% linoleic acid and 44% oleic acid with less (approximately 23%) saturated fatty acid. Apart from the better quality fatty acid profile, RBO also contains gamma-oryzanol, vitamin E, and phytosterols, which have higher antioxidant potentials compared to the other available cooking oils (Latha & Nasirullah, 2014). However, it is still uncertain whether RBO administration can be an effective strategy for preventing CVD and its associated co-morbidities. The present study aimed to elucidate the impact of RBO on lipid profiles, liver enzymes, and organ-specific fat deposition in obese Long Evans rats.

MATERIALS AND METHODS

Experimental animals

Twenty-four (12 males and 12 females) Long Evans (*Rattns norvigicus*) rats (4–5 weeks of age, body weight ~80 g) were obtained from the Animal House, International Center for Diarrheal Disease Research (ICDDR, B) Dhaka, Bangladesh. Rats were housed in a temperature-controlled laboratory room at 23±5°C with a 12:12 hour light–dark cycle and allowed to adapt to laboratory conditions for a week. All rats were maintained in the animal care facilities according to animal care and use guidelines. All experiments conducted in this study were approved by the Ethical Review Committee for the protection of human and animal subjects at the Department of Food Technology and Nutritional Science in Mawlana Bhashani Science and Technology

University, which gave full approval with the ethical approval number MBSTU/FTNS/42/2022/14.

Study design

The rats were randomly divided into two groups, namely control group (*n*=6) and high-fat diet (HFD) group (*n*=18), and were fed with a control diet and HFD, respectively, as described in Table 1. After six weeks of feeding, six rats from each of the control group and HFD group were sacrificed. In the next phase, according to Lee index (Bernardis, 1970), the obese rats from the HFD group (*n*=12) were further divided into two study groups. One group continued to receive HFD (continuing HFD group) and for the other group, dietary fat was replaced with RBO (RBO group). The rats from both groups were sacrificed after 6 weeks of feeding with respective diets. Figure 1 presents the design of the study and experimental protocols.

Preparation of control and experimental diets

Table 1 lists all the ingredients used to prepare the control and experimental diets. The ingredients were purchased from the local market and the diet was formulated following a standard protocol (Mridha *et al.,* 2010). RBO was purchased from a local market (Saffola Active, Marico Bangladesh Ltd, Dhaka, Bangladesh). According to the manufacturer's instructions, Saffola Active RBO contains saturated fatty acids 20%, monounsaturated fatty acids 39%, PUFA 40%, alpha-linolenic acids 2.4%, oryzanol 400 mg/100 g, vitamin E 25 mg/100 g, and energy 900 kcal/100 g, with no protein and carbohydrate. Rats had *ad libitum* access to water and diet.

Measurement of body weight

Body weight of experimental rats was recorded in triplicate at baseline

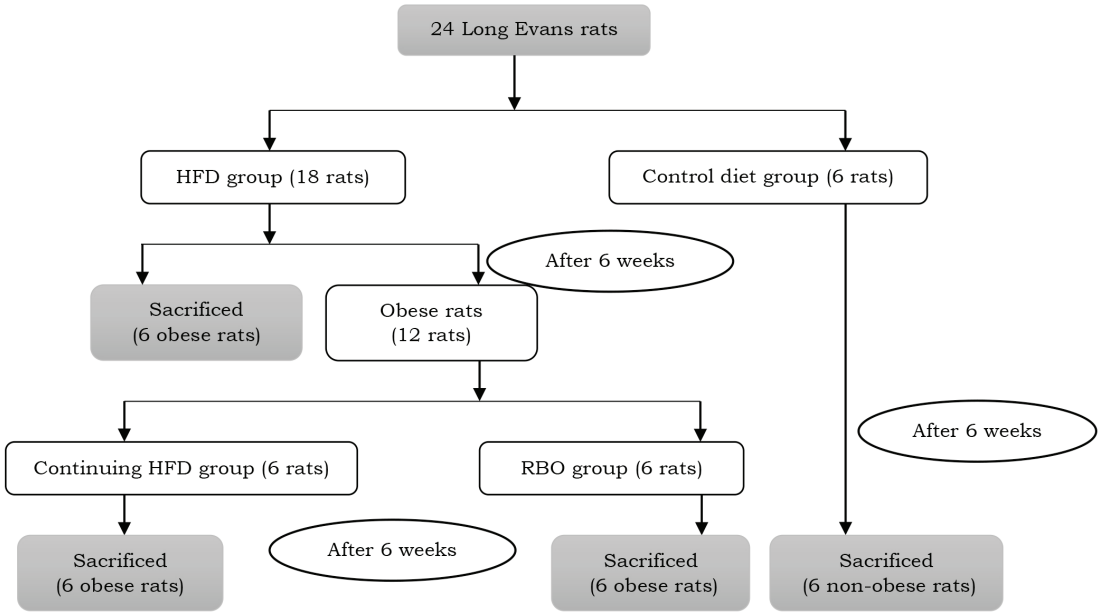

Figure 1. Flowchart of experimental protocols

Table 1. Composition of experimental diets (g/100 g) (Mridha *et al.,* 2010)

Ingredients	*Control diet (g)*	*High-fat diet (HFD) (g)*	*Rice bran oil (RBO) diet (g)*
Wheat	30.0	30.0	30.0
Wheat bran	25.0	25.0	25.0
Rice polish	25.0	25.0	25.0
Ghee	0.0	10.0	0.0
Egg yolk	0.0	5.0	0.0
Rice bran oil	0.0	0.0	15.0
Fish meal	7.0	7.0	7.0
Soybean cake	7.0	7.0	7.0
Vitamin GS	0.5	0.5	0.5
Soybean oil	1.5	1.5	1.5
Salt	1.5	1.5	1.5
Molasses	2.5	2.5	2.5

and weekly until the last day of the experiment. Average weight was used to calculate body weight gain per week.

Serum separation

At the end of each treatment period, rats were fasted for 12 hours and placed in metabolic cages. The rats were then insensible to an intraperitoneal injection of ketamin K (5 mg/100 g body weight; Abbott, IL, USA). Blood was sampled from the abdominal aorta into a sterile syringe and then into a tube, and allowed to clot at room temperature. The tubes were then centrifuged at 3000 rpm for 10 minutes, maintaining the temperature at 4°C (Clay-Adams Co. Inc. centrifuge, New York, USA). Serum was stored at -40°C until analysis.

Tissue isolation

Three regions of adipose tissue were carefully dissected, and interscapular brown adipose tissue (IBAT) was dissected as brown adipose tissue (BAT); dorsolumber, inguinal, and gluteal posterior subcutaneous depots were dissected as abdominal adipose tissue (AAT); and mediastinic, retroperitoneal, gonadal, and perirenal visceral depots were dissected as epididymal adipose tissue (EAT). For collecting IBAT, the rat was placed on its abdomen, with its head towards the investigator. To moisten the coat and avoid contaminating the samples with hair, the shoulder region was thoroughly rinsed with 70% ethanol. The skin was gripped with a tong and incised from the center of the head to the middle of the back revealing the butterfly shape of IBAT which was then carefully dissected as IBAT (Casteilla *et al.,* 2008). For collecting AAT, the rat was placed on its back with its tail towards the investigator. The abdomen was rinsed with ethanol, and the skin was widely incised. After removing the pad, the lymph nodes present among the fat were then discarded and dissected to harvest the AAT. After removing the AAT, the abdominal wall was opened to extract the genitals (ovaries or testes, according to sex) from the abdominal cavity. EAT was collected carefully by dissecting and gently pulling the fat tissue surrounding the gonadal tract and other tissues. All tissues were collected in warm saline, blotted, and weighed to the nearest milligram (Casteilla *et al.,* 2008).

Measurement of liver fat

Liver fat was determined following standard protocol (Domínguez-Avila *et al.,* 2015). Approximately 11 g of liver was sampled from each rat. The liver sample was then dried in an air oven

at 105°C for 24 hours. The dry weight was recorded and the percentage of water in the liver was calculated from the initial and final weights. The dried livers were crushed and inserted into pre-weighed cellulose thimbles and lipids were extracted for four hours in a Soxhlet apparatus using hexane as the solvent. Hexane was evaporated using a rotary evaporator and the flask was dried to a constant weight. The amount of lipids recovered was then calculated by measuring the difference in weight between the empty flask and its weight after the extraction was completed. This process was repeated three times and the mean value was recorded.

Biochemical measurements

Serum total cholesterol (TC), triacylglyceride (TAG), and high-density lipoprotein cholesterol (HDL-C), ALT,

Table 2. Obesity development phase (0–6 weeks)

A) Effect of high-fat diet (HFD) on body weight (Mean±SEM)

Group	*Initial body weight (g)*	*Final body weight (g)*	*p-value within the group*	*p-value between the groups*
Control diet group	59.0±4.4	207.0±14.0	<0.001	0.204
HFD group	55.0±3.3	231.0±9.5	<0.001	

B) Effect of high-fat diet (HFD) on the weight of different organs, lipid profile, and functional liver enzymes (Mean±SEM)

Parameter	*Control diet group*	*HFD group*	*p-value*
Liver (g)	10.1±0.8	10.2±0.7	0.924
Heart (g)	0.8±0.0	0.9±0.1	0.317
AAT (g)	1.6±0.2	3.8±0.2	<0.001*
EAT (g)	1.2±0.2	2.2±0.2	0.004*
IBAT (g)	0.3±0.1	0.6±0.0	0.001*
Liver fat (%)	2.2±0.1	2.6±0.1	0.010*
TC (mmol/L)	5.7±0.8	6.2±0.7	<0.001*
TAG (mmol/L)	6.4±1.2	6.8±0.8	0.003*
HDL-C (mmol/L)	2.4±0.6	1.8±0.7	<0.001*
LDL-C (mmol/L)	2.1±0.9	3.1±0.2	<0.001*
VLDL-C (mmol/L)	1.3±0.3	1.3±0.2	0.003*
TC/HDL-C	2.4±0.1	3.5±0.0	<0.001*
LDL-C/HDL-C	0.9±0.0	1.7±0.0	<0.001*
ALT (IU/L)	35.0±0.1	52.0±0.1	<0.001*
AST (IU/L)	117.0±0.1	142.0±0.1	<0.001*
ALP (IU/L)	196.0±0.0	202.0±0.1	<0.001*
GGT (IU/L)	5.8±0.1	6.9±0.1	<0.001*

AAT: abdominal adipose tissue; EAT: epididymal adipose tissue; IBAT: interscapular brown adipose tissue, TC: total cholesterol; TAG: triacylglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein cholesterol; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase

Data are expressed as mean±SEM

*p<0.05 when comparing control diet to HFD

AST, alkaline phosphatase (ALP), and GGT were determined using standard clinical methods (enzyme colorimetric and enzyme kinetic methods) with a biochemical autoanalyser (Technicon Instruments Corporation, Tarrytown, NY, USA) using commercial kits (RANDOX kits; Randox Laboratories, Ltd., Antrim, UK). Low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were determined using the Friedwald equations (Friedewald, Levy & Fredrickson, 1972), LDL-C (mg/dL) = TC (mg/dL) – HDL-C (mg/dL) – TAG (mg/dL)/5; VLDL = TAG/5.

Statistical analysis

Descriptive statistics were calculated for all variables by using the IBM SPSS Statistics for Windows version 25.0 (IBM Corp., Armonk, New York, NY, USA) and all values were expressed as mean±SEM. The significance of difference between the means of two groups was determined by independent sample Student's *t*-test. Differences were considered significant at $p<0.05$.

RESULTS

Obesity development phase (0–6 weeks)

Effect of HFD on body weight

Feeding the rats with HFD for six weeks resulted in increased body weight in both the control and HFD groups ($p>0.05$). However, it did not differ significantly between groups ($p<0.05$), as shown in Table 2A.

Effect of HFD on the weights of liver, heart, adipose tissue, and liver fat

HFD caused an insignificant ($p>0.05$) elevation in the weights of the liver and heart when compared to the control diet group in the first phase. The good physiological conditions had mildly increased the weight of the liver. Conversely, the weights of AAT, EAT, BAT, and liver fat increased significantly ($p<0.05$) than the control diet group, as presented in Table 2(B).

Hyperlipidaemic effect of HFD

Six-week feeding of HFD demonstrated a hyperlipidaemic effect in the HFD group and caused a significant elevation in serum TC, TAG, LDL-C, and VLDL cholesterol, but induced a significant reduction in serum HDL-C level compared to the control diet group ($p<0.05$). Lipid profile at week 6 is presented in Table 2(B).

Effect of HFD on functional liver enzymes

Besides developing hyperlipidaemia, HFD feeding for six weeks significantly increased the circulatory levels of functional liver enzymes (ALT, AST, ALP, and GGT) compared to the control group ($p<0.05$). Serum levels of liver enzymes at week 6 are presented in Table 2(B).

Experimental phase (6–12 weeks)

In the second phase, the remaining 12 rats from the HFD group (which became obese) were divided into two groups of similar average body weight; one group of rats continued the HFD (continuing HFD group), whereas the other group of rats were fed with a RBO diet (RBO group). After another six weeks of feeding with respective diets, all the rats were sacrificed and their serum, adipose tissue, and other organs were collected for analysis as described in the first phase.

Effect of RBO diet on body weight of obese rats

Both the HFD and RBO diet caused a continuous increase in body weight at the experimental phase (6–12 weeks) for six weeks in obese rats. This increase in body weight did not differ between groups, as shown in Table 3A.

Effect of RBO diet on the weights of liver, heart, adipose tissue, and liver fat

In the second phase (6–12 weeks), feeding of RBO diet in obese rats caused a non-significant reduction in the weights of the liver and heart compared to the continuing HFD group. Conversely, feeding on a diet with RBO caused a significant decrease (p<0.05) in the weights of AAT, EAT, IBAT, and liver fat compared to the continuing HFD group (Table 3B).

Hypolipidaemic effect of RBO diet on high fat-induced obese rats

A six-week feeding (6–12 weeks) of RBO diet on high fat-induced obese rats caused a significant reduction (p<0.05) in serum TC, TAG, LDL-C, and VLDL-C levels in the RBO diet group compared to the continuing HFD group. On the other hand, the RBO diet significantly (p<0.05) increased serum HDL-C in the RBO group in comparison to the continuing HFD group, as presented in Table 3B.

Table 3. Experimental phase (6–12 weeks)

A) Effect of rice bran oil (RBO) diet on body weight (Mean±SEM)

Group	*Initial body weight (g)*	*Final body weight (g)*	*p-value within the group*	*p-value between the group*
RBO diet group	230.0±7.4	326.0±17.6	0.003	0.744
Continuing high-fat diet group	218.0±11.2	335.0±18.9	<0.001	

B) Effects of continuing high-fat diet (HFD) and rice bran oil (RBO) diet on organ weight, lipid profile, and functional liver enzymes (Mean±SEM)

Parameters	*Continuing HFD group*	*RBO diet group*	*p-value*
Liver (g)	11.9±0.4	11.8±0.3	0.974
Heart (g)	1.0±0.1	0.9±0.0	0.531
AAT (g)	9.7±0.5	5.7±0.3	<0.001*
EAT (g)	7.1±0.6	5.1±0.2	0.008*
IBAT (g)	1.9±0.2	1.2±0.1	0.006*
Liver fat (%)	4.5±0.1	2.5±0.1	<0.001*
TC (mmol/L)	8.2±1.5	5.4±1.6	<0.001*
TAG (mmol/L)	8.8±9.9	5.1±1.4	<0.001*
HDL-C (mmol/L)	2.7±2.3	3.0±0.9	0.038*
LDL-C (mmol/L)	3.8±4.2	1.4±0.9	<0.001*
VLDL-C (mmol/L)	1.8±2.0	1.0±0.3	<0.001*
TC/HDL-C	3.1±0.1	1.8±0.0	<0.001*
LDL-C/HDL-C	1.4±0.1	0.5±0.1	<0.001*
ALT (IU/L)	56.0±0.0	39.0±0.0	<0.001*
AST (IU/L)	147.0±0.2	121.0±0.1	<0.001*
ALP (IU/L)	209.0±0.1	222.0±0.1	<0.001*
GGT (IU/L)	7.3±0.0	6.1±0.0	<0.001*

AAT: abdominal adipose tissue; EAT: epididymal adipose tissue; IBAT: interscapular brown adipose tissue, TC: total cholesterol; TAG: triacylglyceride; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; VLDL-C: very low-density lipoprotein cholesterol; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase

Data are expressed as mean±SEM

*p<0.05 when comparing continuing HFD to RBO diet

Effect of HFD on functional liver enzymes
Continuous feeding of HFD for another six weeks increased the circulatory levels of functional liver enzymes. However, rats fed with RBO diet resulted in reduced ALT, AST, and GGT levels, except ALP ($p<0.05$), compared to the continuing HFD rats. Serum levels of liver enzymes at week 12 are presented in Table 3B.

Efficiency of RBO diet on improving lipid profile of high fat-induced obese rats
RBO supplementation significantly improved the lipid profile of obese rats. RBO diet in comparison to the continuing HFD decreased 45.0% TC, 55.7% TG, and 18.8% LDL-C, and increased 76.3% HDL-C serum concentration, as presented in Table 4.

DISCUSSION

To conduct the present investigation, Long Evans rats were subjected to a controlled HFD for six weeks to promote adiposity and induce an imbalance in their lipid profile. Subsequently, obese and hyperlipidaemic rats were further fed with a diet supplemented with RBO for an additional six weeks and their effects on organ-specific fat deposition and regulation of hyperlipidaemia were compared with those rats that continued with HFD. Our results revealed that a RBO-supplemented diet for six weeks led to favourable outcomes, including maintenance of body weight, reduced organ-specific fat deposition, improved regulation of hyperlipidaemia, and restoration of liver function in obese rats.

In the present study, we observed an increase in food intake among all rats (control and HFD groups) during the initial six weeks of the study. Incorporation of high-fat increased the diet's palatability and calorie content, leading to altered body fat distribution and lipid profile in comparison to the control group. The present findings demonstrated a significantly higher AAT, EAT, IBAT, and hepatic fat, as well as an abnormal lipid profile with significantly higher TC, TAG, LDL-C, VLDL-C, and lower HDL-C in HFD rats compared to the control group. Although the HFD-fed rats showed a significantly greater fat mass than the control group, we observed a slightly greater body weight in HFD-fed rats, which may be attributed to the short-term feeding period. However, the achievement of targeted alterations in fat depots and lipid profiles in HFD rats provided a suitable pre-clinical model for evaluating the impact of RBO diet over HFD.

Energy-dense diets are considered a major factor in the development of obesity. Previous research supports that feeding animals with HFD results in a higher proportion of visceral adipose tissue compared to animals fed with a low-fat diet for the same period (Hariri & Thibault, 2010). During the experimental phase, feeding rats with fats from different sources (either RBO or continuing HFD) for six weeks resulted in a non-significant difference in body weight. Results related to the

Table 4. Efficiency of rice bran oil (RBO) diet over high-fat diet (HFD) in improving lipid profile

Group	*TC (%)*	*TAG (%)*	*LDL-C (%)*	*HDL-C (%)*
Continuing HFD to obese rats	33.3 ↑	30.3 ↑	23.6 ↑	50.0 ↑
RBO diet to obese rats	11.7 ↓	25.4 ↓	52.7 ↓	68.8 ↑

↑ indicates increase
↓ indicates decrease

impact of dietary fat content or total energy consumption on adiposity and body weight are inconsistent. In our study, the absence of a difference in body weight might be related to identical total energy consumption between the experimental groups. However, we have shown that feeding obese rats with RBO diet significantly reduced fat storage in AAT, EAT, and IBAT compared to HFD-fed rats. These outcomes align with a previous study that reported reduced epididymal fat pads in rat models after feeding with a diet supplemented with RBO compared to rats fed with a high-cholesterol control diet (Ha *et al.,* 2005). Contrary to our findings, another study demonstrated no significant changes in fat deposition in different fat depots when lean and obese rats were fed with high butter fat (Rolland *et al.,* 2002). Further reports suggested that mice fed with RBO prevented the growth of white adipose tissue and enhanced lipid metabolism (Al-Okbi *et al.,* 2014). Such observations correspond to the concept that although the energy density of a diet impacts body weight, it is the type and quality of fats in the diets that affect organ-specific fat deposition.

Accumulation of abdominal fat is associated with the development of insulin resistance, hyperlipidaemia, and metabolic syndrome in humans and rodent models (Wajchenberg *et al.,* 2002). In obese rats, surgical removal of EAT has been shown to enhance insulin action (Gabriely *et al.,* 2002). As reflected from the outcomes of our study, the reduction of fats from various fat depots in response to feeding on a RBO diet might play a protective role against the development of cardiometabolic diseases. Additionally, progressive hepatic fat accumulation is a risk factor for NAFLD, the leading cause of liver disease in the United States (Provencher, 2014), affecting 10%–35% of the adult population globally. In our study, feeding obese rats a RBO diet for six weeks significantly reduced liver fat content compared to the continuing HFD-fed rats, which is in line with a previous study (Al-Okbi *et al.,* 2014). Fat deposition in major fat depots, such as visceral fats, are shown to be linked with metabolic disorders and increased GGT and ALT levels (Liu *et al.,* 2013). Interestingly, our study demonstrated that feeding RBO resulted in enhanced serum levels of major functional liver enzymes (ALT, AST, and GGT) when compared with HFD-fed rats. These findings support the protective effect of RBO against the progression of fatty liver disease, which may be due to the presence of beneficial bioactive compounds.

In the present study, feeding obese rats with a diet containing RBO for six weeks demonstrated a robust hypolipidaemic effect by significantly reducing blood TC, TAG, and LDL-C levels compared to rats fed with HFD. Consistent with current findings, previous animal studies have reported similar reductions in serum cholesterol levels through RBO supplementation (Al-Okbi *et al.,* 2014). In a study comparing RBO to coconut oil, rats fed with RBO had a 35.5% reduction in serum cholesterol concentration (Reena & Lokesh, 2007). Furthermore, feeding rats with blended oils containing balanced fatty acids has been shown to lower serum and liver lipids. Another study reported significant reductions in serum TC, LDL-C, and TAG levels (23.8%, 32.4%, and 13.9%, respectively) in rats fed with coconut oil and RBO compared to rats given coconut oil alone (Reena & Lokesh, 2007). Although there is no significant difference in cholesterol-lowering potency between wheat bran oil and RBO, higher cholesterol-lowering potency was observed with RBO over high-cholesterol diets in rodents (Lei *et al.,* 2018). RBO is a rich source of bioactive compounds like γ-oryzanols,

tocopherols, phytosterols, tocotrienols, ferulic acid, and fatty acids (Latha & Nasirullah, 2014). Oleic acid and linoleic acid, which are the major fatty acids present in RBO, have been shown to play a crucial role in reducing blood cholesterol in both humans and rats (Barakat & Mahmoud, 2011). Tocotrienols have been shown to reduce cholesterol biosynthesis, whereas γ-oryzanols suppress cholesterol absorption, enhance faecal sterol excretion, and lower the levels of triglycerides and phospholipids, according to reports from animal and human studies (Chithra *et al.,* 2015).

Besides that, the RBO diet significantly increased serum HDL-C concentration in obese rats in comparison with continuing HFD-fed rats. These results are in agreement with previous animal studies that reported that HDL-C increased by 20% within 18 weeks in rats receiving RBO at the level of 20% in their diet in comparison to rats fed with peanut oil (Liang *et al.,* 2021). Numerous observational and intervention studies have shown the effect of HDL-C on reversing cardiovascular diseases (Siddiqi, Kiss & Rader, 2015). The ratios of TC/HDL-C and LDL/HDL-C are considered as more sensitive and specific indices of cardiovascular risk than total cholesterol alone. These ratios, known as atherogenic indices, are better predictors of cardiovascular risk than LDL cholesterol alone (Ridker *et al.,* 2005). Our results are consistent with previous studies that reported a significant reduction in the TC/HDL-C and LDL-C/HDL-C ratios of obese rats fed with RBO compared to those fed with continuing HFD (Chithra *et al.,* 2015). Based on our findings, we suggest that RBO may improve lipoprotein profile and reduce the risk of metabolic diseases and CVD in obese rats.

Some limitations in our study should be acknowledged. Firstly, we did not measure regular food intake, as rats had *ad libitum* access to food. This may have resulted in higher dietary energy intake for the HFD group compared to the RBO diet group, as reported in a previous study (Miras *et al.,* 2014). However, the lack of a significant difference in body weight between groups suggests the rats had similar food and energy intakes. Secondly, we did not analyse our findings by gender of the rats, which may affect the deposition of fat in specific organs (Hariri & Thibault, 2010). Finally, we did not assess the fatty acid composition of HFD or RBO diets. Moreover, the fatty acid profile of adipose tissues was not assessed, limiting our ability to describe the contribution of dietary fat composition to the deposition of fat in specific organs. To gain better understanding of the underlying mechanisms, these limitations should be addressed in future studies.

CONCLUSION

In conclusion, the study has shown that RBO induced a depot-dependent reduction in adiposity without changes in the body weight of rats. Similarly, these achieved improvements in reducing adiposity demonstrated a remarkable impact on regulating lipid profiles and restoring healthy liver functions in hyperlipidaemic rats. Although these findings are promising, it should be noted that the study was conducted on animals and may not necessarily extrapolate directly to humans. RBO's hypolipidaemic effect might have an effect on lowering cholesterol levels, regulating lipid profiles, and improving liver functions in people. Further studies are required to explore the effects of RBO on lipid metabolism and liver function in humans.

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Authors' contributions

Alam MJ, Hasan MK and Alim MA, responsible for methodology, clinical trial, rat management and feeding, formal analysis, data curation, writing original draft; Akter S, led the preparation of the draft and reviewed the manuscript; Linkon KMMR and Prodhan UK, accountable for supervision, conceptualisation, methodology, assisted in drafting, reviewing and editing.

Conflicts of interest

The authors have no conflicts of interest to declare. The authors provided personal resources for funding this project.

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Association between 25-hydroxyvitamin D levels and incidence of allergy and infection in infants aged 0–6 months

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ABSTRACT

Introduction: Vitamin D levels are known to be related to prevalence of allergy and infection in children. However, vitamin D levels in infants' umbilical cord blood need to be investigated. Therefore, this study aimed to determine association between 25-hydroxyvitamin D [25(OH)D] levels and incidence of allergy and infection in children. **Methods:** A longitudinal study involving 38 full-term newborns was conducted. Serum 25(OH)D levels in infants' umbilical cord and venous blood were measured at birth and six months, respectively. 25(OH)D levels were classified as insufficient (<20 ng/mL) and sufficient (>20 ng/mL). Parents filled out questionnaires about their children's allergy and infection symptoms. Paired *t*-test was performed to compare the 25(OH)D levels at birth and at six months. Chi-squared test was conducted to determine relationship between 25(OH)D levels and incidence of infection and allergy in children. **Results:** 25(OH)D levels in venous blood of 6-month-old infants were significantly higher than in umbilical cord blood (50.44±13.59 ng/mL vs. 20.70±6.60 ng/mL, $p<0.001$). In addition, 25(OH)D level insufficiency in umbilical cord blood was associated with infection ($p<0.05$). However, there was no incidence of allergy, and exclusive breastfeeding and sun exposure were not associated with vitamin D levels in 6-month-old infants. **Conclusion:** We conclude that 25(OH)D level insufficiency in umbilical cord blood was associated with incidence of infection in the first six months of life.

Keywords: 25(OH)D, allergy, infants, infection, umbilical cord blood

INTRODUCTION

Vitamin D and calcium deficiencies are widespread, making them global health concerns, particularly for infants, children, and adolescents (Amrein *et al.*, 2020). A systematic review in the South-East Asia region showed that vitamin D deficiency among healthy children is common. Its prevalence ranges from 0.9% to 96.4%, with >50% of newborns suffering from vitamin D deficiency (Oktaria *et al.,* 2022). Data from Yogyakarta, Indonesia, revealed that vitamin D deficiency was detected in 90% of umbilical cord blood samples and 13% of venous blood samples obtained from 6-month-old infants (Oktaria *et al.*, 2020).

It has been hypothesised that vitamin D deficiency is related to acute and

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chronic diseases, including infectious diseases, allergies, autoimmune and neurological disorders, dental caries, cardiovascular disease, type 2 diabetes, and cancer (Holick, 2017). The role of vitamin D in modulating the immune system against various pathogens is through cathelicidin, which activates autophagy and enhances antimicrobial function against pathogens and inflammation (Chung *et al.*, 2020).

Allergic diseases are relatively common in developed countries, affecting four to five individuals (Wang, 2011). A study conducted on a group of patients aged 0–18 years with atopic dermatitis, asthma, and food allergies demonstrated that 48% of them had insufficient serum 25-hydroxyvitamin D [25(OH)D] levels (<30 ng/mL) (Searing & Leung, 2010). In addition, a study conducted in Taiwan showed that low serum 25(OH) D level in the umbilical cord blood was associated with a high risk of respiratory tract infection in infants aged below six months (Lai *et al.*, 2017).

The correlation between vitamin D levels in umbilical cord blood and allergic and infectious diseases has yet to be adequately investigated in Indonesia. Therefore, this study aimed to determine the association between serum 25(OH) D level in the umbilical cord blood and the incidence of allergy and infection in the first six months of life. Further, it aimed to analyse the difference between serum 25(OH)D levels in umbilical cord blood and in venous blood, as well as the effect of exclusive breastfeeding and sun exposure on serum 25(OH)D levels.

MATERIALS AND METHODS

Study design

This longitudinal study was conducted in Semarang, Central Java, Indonesia, from May 2017 to August 2018. The inclusion criteria for the infants were full-term, singleton, vigorous, and without a history of asphyxia. Meanwhile, the inclusion criteria for the mothers were age <40 years, no pregnancy complications, and willingness to participate in the study from the third trimester until the first six months post-delivery. Sample size was calculated by comparing two proportions formula (Wang & Chow, 2007), with confidence level of 95%, α=0.05, critical value of 1.96, and power=80%. With p1 and p2 as the expected sample proportions of the two groups, the minimum sample calculated was 35 subjects.

All subjects were asked for written informed consent. Umbilical cord blood samples of 50 infants were obtained from hospitals and primary health care centres. During the study period, several infants were excluded: three, who stopped study participation; eight, whose parents refused blood collection; and one, who died. Thus, only 38 infants (22 boys and 16 girls) were included in the final analysis. However, the required minimum sample size was still met. The protocol for this study was approved by the Faculty of Medicine, Diponegoro University ethics committee (Approval no.: 18/EC/FK-RSDK/I/2017).

Allergic and infectious diseases

Allergic manifestations in the gastrointestinal tract (diarrhoea), skin (atopic dermatitis, eczema, urticaria, and angioedema), and respiratory tract (wheezing, nasal hypersecretion, bronchial hypersecretion) were obtained from the questionnaires the parents had answered. In addition, infections consisting of fever with symptoms in the respiratory tract, gastrointestinal tract (diarrhoea), and skin infection were reported by parents in the questionnaires.

Breastfeeding

Exclusive breastfeeding was defined

as feeding an infant with breast milk without any additional food/drink, not even water (WHO and UNICEF, 2021).

Sun exposure

The length of sun exposure was calculated in minutes/week. The infants were exposed to the sun wearing minimal clothing (>50% exposure) and with no sun protection, such as umbrellas, shades, and sunblock, at 8 to 10 a.m. in the morning. The length of sun exposure was categorised as >30 minutes/week and <30 minutes/week, and the exposure was evaluated for six months (Anusha *et al.*, 2019).

Blood samples

Blood sample was collected from the umbilical cord after the infant was born, before the placenta was taken away. Blood (3 mL) was taken by placing two clamps of 10±15 cm on the umbilical cord. Blood from the umbilical vein was collected using a 5-mL syringe and then injected into a non-ethylenediamine tetra acetic acid tube. Blood samples of the 6-month-old infants were obtained from the vein. All samples were brought to the Dr. Kariadi Hospital, Semarang, Indonesia. Samples were centrifuged at 3000 rpm for 10 minutes, and the serum was separated and stored in a freezer at -20°C. After the specimen was collected using a packed cold chain method and stored in a cool box, it was delivered to the GAKI Laboratory of Diponegoro National Hospital, Semarang, Indonesia for the analysis of 25(OH)D level.

Serum 25(OH) D analysis

The serum 25(OH)D levels at birth (from the umbilical cord blood) and six months of age were measured via enzyme-linked immunosorbent assay and using the ELX-800 microplate reader (BioTek Instruments, Inc., Winooski, USA). The 25(OH)D levels were categorised as sufficient (>20 ng/mL or >50 nmol/L) and insufficient (≤20 ng/mL or ≤50 nmol/L) (Amrein *et al.*, 2020).

Data calculation and statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics for Windows version 25.0 (IBM Corporation, Armonk, New York, USA). A normality test was conducted to determine the distribution of numeric variables before analysis. Normally distributed numeric variables were expressed as mean (standard deviation), whereas non-normally distributed ones were expressed as median (minimum and maximum). Categorical variables were presented in a frequency distribution. The difference between serum 25(OH)D levels in umbilical cord blood of newborns and serum 25(OH)D levels in venous blood of 6-month-old infants was evaluated using paired *t*-test. Chi-squared test was employed to analyse the association between 25(OH)D levels with allergic and infectious diseases in infants up to six months old.

RESULTS

The characteristics of the infants were as follows: 26 (68.4%) were born through normal deliveries, 18 (47.4%) were exclusively breastfed (EBF), 36 (94.7%) were exposed to the sun for more than 30 minutes/week, and all infants received complete immunisations according to their age.

Mean 25(OH)D levels in the umbilical cord blood and venous blood of 6-month-old infants are presented in Table 1. Of the 38 umbilical cord blood samples, 50% were found to have sufficient 25(OH) D levels, whereas 50% had insufficient 25(OH)D levels. Contrarily, all samples from 6-month-old infants had sufficient 25(OH)D levels. Furthermore, the 25(OH) D levels in venous blood of 6-month-old infants were also higher than those

Table 1. Comparison of 25(OH)D levels in the umbilical cord blood and at six months

Vitamin D level	*n*	25(OH)D level *(Mean±SD)*	*p*
Umbilical cord blood (ng/mL)	38	20.70±6.60	<0.001†*
6 months (ng/mL)	38	50.44±13.59	

†Paired *t*-test
*Significance, $p<0.05$

Table 2. Association between insufficient 25(OH)D levels in the umbilical cord blood and infection

	25(OH)D insufficient n (%)	*25(OH)D sufficient n (%)*	*p*
Infections (URI and diarrhoea)			
Yes	10 (71.4)	4 (28.6)	0.044†*
No	9 (37.5)	15 (62.5)	
URI			
Yes	8 (66.7)	4 (33.3)	0.163†
No	11 (42.3)	15 (57.7)	
Diarrhoea			
Yes	2 (100.0)	0 (0.0)	0.486‡
No	17 (47.2)	19 (52.8)	

URI: upper respiratory infection
†Pearson's chi-squared test
‡Fisher's exact test
*Significance, $p<0.05$

Table 3. Comparison of 25(OH)D levels (ng/mL) among 6-month-old infants according to breastfeeding status and sunlight exposure

Variables	*n*	25(OH)D level *(Mean±SD)*	*p*
Exclusively breastfed	18	48.52±9.52	0.406†
Non-exclusively breastfed	20	52.16±16.50	
Sunlight exposure			
>30 min/week	36	51.00±13.53	0.283†
<30 min/week	2	40.80±14.89	

†*t*-test

of newborns (20.70±6.60 ng/mL vs. 50.44±13.59 ng/mL, $p<0.001$) (Table 1).

There were no reports of infants experiencing allergic symptoms, but seven infants (18.4%) had a family history of allergy. Infectious diseases, such as upper respiratory infection (URI) and diarrhoea, occurred in 14 infants, of whom 10 had insufficient 25(OH)D levels in the umbilical cord blood (Table 2). However, no skin infection occurred in the infants and none of them were hospitalised. In addition, a significant association was observed between insufficient 25(OH)D levels in the umbilical cord blood and infection.

However, there was no significant association between 25(OH)D levels in the umbilical cord blood and URI or diarrhoea *per se* (Table 2).

Although the 25(OH)D levels of EBF infants were lower than those of non-EBF infants at six months of age, the 25(OH)D levels were higher in those who were exposed to the sun for more than 30 minutes/week than in those who were exposed for less than 30 minutes/week; however, the differences were not statistically significant (Table 3).

DISCUSSION

In this study, the 25(OH)D levels in the umbilical cord blood of 19 infants (50%) were insufficient. This finding is consistent with that from a previous study on South-East Asian newborns, which indicated that 20.2% of newborns were categorised as vitamin D deficient (<12 ng/mL) and 69.1% as vitamin D insufficient (12–20 ng/mL) (Ariyawatkul & Lersbuasin, 2018). A meta-analysis revealed that vitamin D deficiency in Turkey varied from 58.9% to 66.6% and that the risk was higher among neonates, pregnant women, and adult women. It also showed that 86.6% of infants had vitamin D deficiency (Alpdemir & Alpdemir, 2019). Infants born to mothers of non-white race/ethnicity have a higher risk of vitamin D deficiency (Eldjerou *et al.*, 2015). Mean 25(OH)D levels in 100 umbilical cord blood samples according to maternal ethnicity were reportedly higher in Caucasians than in Asians, Hispanics, Pacific Islanders, and African Americans (Halm *et al.*, 2013).

This study found that EBF infants had lower vitamin D levels at six months old than non-exclusively breastfed infants. However, both groups still had sufficient vitamin D levels (>20 ng/mL). This finding is inconsistent with that from a study conducted in Indonesia, which indicated that EBF infants were twice as likely to have vitamin D deficiency at six months of age (Oktaria *et al.*, 2020). Furthermore, a significant difference in the vitamin D levels between newborns and 6-month-old infants was observed ($p<0.001$). A previous study in Denmark reported that 4-month-old infants had vitamin D levels of 94.1±24.2 nmol/L, whereas 9-month-old infants had 82.2±18.9 nmol/L; more than 90% of the infants received vitamin D supplementation (Streym *et al.*, 2013). Contrarily, a study in South Korea reported that vitamin D deficiency (<20 ng/mL) occurred in 48.7% of infants aged 1–6 months. It also showed that the most significant risk factor was exclusive breastfeeding (Choi, Kim & Jeong, 2013). Breast milk and formula milk contain a very small amount of vitamin D. Breast milk contains only <20 IU/L of vitamin D and even less if the mother has low exposure to the sun, which is insufficient to meet the infant's growing needs. Thus, without fortification, EBF infants will receive <20% of their daily needs (Ruangkit *et al.*, 2021). 25(OH)D concentration is found to be higher in hindmilk than in foremilk [2.10% (1.63%–2.65%) vs. 1.35% (1.04%–1.84%), respectively]. 25(OH)D concentration in breast milk is also correlated with maternal plasma concentration; mothers who received vitamin D supplementation have higher 25(OH)D concentrations in their breast milk than those who did not (Streym *et al.*, 2016).

In our study, neither the vitamin D insufficient group nor the vitamin D sufficient group reported any incidence of allergic diseases. This finding is in line with that from a study in Australia, which administered 400 IU of vitamin D supplementation to children aged 1–2.5 years who had familial history of allergy; the results indicated that vitamin D supplementation did not reduce the

development of allergies (Rueter *et al.*, 2020). Another study conducted on children with persistent asthma and vitamin D deficiency found no clinical differences in asthma attacks between the experimental and control groups (Forno *et al.*, 2020).

A significant association was observed between insufficient 25(OH) D levels in the umbilical cord blood and infection. A study on 122 infants in Taiwan found that a serum 25(OH)D level below 13.7 ng/mL in the umbilical cord blood was correlated with a higher risk of respiratory tract infection before the infants reached six months old (Lai *et al.*, 2017). Furthermore, it was reported that infants with a low 25(OH)D level had a higher risk of respiratory tract infection before reaching three months old (Camargo *et al.*, 2011). Similarly, our study demonstrated that infants with insufficient 25(OH)D levels in the umbilical cord blood had a 4.2 times higher risk of URI. Similar results were obtained from a study in Saudi Arabia, whereby low 25(OH)D levels in the umbilical cord blood also increased the risk of lower respiratory tract infection in children at two years of age (Mohamed & Al-Shehri, 2013). Another study in Indonesia found that 19% of infants and children with pneumonia had vitamin D deficiency; however, it was not related to the severity of pneumonia (Oktaria *et al.*, 2021).

A previous study in Tanzania reported that the prevalence rates of vitamin D deficiency and insufficiency in 188 children aged <5 years were relatively high, at 53.7% and 34%, respectively; however, no association was observed between vitamin D level and the incidence of diarrhoea (Hassam *et al.*, 2019). Similarly, our study found no association between the incidence of diarrhoea and insufficient 25(OH)D levels in the umbilical cord blood, with just two of our infants having diarrhoea.

Despite the sun being the main source of vitamin D, there are concerns regarding excessive sun exposure as one of the risk factors for skin cancer. The American Academy of Pediatrics recommends that infants below the age of six months not be exposed to direct sunlight (Long, 2017). In this study, data about infants' sun exposure were obtained. We found that 6-month-old infants with sun exposure <30 minutes/ week and those with sun exposure >30 minutes/week had sufficient vitamin D levels (>20 ng/mL). This finding is supported by a study in Sri Lanka, which reported that sun exposure >30 minutes/week was significantly associated with higher vitamin D levels in infants aged 4–6 weeks (Anusha *et al.*, 2019). In addition, a study in India also reported that sun exposure of at least 30 minutes/week for at least 16 weeks achieved sufficient vitamin D levels (>20 ng/mL) in 6-month-old infants (Meena *et al.*, 2017). Our data indicated that 94.7% of infants received sun exposure >30 minutes/week.

A study on sun exposure in South-East Asia has reported that the highest intensity of ultraviolet B is from 10 a.m. to 3 p.m., when the sun is directly above the head. A previous study conducted in Semarang, Indonesia, on 109 infants aged 7–12 months has reported that sun exposure from 10 a.m. to 2 p.m., three times per week for 5 minutes (50% of body surface area) for two months, can increase serum 25(OH)D levels by 8.9 ng/mL (Pratiwi, 2019).

The limitations of this study were that several parents refused to allow their child's blood to be taken at six months of age, and vitamin D levels in the mothers' blood were not measured. However, to the best of our knowledge, this is the first study in Indonesia that has analysed the association of vitamin D levels in the venous blood and umbilical cord blood of 6-month-old infants with

the incidence of allergic and infectious diseases.

CONCLUSION

The incidence rate of 25(OH)D level insufficiency in the umbilical cord blood was 50%. The 25(OH)D level in the venous blood of 6-month-old infants was higher than that in the umbilical cord blood of newborns. Significant association was observed between 25(OH)D level insufficiency in the umbilical cord blood and infection. However, allergic diseases did not occur in both the insufficient and sufficient 25(OH)D groups. Further study is needed to analyse the vitamin D levels of infants diagnosed with allergic and infectious diseases.

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Authors' contributions

Mexitalia M, conceived and designed the study, analysed and interpreted literature data, and wrote the first drafts of the manuscripts, and approved the final version as submitted; Awang YK, contributed to the study design, analysed the data, edited references, and approved the final revision as submitted; Pratiwi R, contributed to the study design, helped with the interpretation of data, reviewed the manuscript; Utari A, contributed to the study design, analysed the data, interpreted literature data, reviewed the manuscript, and approved the final version as submitted.

Conflicts of interest

The authors declare that there is no conflict of interest.

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Validation of selected 2021 infant and young child feeding indicators for appropriate complementary feeding in relation to dietary adequacy and anthropometric status

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ABSTRACT

Introduction: With the new set of 2021 infant and young child feeding (IYCF) indicators released by WHO and UNICEF, there is a need for its validation in the Philippine setting. The study evaluated the validity of cut-off points used for minimum dietary diversity and minimum feeding frequency in terms of micronutrient and energy intake adequacy, respectively, and minimum acceptable diet (MAD) in relation to anthropometric status. **Methods:** The study covered 8360 infants and young children aged 6-23.9 months with complete information on IYCF, anthropometric measurements, maternal information, and household characteristics from the 2018-2019 Expanded National Nutrition Survey (ENNS). Bivariate and correlation analyses using STATA version 15 (Corp LLC, Texas, USA 2017) were performed to determine the association of specific IYCF indicators: dietary diversity and feeding frequency with nutrient and energy intake adequacy, and MAD with anthropometric status. Sensitivity and specificity analyses were performed to evaluate the accuracy of dietary diversity score (DDS) and feeding frequency in identifying children with adequate nutrient and energy intakes, respectively. **Results:** Significant associations were found between DDS and micronutrient adequacy, and between feeding frequency and energy intake adequacy, regardless of breastfeeding status. A DDS of 5 and 6 and feeding frequency of 4-5 and 8 maximised sensitivity and specificity in identifying breastfed and non-breastfed children meeting 100% nutrient and energy adequacy, respectively. **Conclusion:** The selected 2021 complementary feeding indicators are practical guidelines to reflect dietary adequacy, but may not capture the entire process related to nutritional outcomes, especially stunting.

Keywords: complementary feeding, dietary diversity, meal frequency, minimum acceptable diet, nutritional status

INTRODUCTION

In the Philippines, half (49.8%) of the Filipino children aged 0-23 months are appropriately breastfed while receiving timely complementary foods (DOST-FNRI, 2022). Based on the 2018-2019 ENNS, only 11.7% of children 6.0-23.9 months old met the minimum acceptable diet (MAD), while only 21.6% met the minimum dietary diversity.

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Complementing these poor feeding practices, majority of infants 6-23 months old are inadequate in energy, iron, and vitamin A. Results revealed significant linear growth faltering and micronutrient deficiencies during the second six months of infancy when complementary foods are essential to provide sufficient diet for growth and development (DOST-FNRI, 2022).

Infant and young child feeding (IYCF) is a major component of the Philippines' nutrition programme as embodied in the Department of Health IYCF 2030 Strategic Plan and the Philippine Plan of Action for Nutrition (NNC, 2017). To assess and monitor the progress of feeding practices at the national and local levels, the Philippines' IYCF 2030 Strategic Plan included three IYCF core indicators of appropriate complementary feeding based on the 2008 guidelines: minimum dietary diversity (MDD), minimum meal frequency (MMF), and MAD. The MDD is intended as a proxy indicator for micronutrient adequacy, while the MMF is a proxy indicator for dietary energy adequacy.

However, in 2021, the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) released an updated version of the IYCF indicators. Among the revisions made was to include breast milk as an eighth food group in the MDD indicator. The 7-group MDD indicator in the 2008 version was technically correct in its focus on complementary foods, but required separate estimates for breastfed and non-breastfed children. Consequently, the dietary diversity score (DDS) cut-off was increased to five groups compared to the 2008 version, which used four out of seven food groups (WHO, 2021).

With this context, there is a need to evaluate the validity of these three selected 2021 complementary feeding indicators to ensure their sensitivity and specificity as they are currently used to set the national IYCF targets. Moreover, the use of the modified dietary diversity as an indicator of adequate nutrient intake remains under evaluation, particularly in developing countries like the Philippines. Evidence showed inconsistent results on the associations between complementary feeding indicators and child nutritional status (Ruel, 2017). Understanding the extent to which these complementary feeding indicators can predict nutritional outcomes may lead to a more accurate identification of young children at-risk.

Considering that MDD and MMF are used as proxy indicators, this study evaluated the new MDD, MMF, and MAD complementary feeding indicators in assessing micronutrient and energy adequacy of complementary food intake among Filipino children 6.0-23.9 months old. Specifically, it evaluated the validity of cut-off points used for MDD and MMF in terms of micronutrient and energy intake adequacy, respectively, and evaluated the MAD in relation to anthropometric status.

MATERIAL AND METHODS

Study design and study participants

The study analysed the existing IYCF data of children 6.0-23.9 months old from the 2018-2019 ENNS, which covered 79 provinces and highly urbanised cities (HUCs). The ENNS had a cross-sectional survey design which utilised the 2013 Master Sample of the Philippine Statistics Authority as its sampling design, which has been described in detail elsewhere (DOST-FNRI, 2022).

A total of 8360 infants and young children aged 6-23.9 months (4287 breastfed and 4073 non-breastfed children) with complete anthropometric, biochemical, dietary, and IYCF data were included in the study.

Measured outcomes

Complementary feeding practices

Three modified 2021 complementary feeding indicators were evaluated based on the dietary intake of the previous day: DDS/MDD, meal frequency/MMF, and MAD. DDS was calculated by adding up the number of food groups consumed by the child in the past 24-hour period. Meeting the MDD indicator was based on the consumption of at least five of the following eight food groups: 1) grains, roots and tubers; 2) legumes and nuts; 3) dairy products; 4) flesh foods; 5) eggs; 6) vitamin A-rich fruits and vegetables; 7) other fruits and vegetables; and 8) breast milk. To achieve MMF, breastfed infants aged 6-8 months and 9-23 months must have received solid, semi-solid or soft foods at least two and three times, respectively, in the previous day; while non-breastfed children 6-23 months old must have received solid, semi-solid, soft foods, or milk feeds at least four times the previous day. The MAD indicator is a composite of the MDD and MMF indicators. Breastfed children must have received MDD and MMF the previous day, while non-breastfed children must have received at least two milk feedings and met the MDD and MMF during the previous day.

Individual dietary intake

A 24-hour food recall was conducted among the mothers or caregivers of children 6-23.9 months old via face-to-face interview to recall all foods and beverages that the child consumed on the previous day. To estimate the amount of each food item or beverage consumed, measuring tools such as cups, tablespoons, wooden matchboxes, and plastic circles were utilised. The Individual Dietary Evaluation System containing the updated Food Composition Table, which includes human milk (DOST-FNRI, 2019), was used to estimate the energy, protein, and micronutrient (iron, zinc, calcium, vitamin A, vitamin C, thiamin, riboflavin, and niacin) intakes of each child.

Dietary energy, protein, and micronutrient adequacies of each child's intake on the previous day were assessed using the 2015 Philippine Dietary Reference Intakes (PDRI) (DOST-FNRI, 2015). For iron, zinc, calcium, vitamin A, vitamin C, thiamin, riboflavin, and niacin, an aggregated mean percent micronutrient adequacy was obtained by adding the mean percent adequacy of each of the eight micronutrient adequacy and divided by eight micronutrients.

Child anthropometry

Anthropometric measurements (weight and height/length) were assessed using the WHO Child Growth Standards to determine the nutritional status of children (WHO, 2006).

Socioeconomic and demographic variables

The ENNS data on household economic status, place of residence, age, education, and working status of mothers were culled to describe the children's households. Wealth index was determined through principal component analysis of variables such as household characteristics, household assets, infrastructure factors, and utility access. Food security status was assessed using the Household Food Insecurity Access Scale questionnaire.

Statistical analysis

Statistical Analysis Stata 15 (Stata Corporation, Texas, USA 2017) was used for all statistical analyses. Mean and standard errors of selected complementary feeding indicators (DDS, meal frequency), energy, and nutrient intakes of children aged 6-23 months were estimated. The proportion of children meeting the MDD, MMF, MAD, recommended energy intake

(REI), nutrient's estimated average requirements (EAR), prevalence of undernutrition, and household food insecurity were generated.

Normality test was done for all continuous variables using the Shapiro-Wilk test. Since data on mean percentage adequacy (MPA) and energy adequacy were not normally distributed, correlation coefficients between dietary diversity vs. MPA and meal frequency vs. percent energy adequacy were assessed using Spearman's rank correlation test. Mann-Whitney U and Kruskal-Wallis tests were computed to determine the difference between medians from two and more than two independent groups/ categories, respectively.

MPA was used as the gold standard to evaluate DDS for sensitivity and specificity, while energy adequacy was used for meal frequency. Sensitivity and specificity analyses were performed to determine the accuracy of DDS and meal frequency in correctly classifying children with high MPA values and percent energy adequacy, respectively. To show what the generated DDS and MMF cut-off points could achieve in terms of nutrient and energy adequacy, three MPA and percent energy adequacy cut-off values were used in this analysis: 50%, 75%, and 100%. DDS and meal frequency cut-off points were identified based on the maximum sensitivity and specificity values. Area under the receiver operating characteristic curve (AUC) was computed using the ROCTAB command of Stata. The AUC was used to measure the accuracy of the diagnostic tests performed and AUC >0.7 was considered acceptable.

P-values <0.05 were considered statistically significant. All analyses were accounted for sampling weights to reflect nationally representative results.

Ethical approval

The study was conducted in accordance with the declaration of Helsinki and the protocol was approved by the Department of Science and Technology-Food and Nutrition Research Institute Ethics Review Committee on July 9, 2021 with FIERC#2021-022. All surveyed households including their members provided informed consent prior to participation.

RESULTS

General characteristics, feeding practices, and dietary intake of study population

Table 1 shows the characteristics of study children and their mothers, including their households' socio-demographic and economic status, as well as their feeding practices and dietary intake. The mean age of children was 15.3±0.1 months, of which breastfed and non-breastfed children had mean ages of 14.2±0.1 months and 16.4±0.1 months, respectively. There was an equal proportion of children between the age groups of 6-11 months (31.3%), 12-17 months (33.4%), and 18-23 months (35.3%). Majority (87.0%) of children had normal birth weight. There was an almost equal representation of boys and girls. By nutritional status, 28.1% were stunted, 16.8% were underweight, while 6.1% were wasted. There were more stunted, underweight, and wasted breastfed than non-breastfed children, while there were more overweight non-breastfed than breastfed children.

The mean age of mothers was 29.6±0.1 years. Most mothers were ≥20 years old (94.2%). More than half (56.7%) reached at least secondary level of education. Majority of mothers (75.5%) were not working at the time of survey. There was a higher proportion of non-breastfed children than breastfed children whose mothers had reached at least tertiary education (29.0% vs. 18.4%) and were working (33.1% vs. 16.9%). There were

Table 1. General and household characteristics of children 6-23 months old and their mothers, and feeding practices, food consumption, and dietary intake adequacy of children, Philippines: 2018-2019

Variables	*All children (n=8,360)*		*Breastfed children (n=4,285)*		*Non-breastfed children (n=4,073)*	
	n	*Proportion/ Mean±SE*	*n*	*Proportion/ Mean±SE*	*n*	*Proportion/ Mean±SE*
Child's characteristics						
Sex						
Boys	4302	50.5±0.6	2154	49.7±0.9	2148	51.2±1.2
Girls	4056	49.5±0.6	2131	50.3±0.9	1925	48.8±1.2
Mean age (in months)	8358	15.3±0.1	4285	14.2±0.1	4073	16.4±0.1
6-11	2643	31.3±0.7	1702	39.4±1.5	941	23.2±0.8
12-17	2751	33.4±0.7	1456	34.3±1.2	1295	32.6±1.0
18-23	2964	35.3±0.6	1127	26.3±0.9	1837	44.2±1.1
Birth weight (g)						
<2500	909	13.0±0.8	442	11.7±0.7	467	14.3±1.1
≥2500	6324	87.0±0.8	3318	88.3±0.7	3006	85.7±1.1
Nutritional status						
Underweight (%)	8358	16.8±0.6	4285	21.3±0.9	4073	12.4±0.8
Stunted (%)	8358	28.1±1.2	4285	31.3±1.5	4073	24.9±1.6
Wasted (%)	8358	6.1±0.5	4285	7.0±0.7	4073	5.1±0.6
Overweight/Obese (%)	8358	2.4±0.3	4285	1.7±0.3	4073	3.1±0.4
Maternal characteristics						
Age (years)	7415	29.6±0.1	4067	29.8±0.1	3348	29.4±0.2
<20	453	5.8±0.3	256	6.0±0.4	197	5.7±0.6
20-29	3692	49.0±0.9	1971	47.5±1.3	1721	50.7±1.4
≥30	3270	45.2±0.9	1840	46.6±1.1	1430	43.6±1.5
Education						
No education	93	1.1±0.2	71	1.6±0.3	22	0.5±0.2
Elementary	1414	17.7±1.2	921	21.2±1.3	493	13.8±1.2
At least high school	4040	56.7±1.1	2266	57.6±1.4	1774	55.7±1.1
At least college	1777	23.4±1.1	761	18.4±1.1	1016	29.0±1.5
Others	91	1.1±0.2	48	1.2±0.2	43	1.0±0.2
Currently working (%)						
Working	1730	24.5±1.4	673	16.9±0.9	1057	33.1±2.1
Not working	5685	75.5±1.4	3394	83.1±0.9	2291	66.9±2.1
Household characteristics						
Residence						
Rural	5483	56.4±3.8	2992	61.1±3.8	2491	51.7±3.9
Urban	2875	43.6± 3.8	1293	38.9±3.8	1582	48.3±3.9
Wealth status						
Poorest	2702	26.8±2.2	1637	32.7±2.4	1065	20.9±2.2
Poor	2055	23.5±1.3	1124	25.7±1.4	931	21.4±1.6
Middle	1510	19.4±0.9	730	18.6±1.1	780	20.3±1.1
Rich	1171	17.0±1.3	470	13.7±1.0	701	20.3±1.7
Richest	910	13.2±1.3	319	9.3±1.2	591	17.1±1.5

Table 1. General and household characteristics of children 6-23 months old and their mothers, and feeding practices, food consumption, and dietary intake adequacy of children, Philippines: 2018-2019 *(continued)*

Variables	*All children (n=8,360)*		*Breastfed children (n=4,285)*		*Non-breastfed children (n=4,073)*	
	n	*Proportion/ Mean±SE*	*n*	*Proportion/ Mean±SE*	*n*	*Proportion/ Mean±SE*
Food security[†]						
Severely food insecure	1645	29.6±1.3	740	26.1±1.4	905	33.0±1.5
Moderately food insecure	837	14.4±0.8	431	14.6±1.0	406	14.3±1.0
Mildly food insecure	2385	38.0±1.6	1296	40.0±1.7	1089	35.9±1.9
Food secure	1100	18.0±1.4	603	19.3±1.4	497	16.8±1.6
Current type of feeding practices						
Breastfeeding+ complementary feeding	3658	41.7±1.2	3658	83.8±0.8	0	0.0
Breastfeeding+ complementary feeding+ other milk	627	8.1±0.4	627	16.2±0.8	0	0.0
Other milk + semi-solid/ soft foods	4073	50.3±1.3	0	0.0	4073	100.0
Dietary diversity score (DDS)						
Mean DDS±*SE*	8358	3.1±0.0	4285	3.3±0.0	4073	2.9±0.0
Meeting minimum dietary diversity, %	8358	11.9±0.6	4285	15.9±0.9	4073	8.0±0.7
Proportion of children by DDS, %						
1	248	2.7±0.3	31	0.5±0.1	217	4.9±0.4
2	2735	32.1±0.9	1322	29.5±1.3	1413	34.6±1.2
3	2699	32.0±0.8	1377	31.6±1.3	1322	32.3±0.9
4	1706	21.3±0.8	920	22.5±0.9	786	20.2±1.1
5	734	8.8±0.4	463	11.2±0.6	271	6.5±0.5
6	220	2.9±0.2	159	4.4±0.4	61	1.4±0.3
7	16	0.2±0.1	13	0.4±0.1	3	0.1±0.0
8	0	0.0	0	0.0	0	0.0
Meal frequency						
Mean meal frequency±*SE*	8358	5.8±0.1	4287	4.0±0.0	4073	7.6±0.1
Meeting minimum meal frequency, %	8358	93.0±0.6	4285	89.3±1.2	4073	96.6±0.4
Proportion of children by meal frequency, %						
2	574	7.3±0.6	563	14.4±1.3	11	0.4±0.1
3	1428	15.1±0.9	1277	27.3±1.4	151	3.0±0.4
4	1387	16.0±0.8	1116	25.7±1.0	271	6.4±0.5
5	1259	14.6±0.7	754	17.7±0.9	505	11.5±1.1
≥6	3710	47.0±1.7	575	14.9±0.9	3135	78.8±1.7

Table 1. General and household characteristics of children 6-23 months old and their mothers, and feeding practices, food consumption, and dietary intake adequacy of children, Philippines: 2018-2019 *(continued)*

Variables	*All children (n=8,360)*		*Breastfed children (n=4,285)*		*Non-breastfed children (n=4,073)*	
	n	*Proportion/ Mean±SE*	*n*	*Proportion/ Mean±SE*	*n*	*Proportion/ Mean±SE*
Meeting minimum acceptable diet (MAD), %	8358	11.0±0.6	4285	15.7±0.9	4073	6.4±0.6
6-8 months	1231	1.5±0.4	800	2.1±0.6	431	0.5±0.3
9-23 months	7127	12.6±0.6	3485	18.7±1.1	3642	7.1±0.7
Consumption across food groups						
Grains, %	8358	96.7±0.5	4285	97.8±0.5	4073	95.7±0.5
Legumes, %	8358	5.6±0.6	4285	5.8±0.7	4073	5.3±0.5
Milk and dairy, %	8358	64.2±1.2	4285	34.9±1.3	4073	93.2±0.7
Flesh meat, %	8358	40.0±1.0	4285	37.4±1.1	4073	42.5±1.6
Eggs, %	8358	19.7±0.8	4285	18.4±1.0	4073	20.9±0.8
Fruits and/or vegetables, %	8358	29.3±0.8	4285	28.8±0.9	4073	29.8±1.2
Eggs, and/or flesh foods, %	8358	49.1±1.1	4285	46.4±1.2	4073	51.7±1.4
Consumption of unhealthy food						
Sweetened beverages, %	8358	5.0±0.4	4285	4.3±0.5	4073	5.7±0.6
No fruits and vegetables, %	8358	70.7±0.8	4285	71.2±0.9	4073	70.2±1.2
Dietary adequacy						
Energy						
Mean intake (kcal)	8358	777±8.0	4285	688±7.0	4073	865±11
Mean adequacy, %	8358	90.6±0.9	4285	82.6±0.9	4073	98.5±1.4
Meeting 100% adequacy, %	8358	31.0±0.9	4285	22.0±1.0	4073	39.8±1.4
Protein						
Mean adequacy, %	8358	169.8±3.0	4285	113.6±1.8	4073	225.3±3.7
Meeting 100% adequacy, %	8358	64.0±1.0	4285	43.5±1.3	4073	84.3±1.0
Iron						
Mean adequacy, %	8358	61.5±2.3	4285	36.0±1.2	4073	86.8±3.1
Meeting 100% adequacy, %	8358	20.3±1.3	4285	6.6±0.7	4073	33.8±1.7
Calcium						
Mean adequacy, %	8358	147.7±4.7	4285	70.5±1.7	4073	224.1±6.2
Meeting 100% adequacy, %	8358	44.8±1.4	4285	17.7±0.9	4073	71.7±1.7
Vitamin A						
Mean adequacy, %	8358	183.6±4.7	4285	134.2±4.2	4073	232.5±8.0
Meeting 100 % adequacy, %	8358	50.0±1.0	4285	43.4±1.3	4073	56.5±1.2

Table 1. General and household characteristics of children 6-23 months old and their mothers, and feeding practices, food consumption, and dietary intake adequacy of children, Philippines: 2018-2019 *(continued)*

Variables	*All children (n=8,360)*		*Breastfed children (n=4,285)*		*Non-breastfed children (n=4,073)*	
	n	*Proportion/ Mean±SE*	*n*	*Proportion/ Mean±SE*	*n*	*Proportion/ Mean±SE*
Vitamin C						
Mean adequacy, %	8358	185.7±8.9	4285	62.9±3.3	4073	307.2±13.2
Meeting 100 % adequacy, %	8358	45.0±1.4	4285	17.8±0.8	4073	71.8±1.7
Zinc						
Mean adequacy, %	8358	119.8±3.6	4285	65.3±1.6	4073	173.7±4.5
Meeting 100% adequacy, %	8358	42.1±1.5	4285	17.5±1.0	4073	66.4±1.7
Thiamin						
Mean adequacy, %	8358	105.3±2.5	4285	55.0±2.1	4073	155.1±2.7
Meeting 100% adequacy, %	8358	38.9±1.4	4285	14.5±0.9	4073	63.0±1.6
Riboflavin						
Mean adequacy, %	8358	201.7±5.1	4285	78.8±2.5	4073	323.3±8.5
Meeting 100% adequacy, %	8358	51.4±0.9	4285	22.9±1.0	4073	79.6±1.4
Niacin						
Mean adequacy, %	8358	122.1±4.7	4285	63.5±2.0	4073	180.1±7.1
Meeting 100% adequacy, %	8358	37.8±1.4	4285	18.5±0.9	4073	56.8±2.4
Mean percentage adequacy						
Mean adequacy, %	8358	65.6±0.8	4285	49.5±0.7	4073	81.5±1.0

[†]Food security was assessed using Household Food Insecurity Access Scale (HFIAS)

more children from rural (56.4%) than urban (43.6%) households. About one-third (29.6%) of children belonged to severely food insecure households and half of the study children were from the poor (23.5%) and poorest households (26.8%).

Majority (83.8%) of breastfed children were currently receiving complementary foods, while 16.2% were given complementary foods plus breast milk substitute. All non-breastfed children received semi-solid or soft foods at the time of survey. The mean DDS was 3.1±0.0, regardless of breastfeeding status. Among breastfed and non-breastfed children, mean DDS were 3.3±0.0 and 2.9±0.0, respectively. Majority (88.1%) of the children consumed less than the recommended five food groups per day, about two-thirds received 2-3 food groups, while only 11.9% met the MDD of five or more food groups in their diets (16.0% among breastfed, and 8.0% among non-breastfed children).

The children received about six (5.8±0.10) feedings per day (including meals plus snacks, but not including breast milk for breastfed children and

including milk feedings for non-breastfed children). Nearly all (93.0%) children met the MMF recommendations; non-breastfed children had a mean feeding frequency of 7.6±0.1 (96.6% met the MMF) and 4.0±0.0 (89.3% met the MMF) among the breastfed. By meal frequency, 14.4% and 27.3% of breastfed children received two and three feedings, respectively, while majority (96.7%) of non-breastfed children received ≥4 feedings per day, implying that most children received more than the recommended feedings.

However, most (89.0%) children failed to receive the MAD. This improved significantly with age from 6-8 months (1.5%) to 9-23 months (12.6%), regardless of breastfeeding status. By breastfeeding status, 15.7% and 6.4% of breastfed and non-breastfed children met the MAD, respectively.

The diet of infants and young children reflected a limited range of food group intakes. Nearly all (96.7%) children consumed foods from the "grain products, roots and tubers" group. Nutrient-dense animal source foods like milk and dairy were consumed higher (64.2%) than meat (40.0%) and eggs (19.7%). Milk and dairy products were consumed by the majority (93.2%) of non-breastfed children, while only 34.9% among breastfed children. Non-breastfed children consumed slightly more meat (42.5%) and eggs (20.9%) than breastfed children (37.4% and 18.4%, respectively). Low consumption of fruits and/or vegetables (29.3%) was observed, with almost similar consumption pattern among non-breastfed and breastfed children. Alarmingly, majority (70.7%) of children did not consume any fruits and vegetables, and about 5.0% consumed sweetened beverages.

In terms of dietary and nutrient adequacies, the mean energy intake of children was 777 kcal/day, with 90.6% mean energy adequacy, but only 31.0% of children met the REI. Protein intake of children had >100% mean adequacy with about two-thirds (64.0%) meeting the protein requirement. The MPA was 65.6%. When disaggregated, mean iron adequacy was only 61.5%, while calcium, vitamin A, vitamin C, zinc, thiamin, riboflavin, and niacin had >100% mean adequacies. However, all eight key micronutrients had low proportion of children meeting the EARs.

By breastfeeding status, there were higher proportions of non-breastfed than breastfed children meeting the daily requirements for energy (39.8% vs. 22.0%), protein (84.3% vs. 43.5%), and eight key micronutrients. Likewise, non-breastfed children had a higher mean energy adequacy (98.5%) than breastfed children (82.6%). Mean protein and micronutrient adequacies were >100% in non-breastfed children, except for iron (86.8%), while only protein and vitamin A had >100% mean adequacies among breastfed children, with iron having the lowest mean adequacy (36.0%). The MPA among breastfed and non-breastfed children were 49.5% and 81.5%, respectively.

Association between dietary diversity and micronutrient adequacy

Table 2 presents the association of DDS and feeding frequency to dietary intakes of study children. Median MPA of all children was 70.3%. Median MPA increased with DDS, and it was significantly higher at DDS ≥5 (DDS 5: 79.2% and DDS >5: 84.8%) than DDS <5 (68.1%; $p<0.001$). This pattern was observed regardless of breastfeeding status. However, higher median MPA was consistently observed among non-breastfed than breastfed children ($p<0.001$). Significant correlation was seen between DDS and MPA; it was positively moderate in breastfed ($r_s=0.55$) and positively very weak in non-breastfed ($r_s=0.08$) ($p<0.001$) children.

To assess whether the 2021 IYCF

Table 2. Associations of dietary diversity score and meal frequency to dietary intakes of children 6-23 months old, Philippines: 2018-2019

Dietary Intake	*All children*				*Breastfed children (Bf)*				*Non-breastfed children (NBf)*				*Bf vs. NBf (p-value)*
	Median	*SD*	r_s [§]	*p-value*	*Median*	*SD*	r_s [§]	*p-value*	*Median*	*SD*	r_s [§]	*p-value*	
Dietary Diversity Score[†]													
Mean percentage adequacy, %	70.3	27.7	0.187	<0.001[§*]	42.6	24.9	0.554	<0.001[§*]	87.0	20.2	0.075	<0.001[§*]	<0.001[b*]
DDS													
<5	68.1	28.2	-	<0.001[a*]	38.3	23.7	-	<0.001[a*]	86.6	20.6	-	0.038[a*]	<0.001[b*]
5	79.2	21.0	-		67.6	21.4	-		90.4	14.8	-		<0.001[b*]
>5	84.8	18.1	-		70.0	18.4	-		92.3	12.8	-		<0.001[b*]
Feeding frequency[‡]													
Energy													
Total intake (kcal)	667	374	0.501	<0.001[§*]	614	259	0.615	<0.001[§*]	761	451	0.430	<0.001[§*]	<0.001[b*]
Feeding frequency													
2	462	98	-	<0.001[a*]	463	90	-	<0.001[a*]	277	290	-	<0.001[a*]	0.005[b*]
3	536	175	-		546	155	-		385	276	-		<0.001[b*]
4	621	241	-		641	211	-		498	321	-		<0.001[b*]
5	700	275	-		736	248	-		637	298	-		<0.001[b*]
≥6	840	445	-		907	337	-		821	461	-		<0.001[b*]
Percent energy adequacy, %													
Age group													
6-23 months	79.3	44.0	0.430	<0.001[§*]	75.7	31.4	0.461	<0.001[§*]	85.2	53.3	0.492	<0.001[§*]	<0.001[b*]
Feeding frequency													
2	64.2	16.3	-	<0.001[a*]	64.4	15.6	-	<0.001[a*]	30.1	30.7	-	<0.001[a*]	<0.001[b*]
3	67.3	24.8	-		69.4	22.5	-		41.6	33.6	-		<0.001[b*]
4	74.0	29.6	-		77.4	26.6	-		51.7	35.0	-		<0.001[b*]
5	80.0	31.9	-		87.0	29.2	-		68.0	32.4	-		<0.001[b*]
≥6	96.0	52.8	-		105.2	43.2	-		94.0	54.3	-		<0.001[b*]

Table 2. Associations of dietary diversity score and meal frequency to dietary intakes of children 6-23 months old, Philippines: 2018-2019 *(continued)*

Dietary Intake	*All children*				*Breastfed children (Bf)*				*Non-breastfed children (NBf)*				*Bf vs. NBf (p-value)*
	Median	*SD*	r_s §	*p-value*	*Median*	*SD*	r_s §	*p-value*	*Median*	*SD*	r_s §	*p-value*	
6-8 months	86.6	48.1	0.480	<0.001§*	82.0	31.4	0.605	<0.001§*	102.4	65.9	0.391	<0.001§*	<0.001[b]*
Feeding frequency													
2	71.0	12.1	-	<0.001[a]*	71.0	12.1	-	<0.001[a]*	00.0	00.0	-	<0.001[a]*	-
3	80.6	19.6	-		80.8	18.9	-		39.7	20.9	-		0.002[b]*
4	87.5	35.6	-		87.8	35.4	-		72.5	29.8	-		0.023[b]*
5	99.6	29.9	-		101.9	24.8	-		80.0	49.5	-		0.006[b]*
≥6	109.1	64.6	-		127.6	53.3	-		103.9	66.0	-		0.002[b]*
9-23 months	77.8	43.0	0.455	<0.001§*	73.8	31.1	0.509	<0.001§*	83.2	51.1	0.492	<0.001§*	<0.001[b]*
Feeding frequency													
2	56.0	16.0	-	<0.001[a]*	56.4	15.0	-	<0.001[a]*	30.1	30.7	-		<0.001[b]*
3	62.4	24.4	-		64.0	21.8	-		41.7	34.0	-		<0.001[b]*
4	71.1	27.9	-		74.6	24.0	-		51.5	35.2	-	<0.001[a]*	<0.001[b]*
5	78.0	31.5	-		84.5	29.1	-		67.4	31.9	-		<0.001[b]*
≥6	94.2	50.5	-		102.6	41.0	-		92.2	52.1	-		<0.001[b]*

[†]Dietary diversity score was determined based on the eight (8) food groups in the 2021 WHO-IYCF guidelines: breast milk, grains, roots and tubers and plantains, dairy products, legumes and nuts, flesh foods, eggs, vitamin-A rich fruits and vegetables, and other fruits and vegetables.

[‡]Feeding or meal frequency is the number of feedings received by the child in the previous day. It includes milk feedings among non-breastfed children.

[§]Based on Spearman's correlation coefficient

[a]Based on Kruskal-Wallis Test

[b]Based on Mann-Whitney U test

*Significant at $p<0.05$

Table 3. Area under the curve (AUC) analysis of dietary diversity score among children 6-23 months old, Philippines: 2018-2019

Dietary diversity score	*Mean percentage adequacy (MPA) cut-off*											
	MPA: ≥50%				*MPA: ≥75%*				*MPA: ≥100%*			
	Cut-off	*Sensitivity*	*Specificity*	*AUC*†	*Cut-off*	*Sensitivity*	*Specificity*	*AUC*†	*Cut-off*	*Sensitivity*	*Specificity*	*AUC*†
All children	4	0.39	0.80	0.59	4	0.36	0.72	0.54	6	0.03	0.97	0.50
Breastfed children	4	0.58	0.79	0.69	4	0.61	0.70	0.65	5	0.31	0.86	0.58
Non-breastfed children	3	0.62	0.56	0.59	4	0.30	0.78	0.54	6	0.02	0.98	0.50

†Area under receiving operating characteristic curve

Table 4. Area under the curve (AUC) analysis of feeding frequency among children 6-23 months old, Philippines: 2018-2019

Feeding frequency	*Meeting the recommended energy intake*											
	Energy intake adequacy: ≥50%				*Energy intake adequacy: 75%*				*Energy intake adequacy: 100%*			
	Cut-off	*Sensitivity*	*Specificity*	*AUC*†	*Cut-off*	*Sensitivity*	*Specificity*	*AUC*†	*Cut-off*	*Sensitivity*	*Specificity*	*AUC*†
All children	4.5	0.62	0.55	0.59	4.5	0.73	0.57	0.65	5.5	0.72	0.67	0.69
Breastfed children	3.5	0.60	0.76	0.68	3.5	0.73	0.60	0.67	4.5	0.63	0.77	0.70
6-8 months	-	-	-	-	2.5	0.87	0.56	0.71	3.5	0.70	0.73	0.71
9-23 months	3.5	0.66	0.76	0.71	3.5	0.80	0.56	0.68	4.5	0.68	0.74	0.71
Non-breastfed children	6.5	0.70	0.69	0.69	7.5	0.61	0.74	0.68	7.5	0.69	0.66	0.67

†Area under receiving operating characteristic curve

DDS cut-off points could correctly identify Filipino children with adequate nutrient intake, sensitivity and specificity analyses were done using 50%, 75%, and 100% MPA. Table 3 shows that the DDS cut-offs that maximised sensitivity and specificity increased with MPA, thus improving nutrient intake adequacy. The cut-offs that maximised sensitivity and specificity in correctly identifying breastfed and non-breastfed children with 100% MPA were DDS 5 (AUC=0.58) and 6 (AUC=0.50), respectively.

Association between feeding frequency and energy adequacy

Feeding frequency was positively associated with energy intake as shown in Table 2. Total energy intake and percent energy adequacy increased with feeding frequency: from 462 kcal and 64.2% for two feedings to 840 kcal and 96.0% for ≥6 feedings daily, respectively (p<0.001). The same pattern was also observed in breastfed and non-breastfed children. By feeding frequency recommendations, breastfed children receiving 2-3 feedings daily had a total intake of 463-546 kcal, while non-breastfed children receiving four feedings had an intake of 498 kcal. The percent energy adequacy of breastfed children aged 6-8 months receiving two feedings daily was 71.0%, while those aged 9-23 months receiving three feedings daily had 64.0% energy adequacy. Non-breastfed children meeting the MMF of five had an energy adequacy of 68.0%.

Total intake and energy adequacy were significantly higher among non-breastfed than breastfed children (761 kcal vs. 614 kcal, and 85.2% vs. 75.7%, respectively; p<0.001), while the opposite was observed in terms of feeding frequency. Feeding frequency and total intake were positively, strongly correlated among breastfed children (r_s=0.62; p<0.001) and positively, moderately correlated among non-breastfed children (r_s=0.43; p<0.001). Percent energy adequacy was positively, strongly correlated with feeding

Table 5. Association of minimum acceptable diet to nutritional status of children 6-23 months old, Philippines: 2018-2019

Complementary Feeding Indicators	*n*	*Nutritional status*					
		Underweight		*Stunting*		*Wasting*	
		%	*p-value*†	*%*	*p-value*†	*%*	*p-value*†
All Children							
MAD							
Not meeting	7463	16.8	0.782	27.6	0.037*	6.3	0.036*
Meeting	895	16.9		32.3		4.2	
Breastfed children							
MAD							
Not meeting	3658	21.5	0.596	30.8	0.080	7.5	0.019*
Meeting	627	19.7		34.1		4.7	
Non-breastfed children							
MAD							
Not Meeting	3805	12.5	0.169	24.7	0.866	5.3	0.174
Meeting	268	9.9		27.8		3.1	

MAD: minimum acceptable diet
†*p*-value based on Chi-square test
*Significant at p<0.05

frequency among breastfed children aged 6-8 months (r_s=0.61; p<0.001) and positively, moderately correlated among breastfed children aged 9-23 months (r_s=0.51) and non-breastfed children (r_s=0.49) (p<0.001).

In evaluating the IYCF feeding frequency recommendations, our results showed that a feeding frequency cut-off of 4 (AUC=0.71) and 5 (AUC=0.71) for breastfed children 6-8 and 9-23 months old, respectively, and a cut-off of 8 (AUC=0.67) for non-breastfed children maximised the sensitivity and specificity in identifying Filipino children meeting the 100% REI (Table 4). Moreover, it can be noted that energy intake adequacy improved from low (50%), better (75%), to high (100%) as feeding frequency cut-offs increased.

Association between minimum acceptable diet and anthropometric status

Table 5 shows the association between the MAD and anthropometric status of study children. Stunting was positively associated with MAD in all children, reflecting a higher prevalence among those who met the recommendation (32.3%; p<0.05). Meanwhile, wasting was negatively associated with MAD in all children and breastfed children, showing higher prevalence among children who failed to achieve the MAD (6.3% and 7.5%, respectively; p<0.05). No significant associations were found between MAD and anthropometric status among non-breastfed children.

DISCUSSION

Findings of this study suggested that complementary feeding practices measured using the selected 2021 IYCF indicators were associated with the nutrient and energy adequacy of diets in children 6-23 months old. Sensitivity and specificity analyses showed that the 2021 DDS cut-off of 5 was a good proxy indicator in identifying breastfed Filipino children with adequate nutrient intake, but a DDS cut-off of 6 was more fitting for non-breastfed children. For feeding frequency, a cut-off of 4 for breasted children aged 6-8 months, 5 for breastfed children aged 9-23 months, and 8 for non-breastfed children identified children with adequate energy intake, which were higher than the WHO and UNICEF recommendations. Lastly, the MAD indicator demonstrated a positive association with stunting in all children and a negative association with wasting in all children and breastfed children.

Dietary diversity as an indicator of micronutrient adequacy

The diet of Filipino children mainly consisted of rice, milk and dairy, meat, eggs, fruits and vegetables, with a mean DDS of 3.1. Similar to the findings of Molani-Gol, Kheirouri & Alizadeh (2023), DDS was positively correlated with micronutrient adequacy. The MDD has the highest contribution to dietary adequacy (Khor *et al.*, 2016), hence a useful proxy indicator in predicting dietary quality regardless of breastfeeding status (Moursi *et al.*, 2008). Dietary diversity was positively associated with dietary quality in developing countries as mean nutrient density adequacy increased with DDS, regardless of quantity consumed (Dewey *et al.*, 2006; Working Group on IYCF Indicators, 2006). Thus, it is worth highlighting the significantly higher median MPA in children with DDS ≥5 than DDS <5 in this study, implying that the new DDS cut-off could predict higher micronutrient adequacy. However, there was a low MPA among breastfed children in this study. Giving nutritionally adequate complementary foods will likely fill the nutrient gap of certain key nutrients (Dewey, 2013). On the other

hand, the very weak correlation between DDS and micronutrient adequacy among non-breastfed children could be influenced by low intake of foods from different food groups (Kennedy *et al.*, 2007), breastfeeding status, energy intake (Wright *et al.*, 2015), age, and sex (Rani, Arends & Brouwer, 2010).

Understandably, the selected 2021 IYCF indicators are still lacking in validation studies. This study showed that a DDS cut-off of 5 and 6 can correctly identify breastfed and non-breastfed children with ≥100% MPA respectively, which may indicate that the new DDS cut-off indicator is appropriate for breastfed, but not non-breastfed children. Meanwhile, in previous validation studies, a DDS cut-off of 6 could only identify non-breastfed Filipino children with ≥75% MPA (Kennedy *et al.,* 2007).

Feeding frequency as an indicator of energy adequacy

Feeding frequency that is less than recommended can compromise energy intake, which may cause growth faltering, stunting, and micronutrient deficiencies (WHO, 2021). Our findings concur with other studies wherein feeding frequency reflected energy intakes (Dewey *et al.,* 2006; Islam *et al.,* 2008; Roche *et al.,* 2017; Working Group on IYCF Indicators, 2006). Achieving the MMF increases the probability of meeting energy requirements, particularly in developing countries with low or average breast milk intake (Roba *et al.*, 2016). However, despite the significant correlation between feeding frequency and total intake and adequacy, breastfed children aged 6-8 months and 9-23 months, and non-breastfed children aged 6-23 months were not able to meet 100% adequacy with the minimum feeding frequency recommendations. Feyisa *et al.* (2020) found that even children who met the MMF had inadequate energy intake, which could be due to low energy density, tied with the consumption of food with limited diversity in small quantities.

The best feeding frequency cut-offs that could identify breastfed children aged 6-8 and 9-23 months meeting the 100% REI were 4 and 5, respectively, while for non-breastfed children, the identified cut-off was 8. These cut-offs were twice higher than the established recommendations. This could explain the high proportion of children meeting the minimum feeding frequency, yet only nearly a third were able to achieve adequate energy intake based on the established MMF. Hence, increasing the MMF cut-offs may be able to classify more Filipino children with 100% energy adequacy.

Association of MAD to anthropometric status

The MAD indicator is a composite of MDD and MMF, which are essential for proper growth and development among children. More than a quarter of children were stunted and about one-fifth were underweight. Similar with a study from Indonesia (Ahmad *et al.,* 2018), this study found no significant difference between MAD status and underweight prevalence. Meanwhile, there was a negative association between MAD and wasting in all children and breastfed children, and a positive association with stunting in all children. In Kenya, MAD was a significant predictor of wasting among children (Korir, 2013).

The three selected complementary feeding indicators best reflect a child's recent diet as they only capture current food intake of the previous day, implying that these indicators may not be sensitive to chronic undernutrition, thus, showing more association with wasting than stunting (Saaka *et al.*, 2015). Moreover,

anthropometric status of children may be influenced by the dietary diversity component of MAD (Jones *et al.*, 2014). Poor dietary diversity simultaneously affects MDD and thereafter MAD, thus affecting child growth and development (Roba *et al.*, 2016).

Limitations

Limitations may include one-day recall bias and error of mothers or caregivers during interview for the 24-hour dietary recall. Furthermore, as DDS, MMF, and MAD were only based on the current intake using the 24-hour food recall method, these indicators may not reflect on the status of stunting as this is an outcome of long-term exposure to various risk factors. Despite this, our study is the only local study to date that attempted to validate the three selected 2021 IYCF complementary feeding indicators. This study utilised data from national and population-based survey and outcomes may represent the entire country's situation.

CONCLUSION

The study findings provided evidence that DDS and feeding frequency of the 2021 IYCF complementary feeding indicators are valid indicators of adequate micronutrient and energy intakes for children aged 6-23 months. Despite the practicality of these selected IYCF indicators in reflecting dietary adequacy, they may not capture the entire process related to the nutritional outcomes of infants and young children, particularly stunting. Other factors not covered in the study may have influenced the increased risk of stunting.

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Authors' contributions

Goyena EA, conceived, carried out the study, reviewed and edited the manuscript; Maniego MLV, analysed and interpreted the data; Cristobal AG, interpreted and assisted in the write-up of the manuscript; Goyena EA, Maniego MLV & Cristobal AG read and approved the manuscript.

Conflict of interest

The authors declare that they have no competing interests. The author(s) received no financial support for the research, authorship, and/or publication of this article.

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Effect of processing on resistant starch content of Indian rice varieties (*Sona Masuri* and *Mogra*) and its impact on postprandial blood glucose levels

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ABSTRACT

Introduction: Resistant starch has elicited new possibilities around the globe due to its plausible health benefits and functional properties. This research aimed to evaluate the effect of processing on the resistant starch content in selected Indian rice varieties and its effect on postprandial blood glucose levels. **Methods:** Two Indian rice varieties (*Sona Masuri* and *Mogra)* were evaluated for resistant starch in raw form, after boiling, steaming, and refrigeration. Thereafter, the increment in resistance starch content by different processing methods was validated by oral glucose tolerance test (OGTT) on ten healthy participants. **Results**: Among the two rice varieties, raw *Sona Masuri* had a higher resistance starch content (10.86%). After processing, resistant starch was observed to be high in steamed *Mogra* variety (3.52%). In the boiling process, *Sona Masuri* developed a higher resistant starch content (2.44%) as compared to *Mogra* variety (1.05%). The assessment done after refrigeration revealed a slight increase in resistant starch content in both rice varieties. *Mogra* variety had higher resistant starch (3.68%) than the other rice variety (2.56%) after refrigeration. Validation of increase in resistant starch content and its effect on blood glucose responses done through OGTT revealed that *Mogra* rice (test food) did not cause a swift spike in blood glucose level compared to glucose (reference food). Differences in blood glucose responses by test and reference food at 0, 30, 60, 90, and 120 minutes were statistically significant. **Conclusion**: Steamed and refrigerated *Mogra* rice did not cause significant increase in blood glucose.

Keywords: blood glucose, processing, resistant starch, rice

INTRODUCTION

Cereals are among the most consumed and economical source of carbohydrates (55–75%) and contribute extensively to energy intake (Vaidya & Sheth, 2011). The amount of cereals consumed in daily diet plays a crucial role in the prevalence of metabolic disorders like diabetes, obesity, and cardiovascular diseases (Rhee, 2015; Van Dam, 2020). Among them, diabetes, a chronic metabolic disorder, has been designated as a 'silent killer' due to its poor rate of detection. Research data have recorded many complications associated with diabetes.

Several researches have been done on the modification of dietary factors. Imposing restrictions on intake of high glycaemic foods like sugar and

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sugary foods, sugary soft drinks, white bread, potatoes, and white rice are one of the solutions given to diabetic patients by healthcare professionals. The elimination of so many foods has resulted in limiting the food options for the diabetic population (Nanri *et al.*, 2010).

Scientists have been working on the modifications of high glycaemic foods, especially rice, through various techniques such as genetic modification, altering its chemical composition in terms of amylose and amylopectin content, processing techniques, and storage conditions. One of them is the incubation of starch with enzymes without dispersing agents, which has given a unique classification of starches; the most popular one is resistant starch (RS). RS is resilient to breakdown by amylase and pullulanase, and is found in cereal grains, seeds, and heated starch or starch-containing foods (Charalampopoulos *et al.*, 2002). The process of retrogradation results in the formation of RS type 3, also known as retrograded starch.

Many researches stated the benefits of RS and its positive impact on prevention of colon cancer, diabetes, and obesity because of its high fibre content and slow digestion property in the intestine. It also acts as a soluble fibre in human body (Mikulíková, Masár & Kraic, 2008). It shows reduction in postprandial glycaemic response, abdominal fat, insulin resistance, and cholesterol, and improvement in the number of gut bacteria (Keenan *et al.*, 2013; Shen *et al.*, 2011).

Rice is a staple food in South Asian countries and because of its high glycaemic index (GI), it has become a source of calories; thus, not suggested for diabetic patients. In order to explore the possibilities for the inclusion of rice in diabetic diet, this study was planned and conducted to determine the impact of processing on RS content in rice. The study also aimed to validate the increment in RS content after processing through its impact on blood glucose levels. Thus, the objectives of this study included the estimation of RS content in selected Indian rice varieties (raw, after boiling, steaming, and refrigeration) and the impact of processing on blood glucose levels.

METHODOLOGY

The methodology opted for the current study can be discussed in two phases (Figure 1): Phase I (estimation of RS in selected Indian rice varieties before and after cooking, and after cooling i.e., by refrigeration) and Phase II (effect of processing on blood glucose level).

Phase I

Sample procurement

For the present study, two Indian rice varieties *(Sona Masuri and Mogra*) were selected. Both low-income and middle-income Indian families relish these rice varieties more because of their reasonable market price (as found during market survey done on 10 local grocery shops and online stores). The market price of *Sona Masuri and Mogra* were 50Rs/kg and 55Rs/kg, respectively, as compared to other rice varieties ranging from 80Rs/kg to 200 Rs/kg. *Sona Masuri* is a non-aromatic, Indian non-basmati rice variety with broken grains, while Mogra is an aromatic Indian basmati rice variety with broken grains. Hence, their market price were lower compared to other rice varieties. Samples of selected varieties of rice were collected from a local grocery store (selling different varieties of rice) in Faridabad, Haryana, India. All grains procured were weighed and collected in clean sample collectors.

The selected samples underwent two moist heat methods of processing, namely boiling and steaming. Boiling was done for 20 minutes in an open pan with filtered tap water (1:3 w/v) and

extra water was drained. Steaming of rice was done in a pressure cooker with filtered tap water (1:2 w/v). RS content was analysed by using the RS Megazyme Assay Kit using spectrophotometer (Thermofisher, USA) (McCleary & Monaghan, 2002).

Statistical analysis revealed that the *Mogra* rice had increased RS content after steaming and the *Sona Masuri* had increased RS content after boiling. Therefore, boiled *Sona Masu*ri and steamed *Mogra* rice were selected for further study and they were stored in the refrigerator at 4^0C for ten hours. After refrigeration, RS content was analysed and steamed *Mogra* rice, which was rich in RS content was used for Phase II.

Phase II

Oral glucose tolerance test (OGTT)

To validate the results of phase I, OGTT was performed. Participants were fed with steamed + refrigerated *Mogra* rice.

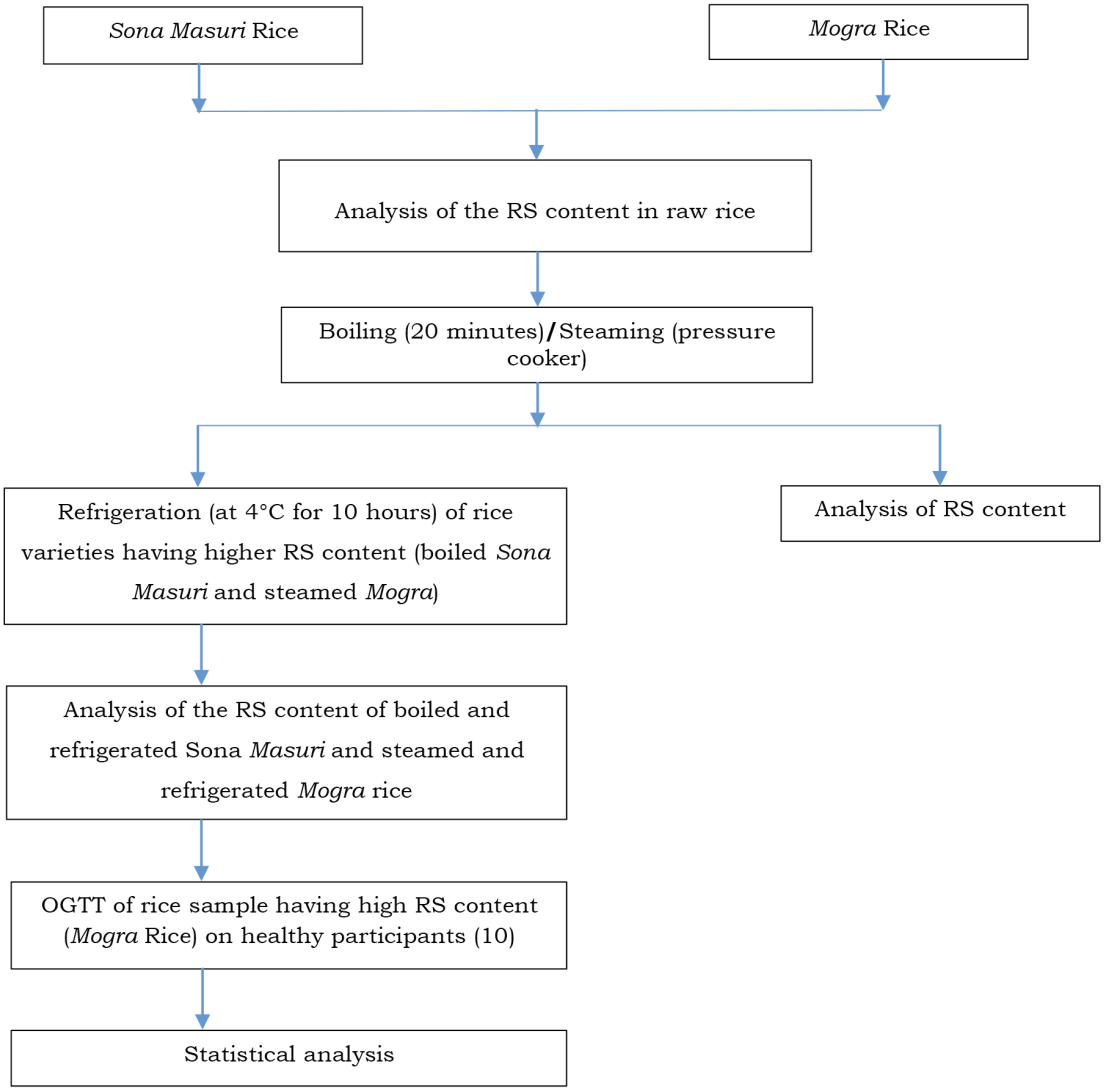

Figure 1. Flowchart of study methodology

For the study, ten healthy volunteers were selected from the Girls Hostel of Manav Rachna Campus. These participants were healthy and aged 20 to 30 years. Before proceeding with the intervention study, written and verbal consents were obtained from the participants.

Glucose solution (75 g of glucose dissolved in 250ml of water) was taken as reference food and rice variety was considered as test food. For blood glucose testing, ten healthy participants (after an overnight fasting of eight hours) were fed with glucose solution. The blood glucose levels of participants were tested after every half an hour i.e., at 0 minute (baseline), 30 minutes, 60 minutes, 90 minutes, and 120 minutes (Nelson & Blauvelt, 2015).

The next day, the same participants were tested first for fasting blood glucose and thereafter, the RS-rich steamed *Mogra* rice was fed to them. The amount of rice fed to the participants was calculated so that it provided 75 g of available carbohydrates. Subsequently, blood glucose levels of the participants were noted at half an hour intervals.

The experimental protocol was approved by the Ethical Committee, Faculty of Allied Health Sciences, Manav Rachna International Institute of Research and Studies (MRIIRS/FAHS/DEC/2021-22/N&D/M035).

Glycaemic Index (GI) of test food

The GI of test food was calculated in the study. The formula used to calculate GI: dividing the incremental blood glucose area of test food by the incremental area of reference food, multiplied by hundred.

Statistical analysis

The IBM SPSS Statistics for Windows version 28.00 (IBM Inc., Armonk, New York, USA) was used for statistical analysis. Study results were expressed as mean±standard deviations (*SD*) for RS content of *Sona Masuri* and *Mogra* rice. To determine the effect of processing on the RS content of raw, boiled, and steamed *Sona Masuri* and *Mogra* rice, one-way analysis of variance (ANOVA) was used; whereas to determine the effect of storage conditions on the RS content of steamed and refrigerated *Mogra* rice, *t*-test was performed.

Comparison of the spike in blood glucose levels by reference and test food against each time interval was done by independent *t*-test. To compare blood glucose responses with time at 0 minute, 30 minutes, 60 minutes, 90 minutes, and 120 minutes in one group (reference or test), one-way ANOVA was used and significance was reported at 5 percent level.

GI was calculated by dividing the incremental blood glucose area of test food by the incremental area of reference food, multiplied by 100. Incremental area under the curve (IAUC) was calculated by Graphpad Prism 9.

RESULTS

Phase I: Effect of processing on the RS content of Indian rice

RS content of raw and cooked rice varieties

Results of the RS content of raw and cooked rice varieties after boiling and steaming are presented in Table 1. Data in the table depicts that RS fraction was significantly ($p \leq 0.05$) influenced by variety.

RS content as analysed in raw rice was higher (10.86) in *Sona Masuri* rice than in the *Mogra* rice variety (3.76). Furthermore, when the raw rice varieties underwent boiling, *Sona Masuri* showed higher RS content (2.44) compared to the latter. After steaming, *Mogra* rice variety showed a higher RS content (3.52) in comparison to steamed *Sona Masuri*. Among all treatments, boiled *Sona Masuri* and steamed *Mogra* rice, due to their higher RS content, were further refrigerated.

Table 1. Effect of cooking and refrigeration on the resistant starch (RS) content of Indian rice varieties

Rice varieties	*Sona Masuri*	*Mogra*
Effect of cooking		
Raw	10.86±0.01	3.76±0.01
Boiled	2.44±0.01	1.05±0.01
Steamed	2.02±0.01	3.52±0.01
F-value	746092.00	67513.00
p-value	<0.001*	<0.001*
One-way ANOVA		
Effect of refrigeration		
Boiled+refrigerated	2.56±0.05	-
Steamed+refrigerated	-	3.68±0.05
t-test	<0.001*	<0.001*

RS values presented as percentages
*Significant at p<0.05

RS content in the rice varieties after refrigeration

The effect of storage conditions on the RS content of Indian rice, *Sona Masuri* and *Mogra*, is depicted in Table 1. The mean values depicted that the steamed and refrigerated *Mogra* rice had significantly higher RS content, i.e., 3.68±0.005 than the boiled and refrigerated *Sona Masuri* variety (2.56±0.005). Considering the result analysis, the *Mogra* rice variety after steaming was further used for Phase II of the study and was analysed for its effect on blood glucose level because of its high RS content.

Phase II

Effect of rice processing on postprandial blood glucose level

Blood glucose concentrations (mmol/L) for reference (glucose) and test (*Mogra* rice) foods at every 30 minutes are shown in Table 2. At 0 minute before intervention, differences in blood glucose spikes by reference and test foods were not significant. However, a sharp spike in the blood glucose level was observed after 30 minutes in the case of reference food. After that, blood glucose levels started to decline.

Table 2. Difference in blood glucose concentrations (mmol/L) after consumption of reference and test foods at 30 minutes intervals

Time	*Reference food (glucose solution)*	*Test food (steamed and refrigerated Mogra Rice)*	*p-value*
0 minute	5.1±0.7 ^{bcd}	5.1±0.7	0.884
30 minutes	8.8±1.6 ^{ade}	5.7±0.9	<0.001*
60 minutes	8.6±1.3 ^{ae}	5.5±0.9	<0.001*
90 minutes	6.9±1.6 ^{ab}	5.2±0.8	0.004
120 minutes	5.8±1.4 ^{bc}	5.1±0.7	0.145
p-value	<0.001**	0.264	

*Data expressed as mean±SEM
**Values in the same row with different superscripts (a,b,c,d,e) are significantly different (p<0.05) by one-way ANOVA and post-hoc test
^{a,b,c,d,e}Means with different superscripts in the same row differ significantly (p<0.05)

Table 3. IAUC and calculated glycaemic index for reference and test foods

Groups	*IAUC±SEM*	*p-value*	*Glycaemic index*
Reference food (glucose)	4979.0±588.5	0.001	-
Test food (steamed and refrigerated *Mogra* Rice)	963.4±483.4		19.34

After intervention, a significant difference ($p<0.05$) was reported in blood glucose responses every 30 minutes. The data revealed that the glucose level of participants administered with test food (*Mogra* rice) had better glucose control throughout the 2 hours as compared to blood glucose levels raised by reference food. The reason for such a difference is because of the property of resistant starch as soluble fibres that slow digestibility in the intestine.

A clear inference can be made from Figure 2, which showed the variation in glycaemic response of glucose solution and *Mogra* rice. The line chart depicted that the glucose solution caused a sharp rise in blood glucose levels, whereas the release of glucose by *Mogra* rice variety was slow and steady, thereby not causing any instant spike in blood glucose levels. The graph also showed that after 120 minutes, blood glucose levels of the participants fed with reference and test foods were almost similar. Further, it can also be inferred from the line graph that glucose solution (reference food) was able to spike blood glucose till 90 minutes of consumption, while blood glucose upsurged by the test food (*Mogra* rice) was limited to 60 minutes with a slow rise.

Changes in blood glucose levels within time intervals for each group (reference and test) were also assessed. Post-hoc test revealed that there was a significant difference ($p<0.05$) in the rise of blood glucose level by reference food at every 30 minutes interval. However, no significant difference was observed in the test food after every 30 minutes of blood glucose analysis.

GI and incremental area under the curve (IAUC)

The study assessed the GI of the rice varieties having maximum resistant starch by calculating the incremental area under the blood glucose response curve (IAUC). The results revealed that

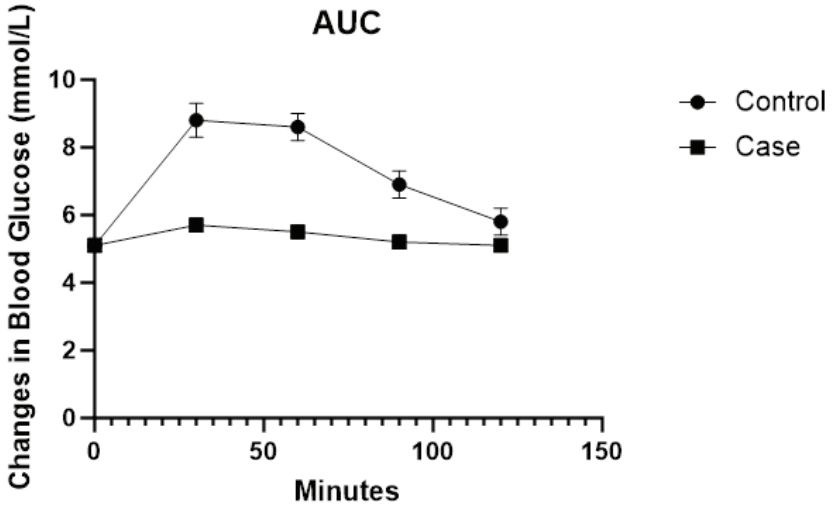

Figure 2. Glycaemic response curves for reference and test foods

GI of *Mogra* rice (steamed and refrigerated) was 19.34 and is represented in Table 3. Table 3 and Figure 2 determined the IAUC for *Mogra* rice (steamed and refrigerated). Significant difference ($p<0.05$) was found in the IAUC between standard and *Mogra* rice (steamed and refrigerated).

DISCUSSION

There was a significant variation in the RS content of different rice varieties, which can be inferred from the values of RS content obtained after analysis with one-way ANOVA. *Sona Masuri* rice was observed with a higher RS content in the raw form than *Mogra* rice. Variation in the RS content of rice varieties in the present study may be caused by changes in starch hierarchical structures, protein compositions and physical barriers, lipid compositions and amylose-lipid complex, cell wall architecture and compositions of the rice grains (Yi & Li, 2021).

The *Mogra* rice variety showed higher RS content after steaming than boiling. On the contrary, *Sona Masuri* rice had greater RS after boiling in contrast to steaming. The reason here could be the destruction of RS structure while steaming, in the case of *Sona Masuri,* that reduced the RS content. Many factors, including amylose chain lengths, granule size, type of crystalline polymorphs, physical insulation of starch by thick-walled cells, porosity, and physical distribution of starch in relation to the dietary fibre components, may have influence over it (Yadav, Sharma & Yadav, 2009). It is not imperative that a rice variety having a higher RS content in the raw form will have a higher RS content post-processing. Although raw *Sona Masuri* rice had a higher RS content, but steamed *Mogra* rice in comparison to steamed *Sona Masuri* rice had a greater RS content after processing. In a study conducted on sweet corn, it was revealed that boiling and steaming did not affect the total starch and water-soluble carbohydrates of sweet corn, but there was a reduction in resistance starch content (Zhang *et al.*, 2022).

A study conducted on resistant starch content after cooling of cooked white rice and its impact on glycaemic response concluded that cooling of rice after cooking can be opted to increase its resistant starch content. This study was conducted in China with three different treatments (treatment 1: freshly cooked rice; treatment 2: cooked rice cooled at room temperature for 10 hours; treatment 3: cooked rice cooled for 24 hours at 4°C then re-heated) (Sonia, Witjaksono & Ridwan, 2015).

Processing techniques, such as baking, boiling, and roasting, can enhance RS content along with shallow frying. Steaming and frying decrease RS content in cereals, specifically wheat, rice, maize, and pearl millet, as verified in another study (Vaidya & Sheth, 2011). Research data have shown that moist heat treatment can have an incremental effect in RS content. On the contrary, the application of dry heat can cause decrease in RS content. A considerable amount of increment in RS content was also seen when rice and grain products were stored overnight (Nigudkar, 2014).

In the current study, the statistical analysis of blood glucose levels by reference and test foods revealed that steaming and refrigeration had an overall positive impact on the rise in blood glucose levels due to the increment in RS content. Application of independent sample *t*-test revealed that the blood glucose levels of participants from both groups significantly increased after 30 minutes and 60 minutes as compared to 90 minutes and 120 minutes. However, a major change in the blood glucose values at 30 and 60 minutes was observed in the case of reference food (glucose solution). It was clear from the one-way ANOVA analysis that RS-rich *Mogra* rice after processing showed a slow and steady rise in blood

glucose levels, while a sharp rise was observed in the case of reference food. There was no clear sudden spike in the blood glucose levels by test food (RS-rich *Mogra),* demonstrating a lower GI due to increment in RS content.

GI was calculated by using IAUC. The results revealed that GI of *Mogra* (steamed and refrigerated) was 19.34, low in comparison to the GI of 5 other Indian rice varieties without any processing treatment (*Sampata*: 56.38; *Dhanrasi*: 59.23; *DRR Dhan* 42: 71.73; *DRR Dhan* 43: 87.40; *Jarava*: 94.05) and thus, can be recommended to diabetic patients (Azam *et al.*, 2020). Variation caused in RS due to the proportion of amylose to amylopectin in grains may decrease total GI in foods after cooking.

The consumption of high GI foods is higher in the South Asian populations, thus making them more prone to type-2 diabetes mellitus and cardiovascular diseases. Factors, such as inherent starch characteristics (amylose: amylopectin ratio), processing techniques after harvesting (particularly parboiling), and cooking methods including storage and then reheating lead to changes in rice, in turn affecting postprandial glycaemic responses in the population. In general, the observed GI of rice ranges from 48 to 93, indicating it as a high-glycaemic food (Blaak *et al.*, 2012). Moreover, the fluctuation in blood glucose levels is further influenced by consumer characteristics like chewing habit and ethnicity. Hence, the cooking time of rice and choosing rice with high amylose content will be beneficial for diabetic people as it will help in bringing a lower postprandial glucose response to the body.

In this study, RS fractions were influenced by the variety of rice, its processing and storage conditions. The combined effect of steaming and refrigeration had a positive impact on the glycaemic response of rice due to increased RS content. Results of the blood glucose level estimation further validated that increased RS content in food varieties can delay glycaemic response in the body.

Although this study had covered vital points with reference to the RS content of rice using two of the most widely used methods of rice preparation, other household methods used to prepare rice like microwaving, soaking then autoclaving, etc. can be used to determine which method yields higher RS content. Other factors, such as botanical source, moisture content, amylose: amylopectin ratio, etc. that have a crucial impact on the RS content of rice should also be considered. The present study estimated postprandial glycaemic response after a half an hour interval. Research data are also available where significant blood glucose spikes were reported within 15 minutes interval. There is a possibility of different inferences in the present study through early inception of postprandial glucose responses in the case of both foods. In this research, for the validation of the variation in increased RS content of rice on blood glucose levels, only female participants were included for the facilitation of the study. However, future research can also include male participants to study any changes on blood glucose response that may be affected by sex differences.

CONCLUSION

The inferences of the results from this study clearly showed that processing methods and low temperature storage of selected rice varieties had an impact on resistant starch content. Furthermore, these methods significantly affected blood glucose levels as observed by OGTT. This study highlighted the possibilities for pre-diabetic and diabetic people to relish white rice by changing its cooking methods, followed by low temperature storage.

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Authors' contributions

Mishra S, performed the lab experiments and clinical study; Awasthi M, supervised the study, data analyses, critically reviewed the manuscript and had overall responsibility; Sharma M, performed the statistical analysis and interpretation.

Conflict of interest

None

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Validation of the Malay version of Food Insecurity Experience Scale (M-FIES) using Rasch analysis

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ABSTRACT

Introduction: Food security can be defined as the availability of and accessibility to food, acquired in an acceptable means at any given time and place in a way that could maintain health and wellbeing. One critical dimension of food security is continued access to adequate food. To measure this dimension, the Food and Agriculture Organization (FAO) developed the latest measurement tool, namely the Food Insecurity Experience Scale (FIES), the first instrument to measure people who experience food insecurity globally. This study aimed to validate the construct validity and reliability of the Malay version of FIES (M-FIES) for Malaysians. **Methods:** This cross-sectional study was conducted among 145 households in Kuantan, Pahang. Rasch analysis was used to analyse the construct validity of FIES. **Results:** FIES met the Rasch model assumptions with all items having an infit value of between 0.7-1.3 and an outfit value of <2.0. The item and person reliability were 0.97 and 0.71, respectively; while the item and person separation were 5.59 and 1.58, respectively. The FIES item severity indicated that the items "few food", "healthy", "skipped", "ate less", and "runout" were disordered. **Conclusion**: The M-FIES is a valid and reliable measurement tool for the food insecurity situation among households based on its construct validity assessed using the Rasch model. Furthermore, the severity of item in M-FIES was different in terms of order from the original FIES, suggesting that the same items may be interpreted differently due to cultural or societal differences.

Keywords: FIES, food insecurity, Rasch measurement, reliability, validity

INTRODUCTION

Food security is a public health issue in both developed and developing countries. Food security exists "when all people, at all times, have physical, social, and economic access to sufficient, safe and nutritious food that meet their dietary needs and food preferences for an active and healthy life" (FAO, 2008). While this concept is widely recognised, there is still a lack of a universal criterion for measuring the frequency and severity of

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food insecurity across nations, languages, and cultures. Moreover, measuring food security seems challenging due to the complexity of the concept and definition. The experience-based food insecurity scale is a commonly used indicator and can be considered as an accurate direct indicator (Pérez-Escamilla, 2012).

These experience-based food security measurement tools have been used for two decades (Radimer *et al.*, 1992). The first experience-based food security scale was the United States Food Security Survey Module (USFSSM), which had been used by the U.S. government to monitor food security and guide policies. Since then, the experience-based food security scale has been widely used to assess food security at the individual and household levels in a variety of countries (Atuoye *et al.*, 2019; Akinboade & Adeyefa, 2018; Mohammadi *et al.*, 2011; Owino, Wesonga & Nabugoomu, 2014) because it allowed researchers to understand the determinants of food insecurity from an individual-, household-, national-, to global level (Smith, Kassa & Winters, 2017).

In 2013, the Food Insecurity Experience Scale (FIES) was developed based on USFSSM, Household Food Insecurity Access Scale (HFIAS), and Latin American and Caribbean Food Security Scale (ELCSA) (Smith *et al.*, 2017; Ballard, Kepple & Cafiero, 2013). The FIES's real innovation is that it gives results that are comparable across countries. According to the Gallop World Poll (GWP) survey, FIES has been used by 153 countries for the purpose of Sustainable Development Goals (SDG2) (Cafiero *et al.*, 2018; Smith, Kassa & Winters, 2017).

FIES can be used as a metric for measuring food insecurity at the household or individual level, depending on people's direct answer of a YES or NO to eight dichotomous questions regarding access to adequate food (Cafiero, Viviani & Nord, 2018). The items are based on reported behaviours and experiences of having to compromise the quality and amount to access food due to lack of financial support at various levels of severity. Besides, FIES also includes the psychological components of anxiety and uncertainty due to inability to obtain sufficient food (Ballard *et al.*, 2013).

The advantage of FIES is that it enables measurement of food insecurity, which can then be analysed together with indicators of its determinants and consequences. The results can inform policy that allows more detailed analysis on the food insecurity situation by gender, income, age, race/ethnicity, geographic location or other policy-relevant characteristics for more effective policies and interventions.

Malaysia is ranked 39th out of 113 countries by the Global Food Security Index 2021 and is committed to improve and combat food insecurity in the country. However, the prevalence of moderate and severe food insecurity is still escalating from 15.1% in 2017 to 18.7% in 2019. The Poverty Line Index (PLI) also increased from 5.6% in 2017 to 8.4% in 2019 (World Bank, 2019). The PLI is an indicator that reflects the sufficiency of income to purchase ample amount of nutritious foods including fish, poultry, meat, cereal, bread, rice, eggs, vegetables, and milk without neglecting other basic needs such as clothing, house rental, transportation, education, and healthcare (EPU, 2019). The current PLI of Malaysia is RM2,208, which means that households in Malaysia with monthly incomes below this level are considered poor (EPU, 2019). Comparing the pattern of PLI and the prevalence of moderate and severe food insecurity, it is shown that the trend of food insecurity is sequential to PLI, which means that more households with food insecurity have been living below PLI. The unprecedented COVID-19 pandemic that spread worldwide in the late 2019 had negative implications on food security and nutrition, where it drove up food insecurity in Malaysia.

Translation of FIES into the Malay language had been done earlier (Roselawati *et al.*, 2021). However, it has not been appropriately validated in the Malaysian population. Therefore, this study aimed to assess the internal validity and reliability of M-FIES for measuring food insecurity among the Malaysian population.

MATERIALS AND METHODS

Sample and population

This cross-sectional study was carried out among 145 households, based on the Rasch sample size calculation with±0.5 logit and confidence at 99% (Azrilah, Mohd Saidfudin & Azami, 2013), in selected urban and rural areas of Kuantan, Pahang. The list of urban and rural areas in Kuantan was obtained from the local government authority – *Majlis Perbandaran Kuantan (MPK*). The study site was randomly selected, but the participants were selected based on purposive sampling. The inclusion criterion was married women of reproductive age between 19 and 49 years old. Women were chosen because they are responsible for food production, purchasing and preparation, and are the key person to household food security (Kardooni *et al.,* 2014). Those who were lactating and pregnant were excluded.

The Food Insecurity Experience Scale (FIES)

The FIES was translated from English to Bahasa Malaysia and these translation steps have been explained thoroughly elsewhere (Roselawati *et al.*, 2021). The FIES consisted of eight questions about the behaviours and experiences regarding food insecurity. Participants were required to answer 'yes' or 'no' to all 8 questions, which were provided with a raw score of 0 for negative response and 1 for an affirmative response. The total FIES score was the sum of scores from all 8 questions and it was then further classified into the following levels of severity: food security (0), mild food insecurity (1-3), moderate food insecurity (4-6), and severe food insecurity (7-8) (Jones, 2017).

Statistical analysis

Rasch model

The Rasch analysis was performed to examine the psychometric properties of M-FIES. Rasch analysis was used to investigate the presence of unidimensionality, item hierarchy, and item appropriateness. The software used in data analysis was based on standard procedure and extreme raw score (0 and 8) was excluded (Bond & Fox, 2007). The Rasch assumptions were: 1) the items discriminate equally, where the items are related to latent trait; and 2) the items are independent and unidimensional, meaning the response to the item is independent and only 1 latent trait is measured (Nord, Cafiero & Viviani, 2016). Rasch transformed raw scores into continuous data with equal interval units called logit, representing the severity of the latent trait measured by the raw score (Schuttle *et al.,* 2016).

The Rasch modelling output included the infit and outfit statistics calculations. The assumption of equal discrimination, primarily the infit value and the outfit value, was used to identify the occurrence of erratic responses. The infit statistic is useful for identifying items that did not perform well in a given population. Infit of <0.7 is considered low, indicating the presence of redundant items, whereas 0.8-1.3 is considered an adequate infit and >1.3 is considered a high infit, which means weaker discrimination. An infit item value of more than 2.0 indicates misfit and an item should be considered dropped from the scale. High infit can be due to problems with translation, suggesting a question was not fully understood. Nevertheless, infit of between 1.3 to 1.5 indicates that items can still be used but need some improvements to the questions. Low infit (below 0.5) indicates item redundancy. While the interpretation of outfit is

almost similar to infit, the former is more sensitive to outliers. An outfit statistic of >2 is considered high. High outfit is due to unusual participant response patterns, for example, misunderstanding of items by respondents. If the infit statistic shows good results, high outfits are usually discarded and not usually considered as criteria for eliminating items.

Reliability in the Rasch analysis (separation index) indicated the 'reproducibility of relative measure location'. In this case, high person or item reliability indicates the likelihood that the persons or items possess high measures compared to persons and items that possess low measures. The ideal value for person reliability should be greater than 0.8 and the value for item reliability should be greater than 0.9 (Fisher, 2007). Meanwhile, the person separation index (PSI) was used to classify a person into high or low performer. A low PSI value indicates that the instrument is not sensitive enough to classify people into low or high performance. The ideal value of PSI should be greater than 2.0 (Fisher, 2007). On the other hand, item separation index (ISI) was used to confirm the existence of item hierarchy in the instrument. A low ISI value indicates a restriction in the sample size to verify the item's difficult hierarchy.

Ethical approval

Ethical approval was obtained from the IIUM Research Ethics Committee (IREC) (IIUM/504/14/11/2/REC 2019-131). In addition, consent was obtained from participants prior to answering the survey.

RESULTS

Fit statistics and overall reliability of FIES

Table 1 shows the infit and outfit statistics of M-FIES. All eight items had acceptable infits ranging from 0.7 to 1.3, generally considered to have met the Rasch assumptions that all items discriminated equally and adequately. For outfit statistic, the items in this measurement also met the assumptions of the Rasch model. All items had an outfit value of <2 (Table 1).

The ordering of the FIES items

The Rasch model analysis was based on eight items (Table 2). The severity parameter was estimated based on the overall respondents' response to the eight items, whether they affirmed or denied the items. The more severe the item, the less likely the respondent would say "YES" to it. The M-FIES item severity parameters indicated that only items 1, 7, and 8 performed as expected. Items 7 and 8 "whole day" and "hungry"

Table 1. The overall fit statistics for FIES

Items	*Infit*		*Outfit*		*Point measure correlation*
	MNSQ	*ZSTD*	*MNSQ*	*ZSTD*	
WORRIED	1.22	1.28	1.14	0.42	0.24
HEALTHY	0.78	-1.24	0.60	-0.77	0.82
FEWFOODS	1.01	0.10	1.22	0.55	0.78
SKIPPED	1.10	0.46	1.75	1.06	0.72
ATELESS	0.88	-0.50	0.57	-0.54	0.80
RUNOUT	0.92	-0.24	0.46	-0.64	0.76
HUNGRY	0.70	-0.77	0.25	-0.65	0.59
WHOLEDAY	1.28	0.59	0.83	0.04	0.17
Mean	0.99	-0.04	0.85	-0.07	

MNSQ=Mean square, ZSTD=Z-standardised

Table 2. Proportion of affirmative responses to FIES items, item severity parameter, and item order

Item order	*Severity±SE*	*Percentage of affirmative (%)*
WORRIED	-4.74±0.36	42.8
FEWFOOD	-2.80±0.32	24.8
HEALTHY	-1.94±0.33	30.3
ATELESS	-0.85±0.37	13.1
RUNOUT	0.37±0.42	18.6
SKIPPED	0.37±0.42	13.1
HUNGRY	3.20±0.59	4.8
WHOLEDAY	6.40±1.07	0.7

SE=Standard error

were the most severe items with item severity of 0.64 and 0.32, respectively. This result also indicated that the items were least likely to obtain a response of "YES" and most respondents rarely experienced both situations in the past 12 months.

The M-FIES item severity (Table 2) indicated that items "few food", "healthy", "skipped", "ate less", and "runout" were disordered. The item "healthy" was indicated as more severe than "few food", while the item "skipped" was more severe than "ate less" and "runout". Our item severity order results were consistent with the results of the response patterns; when the severity of food insecurity increased, the affirmative response decreased.

The eight items' item reliability, person reliability, and separation indexes were excellent. The item reliability was found to be 0.97, while the item separation was 5.59. High reliability showed that items with high measures were confirmed to have higher measures compared with items with low measures. Item separation index was used to verify item hierarchy. An increased item separation index indicated that the study's sample size was adequate to assure the item difficulty hierarchy.

Person reliability and separation indices were 0.71 and 1.58, respectively, indicating good reliability. High person reliability suggested a high probability of the person with a high measure compared to a person with a low measure. The purpose of person separation was to clarify the person. However, the items were not good enough to categorise the person based on different food security statuses.

DISCUSSION

In Malaysia, adult food insecurity was estimated to be within 47% to 100%. These varied and inconsistent findings were due to the different measurement tools used (Norhasmah *et al.,* 2021). The Malaysian sub-populations vulnerable to food insecurity include adults from low-income households, indigenous people, university students, the elderly population, and migrant workers. Thus, a valid and reliable tool that can be used as a standard measurement tool is necessary. This study was among the first study to examine the construct

Table 3. Summary statistics from Rasch analysis

Questions	*Reliability*		*Separation*	
FIES	*Item*	*Person*	*Item*	*Person*
	0.97	0.71	5.59	1.58

validity of M-FIES. The findings of this study provide a significant contribution to the existing measurement tool, especially for the study of food security in Malaysia.

In this study, M-FIES has been shown to be a valid and reliable instrument to measure food security status of Malaysians, even though the tool originated from the United States. Previously, Rasch analysis has been used to validate FIES in other regions, including Sub-Saharan Africa (Na *et al.,* 2019; Sadiddin *et al.,* 2019), Latin America and the Caribbean (Smith *et al.,* 2017), United States, United Kingdom, Australia, New Zealand, as well as East and South Asia (Jones, 2017). Using the same method of Rasch analysis, FIES showed good item infit and outfit, good item and person reliability, and stability of item hierarchy (Na *et al.,* 2019; Sadiddin *et al.,* 2019), thus proving itself to be relevant for assessing food insecurity experiences.

In the present study, all items showed adequate fit, which means that all items were associated with the latent trait and discriminated equally (Argawal *et al.,* 2009), implying that the M-FIES was correctly translated (Roselawati *et al.,* 2021). In this study, the outfit values were within the acceptable range. Some countries reported a high outfit for the item "whole day" and FAO reported identical results in the global 2014 GWP data analysis. This was mainly due to an unusual respondents' response pattern and the items should be attempted through cognitive testing (FA0, 2016). However, even if an item has a high outfit, but its infit is excellent, it is not indicative of any serious violation of the Rasch model assumptions. Our results also indicated a positive point measure correlation with the latent trait that should be measured.

The fundamental feature of M-FIES is that the order of the questions along the scale cannot be considered fixed across populations or countries. In different populations or sub-populations, the severity of the eight items may vary based on the nuances of the translation that the same questions may be interpreted in different ways in different contexts. Moreover, food insecurity conditions are experienced or managed differently in different cultures. According to the severity value calculated by the Rasch model, differences in severity level are based on the affirmative response pattern. Severe item is usually denied compared to less severe item. The items of M-FIES in this study were disordered and it was noted in many countries due to its construct validity. The items "few food", "healthy", "skipped", "ate less", "runout" were disordered. The item "healthy" was indicated as more severe than "few food", while the item "skipped" was more severe than "ate less" and "runout'. This suggests that eating a few types of foods and eating less food is commonly related to the culture in Malaysia. This finding is consistent with a study done using FIES in Sub-Saharan Africa (Wambogo *et al.*, 2018). The item "worried" about food was the least severe item.

According to the list of items in Table 2, the pattern of this result implies that households that first experience food insecurity would first experience anxiety or worries about getting food. Then, the household eats the same kind of food and jeopardises quality. As the situation worsens, they reduce the quantity of food taken. When the household runs out of food as a result of food insecurity, it will be forced to drastically reduce the number of meals and food consumption, experience hunger and will eventually not be able to eat for the whole day. These findings are aligned with the theoretical framework of the experience-based food insecurity as a managed process. However, the process might differ based on cultural and other factors (Radimer *et al.* 1992; Ballard *et al.,* 2013; Coates *et al.,* 2006).

Table 4. The Food Insecurity Experience Scale

Item	*Item code*
You or others in your household worried about not having enough food to eat because of a lack of money or other resources? *Anda atau ahli-ahli dalam isi rumah anda risau tidak mempunyai makanan yang cukup kerana kekurangan wang atau sumber-sumber lain?*	[WORRIED]
Still thinking about the last 12 MONTHS, was there a time when you or others in your household were unable to eat healthy and nutritious food because of a lack of money or other resources? *Masih memikirkan 12 BULAN yang lepas, adakah anda atau ahli-ahli dalam isi rumah anda tidak dapat makan makanan yang sihat dan berkhasiat kerana kekurangan wang atau sumber-sumber lain?*	[HEALTHY]
Was there a time when you or others in your household ate only a few kinds of foods because of a lack of money or other resources? *Adakah anda atau ahli-ahli dalam isirumah anda makan hanya beberapa jenis makanan sahaja kerana kekurangan wang atau sumber-sumber lain?*	[FEWKINDS]
Was there a time when you or others in your household had to skip a meal because there was not enough money or other resources to get food? *Adakah anda atau ahli-ahli dalam isi rumah anda terpaksa meninggalkan satu waktu makan kerana kekurangan wang atau sumber-sumber lain untuk mendapatkan makanan?*	[SKIPPED]
Still thinking about the last 12 MONTHS, was there a time when you or others in your household ate less than you thought you should because of a lack of money or other resources? *Masih memikirkan 12 BULAN yang lepas, adakah anda atau ahli-ahli dalam isirumah anda makan kurang daripada apa yang sepatutnya anda makan kerana kekurangan wang atau sumber-sumber lain?*	[ATELESS]
Was there a time when your household ran out of food because of a lack of money or other resources? *Adakah isi rumah anda kehabisan makanan kerana kekurangan wang atau sumber-sumber lain?*	[RUNOUT]
Was there a time when you or others in your household were hungry but did not eat because there was not enough money or other resources for food? *Adakah anda atau ahli-ahli dalam isi rumah anda berasa lapar tetapi tidak makan kerana tidak mempunyai wang atau sumber-sumber lain yang cukup untuk makanan?*	[HUNGRY]
During the last 12 MONTHS, was there a time whenyou or others in your household went without eating for a whole day because of a lack of money or other resources? *Adakah anda atau ahli-ahli dalam isirumah anda tidak makan sepanjang hari kerana kekurangan wang atau sumber-sumber lain?*	[WHOLEDAY]

(Source: Roselawati *et al.*, 2021)

The limitation of this study was that the selection of study sites for both urban and rural areas was randomised, but the selection of participants was based on purposive sampling, which is not the best method of sampling for representativeness. However, the strength of this study is that it was the first study that translated and validated FIES in a rural and urban setting. The translation and validation of FIES followed the recommended and established guidelines by WHO. The use of Rasch analysis in determining the construct validity of FIES was a novel approach as the validated version can now be used as a tool to measure food insecurity among Malaysians in national surveys. Moreover, it is suggested that the validation of M-FIES should be done in other populations, including Chinese and Indian, with a larger sample size to ensure that the Malay version of FIES is valid and reliable for the Malaysian population.

CONCLUSION

In conclusion, this study revealed that the M-FIES is a valid and reliable tool for measuring the prevalence of food insecurity among households. Furthermore, the item order in evaluating the severity of food insecurity in M-FIES was fundamentally different from the original FIES, suggesting that the same item may be interpreted differently due to cultural or societal differences.

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Authors' contributions

Wan Azdie MAB, principal investigator, conceptualised, designed the study and reviewed the manuscript; Roselawati MY, conducted data collection, ran the analysis and wrote the manuscript; Suriati S, Jamalludin AB, Norhasmah S, Nurul Hazirah J, Noraishah MN, provided advice on data analysis, interpretation of the results and reviewed the manuscript.

Conflict of interest

None declared.

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Development of a plant-based culinary nutrition model for sports science students

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ABSTRACT

Introduction: Food and fitness levels are key factors required to maintain functional life. Hence, nutrition education is crucial in spreading awareness among Sports Science students. There is a paucity of plant-based nutrition knowledge and plant-based culinary skills for fitness locally. Therefore, this study aimed to develop a valid Plant-Based Culinary Nutrition Model (PBCNM) with nutrition knowledge and basic culinary skills to improve fitness among Sports Science students. **Methods:** This exploratory sequential mixed method study consisted of 2 phases. In Phase 1, a three-round modified Delphi method was conducted with 13 experts to generate a questionnaire that was validated. Lawshe worksheet determined the content validity ratio (CVR) for an item. Content validity index (CVI) for each section and the overall instrument was calculated. In Phase 2, quantitative data collection and analysis addressed the research questions and research gaps leading to model development. A total of 271 undergraduate Sports Science students at higher learning institutions were recruited as respondents. **Results:** In Phase 1, CVR of the overall survey was 1.0 and CVI was 0.834. Subsequently, the questionnaire was pilot tested for reliability and a Cronbach's alpha score of 0.836 was obtained for the overall questionnaire. In Phase 2, the respondents' mean and standard deviation score for the model's components and needs was 4.21±0.73 for items 1-15. The integration of experts' perspectives on the need of PBCNM for fitness and feedback from respondents resulted in the development of PBCNM. **Conclusion:** This study highlighted PBCNM as a helpful guideline for better fitness management.

Keywords: Delphi method, education, fitness, nutritional knowledge, plant-based diet

INTRODUCTION

A plant-based diet essentially involves eating fresh or less processed foods closer to nature. Plant-based diets, such as those encompassing vegetables, fruits, and pulses, have been associated with substantial health benefits and overall health improvement, including the potential prevention of chronic diseases such as heart diseases and cancer (Hemler & Hu, 2019; Medawar *et al.*, 2019). The variety of plant-based dietary patterns are lacto-ovo-vegetarian, lacto-vegetarian, and vegan. Vegan excludes dairy and all animal-related products in their lifestyle. Many self-proclaimed vegetarians define a plant-based diet based on their perceptions (Hargreaves *et al.*, 2021).

Transition to a plant-based diet can have a significant impact on the health and fitness of an individual (Adesogan

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et al., 2020). The main concern is lack of nutrients like calcium, iodine, iron, vitamin D, zinc, long-chain omega-3 fatty acids, as well as vitamin B12 even though deficiency can occur across diets (Wirnitzer, 2020). Therefore, the key is well-planned plant-based meals, which could prevent nutritional deficiencies for any age group, including athletes (PCRM, 2014).

A nutrient-dense plant-based diet includes whole foods, which is an evidence-based recommendation, thus should be practised as a health promoting option (Lynch, Johnston & Wharton, 2018). However, studies revealed that those practising a plant-based diet lacked skills in preparing whole food plant-based meals (Tani, Fujiwara & Kondo, 2020). Therefore, the emphasis should be on acquiring nutrition and culinary skills to prepare plant-based meals. The literature review reported the importance of cooking skills in maintaining fitness. One study revealed that learning culinary skills could improve food choices, fitness, and overall health (Knowlden, Robbins & Gardner, 2018). It is therefore crucial for Sports Science students to recognise the relationship between nutrition and culinary skills for fitness and disease prevention.

Prior studies revealed more research on how plant-based diets interact with health, environment, and food businesses (Lynch *et al.,* 2018). Sustainable diets promote a healthy and fit lifestyle for individuals with minimal impact on the environment (Fanzo, 2019). There is evidence from systematic reviews and meta-analyses about the preventive impact of a plant-based diet in the treatment of various chronic diseases; however, clinicians are not trained with the skills and education to prescribe plant-based nutrition (PCRM, 2014). Despite the prejudice, relevant research on athletes' health and fitness is growing. Plant-based diets contain higher carbohydrates and antioxidants, thus may enhance athletic performance, particularly in endurance sports (Devrim-Lanpir, Hill & Knechtle, 2021). The best sources of carbohydrates are those rich in fibre and nutrients, like wholesome vegetables, fruits, whole grains, and legumes (Wirnitzer, 2020). As sports and exercise are vital components in the promotion of health, athletes should be supported with a balanced whole food plant-based diet (Wirnitzer, 2020).

There are many factors involved in the changes of human behaviour. As described by other researchers, such factors can be grouped under the social-cognitive theory (SCT) (Torkan *et al.*, 2018), diffusion of innovation theory, and health belief model (HBM) (Keshani *et al.*, 2019). The SCT encompasses three triads of human behaviour: individual cognitive factors (knowledge, expectations, and attitudes), behavioural factors (skills, practice, and self-efficacy), as well as environmental factors (social norms, access, and influence of others on their environment) (Harris, Carins & Rundle-Thiele, 2021). Some healthy eating studies have used part of the model by choosing one or more of these factors (Cox *et al.,* 2017; Knowlden *et al.*, 2018); we have yet to find studies that applied all nine factors in one research. According to Bandura (2004), the field of health has changed towards health modelling. Thus, the emphasis on the importance of educating people about the benefits of eating whole foods and learning to cook is essential. In this context, the development of a plant-based culinary nutrition education programme (PBCNM) for fitness is an effective way to advocate a plant-based lifestyle for long-term health benefits. Hargreaves *et al.* (2021)'s research supports that similar programme can have a positive impact on health outcomes.

The diffusion of innovation (DOI) theory suggests that new ideas can spread through a society over time,

with different groups of people adopting the behaviour at different rates. DOI emphasises the importance of communication channels and social networks in the process (Mallinson, 2020). Thus, in the context of promoting a plant-based culinary nutrition lifestyle, effective communication can accelerate the adoption of this lifestyle among society. Targeted campaigns and providing easy-to-follow culinary nutrition education could accelerate the adoption process.

Lastly, the HBM centres on the desire to avoid disease and the belief that a particular health action will prevent or cure disease (Lim, Okine & Kershaw, 2021). A person's course of action depends on how he perceives the benefits of adopting a behaviour and the consequences of not changing it. Therefore, an individual's beliefs can influence his/her desired behaviour. HBM was used to identify the perceived benefits and barriers, self-efficacy, severity, and vulnerability to healthy eating (Keshani *et al.*, 2019). In the context of promoting a plant-based lifestyle and developing culinary skills, people may adopt these behaviours to decrease their risk of developing diseases (Keshani *et al.,* 2019).

Overall, these theories provide a strong foundation for promoting the diffusion of plant-based culinary nutrition ideas and increasing the adoption rate among population over time. This study aimed to develop a valid Plant-Based Culinary Nutrition Model (PBCNM), which incorporates the fundamentals of nutrition and culinary skills to improve fitness among Sports Science students. PBCNM was initiated to support a number of initiatives by the Ministry of Health to manage national fitness. Similarly, PBCNM could guide Sports Science students in managing their fitness through plant-based culinary nutrition (Morji, Razak & Hamzah, 2021).

MATERIALS AND METHODS

This study applied the mixed method exploratory sequential design, and included both qualitative and quantitative data collection and analyses. The mixed method can generate comprehensive evidence to address research problems, especially in a health-related context (Almeida, 2018). There were two phases and the study was conducted with the approval of the Universiti Malaya Research Ethics Committee (UM.TNC2/ UMREC–431). The participants provided written consent. Figure 1 shows the overview of the research design.

Phase 1

Phase 1 involved a modified Delphi approach, optimised and tested through the analysis, design, development, implementation, and evaluation (ADDIE) framework. The researchers focused only on the design and development stages of the ADDIE model. The evaluation stage was not planned for this study; it would be for future research. ADDIE was optimised to generate a questionnaire to be used in Phase 2's quantitative data collection. The design and development of the research instrument and research method steered by the ADDIE model was used to develop efficient instructional materials. Three theories – the SCT, the DOI theory, focused on the diffusion of new ideas, and the HBM theory that predicts health-related behaviour were applied to facilitate the research design and analysis process incorporated in both phases.

In Phase 1, a panel of experts was selected based on their expertise in the area of sports nutrition and dietetics, coaching, athletics, academics, sports science, and nutritional sciences (Table 1). This approach ensured that the participants were knowledgeable about the topic being studied and could provide valuable insights and perspectives. There were no strict rules

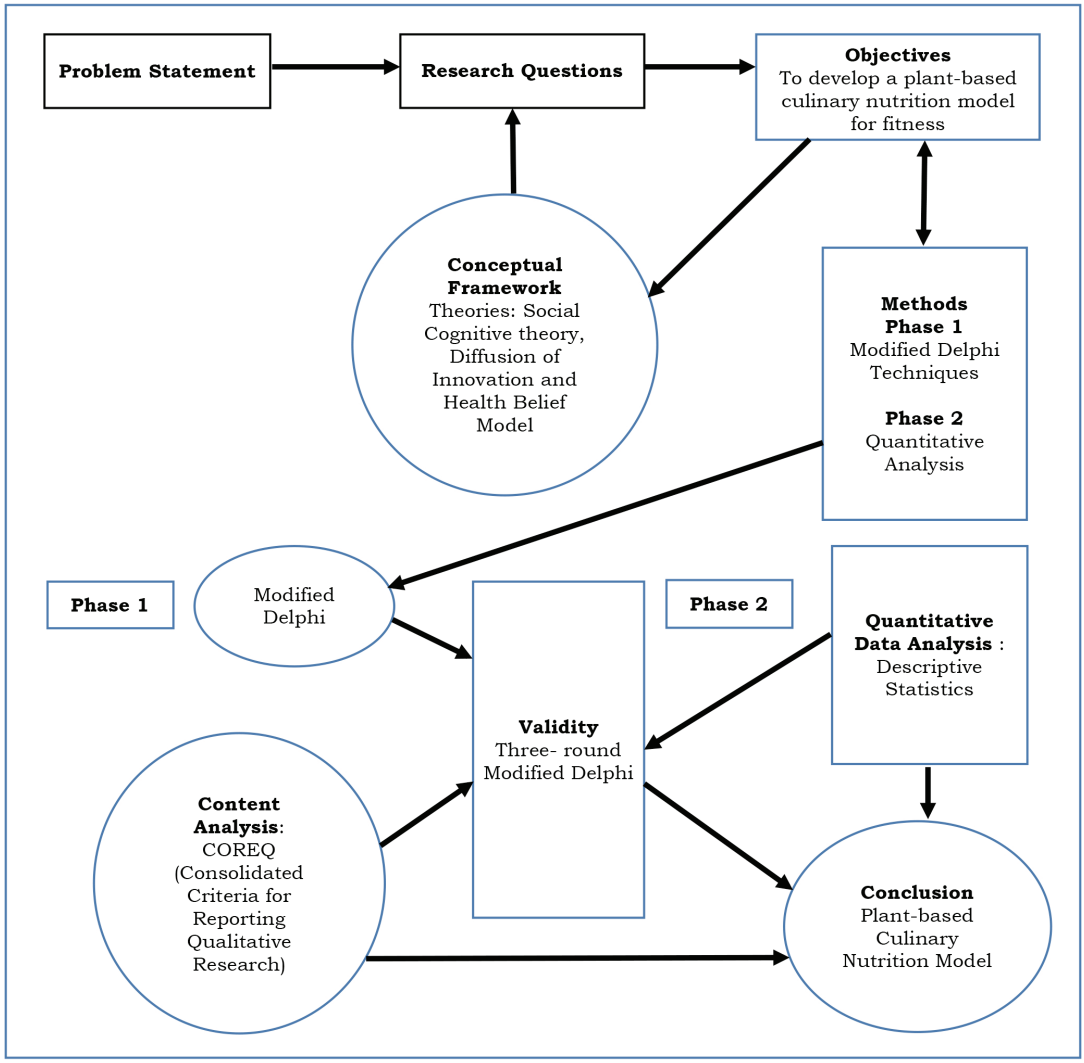

Figure 1. An overview of the study design

on the number of experts required for this study (Tong, Sainsbury & Craig, 2018). Thus, based on the needs and availability of the experts at the point of study, 13 experts were identified and interviewed. The interview process was guided by the Consolidated Criteria for Reporting Qualitative Research (COREQ) (Tong *et al.* 2018). The COREQ is a formal checklist used for focus group discussions and in-depth interviews. This study was reported based on three key areas: reflectivity of the researcher and research team, study design, as well as analysis and results.

Phase 2

Six interview questions were created based on the research questions and reviewed by the experts before the interviews for Phase 2. In the pilot study for Phase 2's quantitative study, 30 Sports Science students pursuing their first degree at a local public university were recruited. Other criteria for selection such as athlete's status, eating

Table 1. The expert panels' experiences and expertise

Expert	*Expertise*	*Years of experience*
1	Dietitian and assistant director (sports performance)	More than 20 years
2	Sports nutrition lecturer	More than 10 years
3	Director, Division of research and innovation	More than 20 years
4	Executive chef,	More than 15 years
5	Sports medicine specialist	More than 20 years
6	Sports coach and tower runner	5 years
7	Endurance sports coach	More than 10 years
8	Sports coach (dodgeball)	More than 10 years
9	Head of sports and exercise science postgraduate studies	More than 20 years
10	Plant-based sports scientist	More than 20 years
11	Emeritus Professor in nutrition	More than 30 years
12	Director of plant-based nutrition education	More than 15 years
13	Advanced sports dietitian	More than 20 years

disorders, food restrictions, recent dieting attempts, and health conditions were excluded. Non-Sports Science students were excluded from this study. In Phase 2, the study population was Sports Science students, which included 271 students from two local public universities offering undergraduate Sports Science programmes. This was the actual sample size retrieved based on the Krejcie & Morgan provisions (Krejcie & Morgan, 1970). The students were between 19-25 years old, pursuing various degree programmes in Sports Science and were in the 1st to 3rd year of their studies at the time of data collection. They were purposively selected due to the reason that PBCNM was targeted to equip Sports Science students with relevant culinary skills applicable for managing fitness.

Instrument development using Delphi technique

The Delphi method was modified for a few reasons. Firstly, the interview was conducted in-person rather than by email in this study. Secondly, external experts were recruited to participate in a few subsequent rounds (Tong *et al.*, 2018). A content analysis of the interview data was performed to identify the themes and level of agreement among experts on the need for PBCNM and the components that should be included in the model. In essence, the in-depth interview was conducted as a needs analysis of this research. The needs analysis was a crucial part of this study as the current problem has not been explored in-depth before in the local setting (Morji *et al.*, 2021).

Based on the analysis of the interview in Round 1 of the Delphi study, a questionnaire was drafted. This questionnaire had two parts: Part I was the Demographics with eight items; and Part II had two sections, Section A with 12 items and Section B with eight items. Part II initially consisted of 20 questions on a 5-point Likert scale. This questionnaire draft was reviewed and further analysed in Rounds 2 and 3 by six experts. After the review, few questions were eliminated and few were merged. Finally, Part I remained with eight questions on Demographics, while Part II contained a total of 15 items – Section A had eight items and Section B had seven items.

There were several steps involved in the development of the instrument. Firstly, a thorough literature review ensured the research was grounded in existing knowledge and the findings were meaningful and relevant to the field. Next,

a needs analysis or an in-depth interview enriched the content area. Experts contributed valuable experiences and expertise in the field. The researchers ensured that the items would reflect the research questions of this study. Any item deemed essential by more than half of the panellists must obtain a certain degree of content validity to quantify the consensus reached. Secondly, the higher the number of panellists who considered an item to be essential, the greater the degree of content validity (Lawshe, 1975). This quantification was determined via content validity ratio (CVR) and content validity index (CVI). CVR can range from 1 to -1; a higher score indicates a higher agreement. For this study, a CVI average of 0.62 for each item was considered as an acceptable threshold.

Statistical analysis

Descriptive statistics from the IBM SPSS Statistics for Windows version 23.0 (IBM Corp, Armonk, New York, United States) were utilised to analyse the data in Phase 2. Sample size calculation for Phase 2 was based on Krejcie and Morgan's table (Krejcie & Morgan, 1970). Reliability was tested using Cronbach's alpha, measuring the internal reliability of an instrument based on the average inter-item correlation (Taber, 2018). Cronbach's alpha was used to run on a sample size of 30 respondents and optimised for reliability. Cronbach's alpha values of >0.9, 0.8, 0.7, 0.6, and 0.5 were deemed excellent, good, acceptable, questionable, and poor, respectively; while values <0.5 were considered unacceptable (Göleç & Maksudunov, 2019). Results of reliability were expressed as mean±standard deviation (*SD*). CVR and CVI calculations, which evaluate the study instrument's content validity, were done using the Lawshe worksheet. Frequency was optimised to analyse Likert scale data. Mean and standard deviation were used to characterise the central tendency and variability of the data.

RESULTS

A three-round modified Delphi method was conducted in Phase 1 of this research. Based on the findings in the first round, the 13 experts agreed to include plant-based culinary nutrition as part of a module in the current Sports Nutrition curriculum. As high as 84.6% or 11 experts were confident this could be done. The experts believed a PBCNM for fitness is essential. To further consolidate the consensus, two more rounds of Delphi were conducted. A total of six experts were recruited, of which three were external experts outside of the university. They believed athletes should learn to cook simple meals and plan meals based on their daily energy requirements. One of the experts mentioned that cooking is not complicated and healthy eating can start with simple tasks such as boiling water for beans and rice. In this study, the experts' suggestions had reinforced the importance of developing the PBCNM.

The consensus among experts was crucial to create a valid survey instrument. Pilot testing was conducted to test its reliability. Reliability was calculated using Cronbach's alpha. For Part I, the need for PBCNM scored a Cronbach's alpha of 0.709. For Part II, the components of the model scored a Cronbach's alpha of 0.832. The overall reliability of the instrument for this research was Cronbach's alpha 0.836. The consensus standardised the survey items. The CVR score and the acceptability or non-acceptability of each item were included. The validated questionnaire comprised of two parts, Part I the Demographics with eight items and Part II with a total of 15 items. The CVR and CVI of these 15 items were based on experts' advice and the scores remained high. The score of CVR was 0.667 and above for each item, and this was accepted as valid. However, one item with the score of only 0.33 was removed due to lack of consensus.

Table 2. Demographics of respondents (*n*=271)

Demographic	*Number of respondents*	*Percentage (%)*
Gender		
Male	162	59.0
Female	109	41.0
Year of study		
1	97	36.0
2	102	38.0
3	72	26.0
Sports played		
Basketball	35	13.0
Football	45	16.6
Futsal	35	13.0
Rugby	5	1.8
Athletics	45	16.6
Ping Pong	33	12.0
Frisbee	1	0.4
Fencing	3	1.1
Javelin	5	1.8
Hockey	35	13.0
Badminton	29	10.7
Level		
National	39	14.0
State	105	39.0
University	95	35.0
Not stated	32	12.0
Cooking ability		
No	194	72.0
Yes	77	28.0
Practise plant-based diet		
No	212	78.0
Yes	59	22.0
Reason to cook		
Health	51	66.0
Love cooking	6	8.0
Limited budget	9	12.0
No reason given	11	14.0

Following experts' suggestions to merge and eliminate few items, consequently, Phase 1 of the study resulted in the development of a questionnaire, which included the needs and components, respectively. 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. Some of the research questions dealt in-depth with the process development of the PBCNM.

Consensus was achieved via a three-round modified Delphi approach that incorporated experts' opinion on the importance and necessity of the PBCNM for the maintenance of fitness. Finally, after the instrument was constructed and the items refined, it was prepared in the form of questionnaire suitable for data collection.

Phase 2

In Phase 2, the quantitative analysis included discussion of the results and expert panels' responses to the four research questions. The questions were: 1) How significant is a plant-based culinary nutrition model for fitness according to experts' perception?, 2)

What are the components of a plant-based culinary nutrition model for fitness?, 3) What is the consensus level of experts on components in a plant-based culinary nutrition model?, and 4) What is the developed model to be incorporated into daily lifestyle?

All participants completed the questionnaire and there were no drop-outs**.** Among these respondents, based on the demographics, 72% of respondents did not cook and if they had to cook, it was because of their love for cooking, health issues or limited budget in campus as students. However, majority of the students, 78% of them did not live a plant-based diet lifestyle. There were only 22% of 271 respondents who practised a plant-based diet (Table 2).

Based on the data collected, 57.9% respondents agreed and 14.8% strongly agreed about the need to have PBCNM for fitness. This made a total of 72.7% respondents eager to have the model. The strong need from respondents compelled the development of components for the PBCNM model. In addition, 71.9% of them agreed that there was a need for a plant-based culinary nutrition programme at higher learning institution. Data analysis conducted for the need and components of items 1-15 yielded in a mean and standard deviation score of 4.21±0.73. While there were differences in the scores, for most items, respondents' viewpoints concurred with the expert panels' viewpoints, hence leading to the development of PBCNM for fitness. In particular, 72.7% of the 271 respondents eagerly anticipated the PBCNM, while 84.6% of the 13 experts were confident that the inclusion of PBCNM in the Sports Nutrition curriculum could be done. Furthermore, the external experts also acknowledged the need for basic culinary skills among athletes; hence, the need for PBCNM in sports nutrition. The standpoint was collective and this research is an original contribution of knowledge.

DISCUSSION

This study aimed to develop a plant-based culinary nutrition model for fitness among Sports Science students. This was a mixed method study that required both qualitative and quantitative data to develop the PBCNM model. The ADDIE process was used to validate the stages in the development of this model. Qualitative data collection and analysis achieved through a modified Delphi process was quantified through quantitative analysis in the next phase using SPSS. The quantitative analysis supported the qualitative part of this research, providing substantial evidence that the PBCNM was needed. The combination of both quantitative and qualitative data provided a robust approach to the development of the PBCNM.

This research was significant because at the point of writing, it was the first local study integrating plant-based nutrition knowledge with culinary skills into a model for fitness improvement among Sports Science students. The mixed method approach applied in the study allowed for comprehensive understanding of the topic, combining quantitative and qualitative data. The expert panel interview was crucial in providing valuable insights into the need for PBCNM, highlighting the importance of including plant-based culinary nutrition in the Sports Nutrition curriculum. The experts' suggestions regarding the inclusion of PBCNM as a course in the Sports Nutrition curriculum further reinforced the importance of this research. Overall, achieving expert consensus on model components was critical in PBCNM development.

The development of PBCNM

This study focused on the development of PBCNM that was intended to fulfil the lack of reference in educational institutions and sports institutions for future plant-based culinary nutrition programme

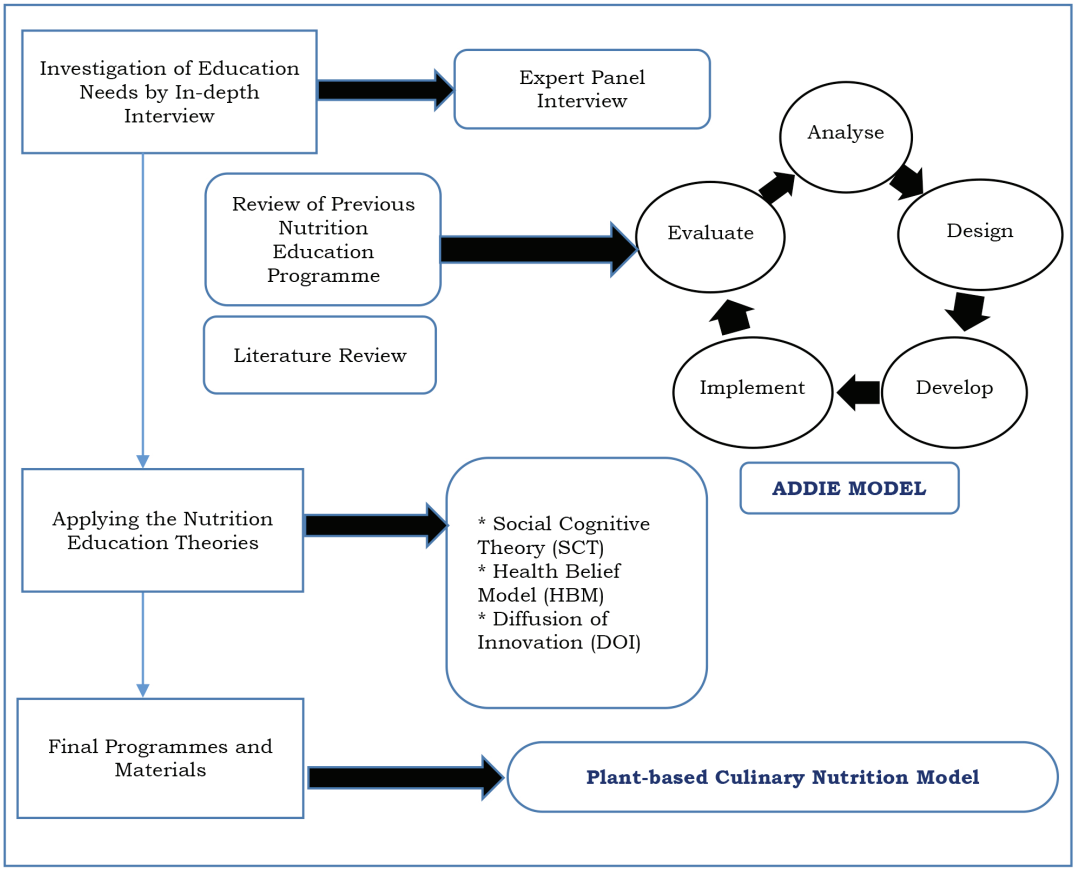

Figure 2. Model development process

development. The conceptual framework was based on three theories commonly used in health promotion research or programme development – SCT, DOI, and HBM, advocating the development of PBCNM for fitness among Sports Science students. The constructs from these theories supported the research findings as shown in Figure 2.

The ADDIE model, a structured approach to human learning and information systems was optimised (Patel *et al.* 2018). The ADDIE framework comprises five phases in developing instructional materials. However, this study utilised the first three steps – analysis, design, and development phases. The implementation and evaluation phases were not optimised.

In addition, this study was based on the premise that prevention is better than seeking cure. The SCT is a guideline providing indicators to enable people to adopt health-promoting behaviours and reduce detrimental behaviours (Oyibo, Adaji & Vassileva, 2018). The Academy of Nutrition and Dietetics stated that a carefully planned vegetarian and vegan diet is nutritionally appropriate, thus, providing health benefits in the prevention and treatment of various diseases (Kahleova & Katz, 2020). Self-efficacy is the confidence a person has in his/her own ability to perform a behaviour successfully. Therefore, mastering basic culinary skills can boost confidence in managing personal health through nutrition. Teachers and

trainers play important roles in helping individuals build confidence to manage their personal health. The PBCNM for fitness in this study was specifically designed for Sports Science students to learn basic culinary skills and master plant-based, home-cooked foods to maintain fitness. Individuals should organise their own daily nutrition routine and incorporate plant-based foods into existing diet to manage their personal health (Fanzo, 2019).

For behavioural modifications, the perceived benefits are positive outcomes related to the behaviour in relation to perceived vulnerability (Glanz, Rimer & Viswanath, 2008). For instance, the perceived susceptibility could be a chronic disease resulting from a sedentary lifestyle and unhealthy eating habits. Plant-based culinary nutrition is the application of behaviours connected to the belief that the health benefits of a plant-based diet could reduce risks of diseases and maintain fitness throughout life. Since PBCNM incorporates culinary sciences in sports nutrition, consequently, it is vital to learn culinary skills to prepare plant-based meals. One expert believed that Sports Science students would benefit greatly from culinary skills and the consumption of whole foods.

On the other hand, HBM is about expectation and threat perception. Most people need to feel personally threatened by diseases before they accept new ideas. The influence of higher authorities, media, family, and friends who live a plant-based lifestyle may convince individuals that prevention is better than cure. The main constructs of the HBM include perceived susceptibility, severity, benefits, barriers, added incentive to act, and self-efficacy. In this study, the focus was on perceived vulnerability and benefits. Accordingly, the DOI theory explains how idea gains momentum and spreads through a particular population or social system. The result of this spread is the adoption of new ideas. Therefore, PBCNM is a progressive step in sports nutrition. The key factors that influenced the development of PBCNM were:

i. Advantage and influence of plant-based diet in the sports environment.
ii. Compatibility of a plant-based diet with the lifestyles of Sports Science students or athletes.
iii. Cooking is possible and pursuing a plant-based diet is straightforward.

There has been research on the connection between food and home cooking, as well as garden-based curriculum, but local studies exploring plant-based culinary nutrition for fitness was none. The wellbeing of Sports Science students is still closely related to their dietary habits and vulnerability to lifestyle diseases. Thus, athletes should learn to cook to maintain long-term fitness. In order to provide the necessary support, the development of PBCNM was conceptualised for Sports Science students, as shown in Figure 3. Eating the least processed food should become a norm in daily food consumption. However, pre-prepared plant-based food could still be a better alternative (Gonera *et al.,* 2021).

The PBCNM consists of three parts. The first part, which is the top, comprises components of the model. The middle circle includes plant-based nutrition, which alongside culinary skills, is the integration of both. This circle is surrounded by seven circles, which are components of the model. These include fundamentals of nutrition, culinary kitchen set up, menu planning, ingredients substitutions, shopping list, cooking methods, and culinary herbs and spices. The fundamentals of nutrition and culinary skills should be applied in daily food consumption to maintain fitness. At the middle part of the model, the Power Plate for Fitness is the circle which represents a healthy plate. Power Plate was created by experts at the Physicians Committee for

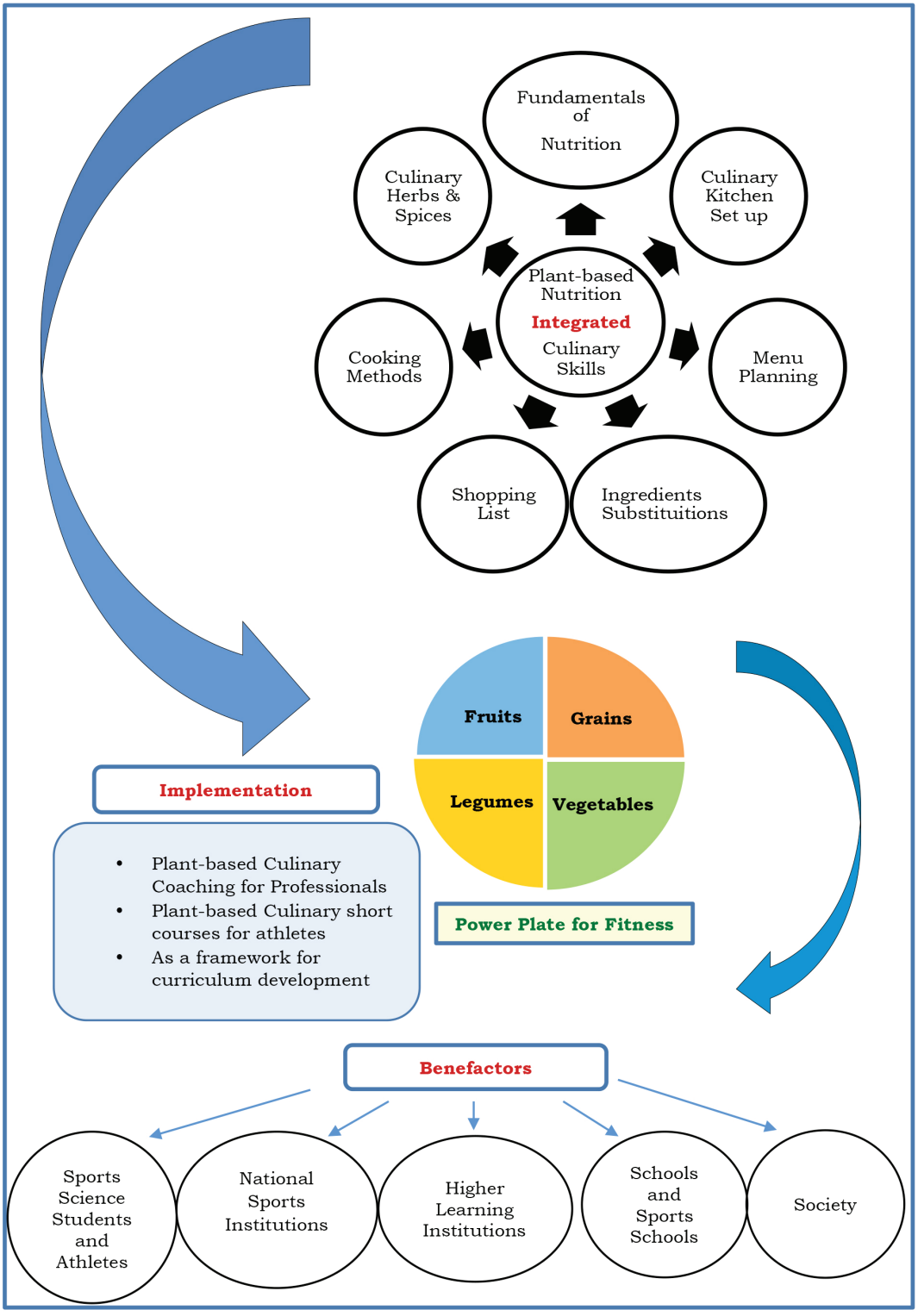

Figure 3: Plant-based Culinary Nutrition Model (PBCNM)

Responsible Medicine (PCRM) to prevent lifestyle diseases such as diabetes, obesity, cardiovascular diseases, and cancers. Accordingly, this Power Plate was appropriately adopted and adapted for this research as the Power Plate for Fitness in PBCNM, featuring four food groups which are essential for health management - vegetables, fruits, whole grains, and legumes. Dietary intake is specific to athletes' requirement for competitions, but generally plant-based portions recommended in the Power Plate will supply a good amount of fibre, protein, calcium, minerals, and vitamins daily. Finally, the third part is the benefactors. PBCNM is particularly developed for Sports Sciences students and athletes. Other beneficiaries are trainers, nutritionists and dietitians, culinary experts, and sports coaches. This model could guide people in warding off obesity, type 2 diabetes, heart diseases, cancers, and stroke if followed closely.

In modern times, globalisation and advances in communication technology have accelerated innovation. The new motto of lifestyle medicine spurred the acceptance of plant-based nutrition and home cooking for health maintenance (PCRM, 2014). This study focused on imparting the necessary culinary skills to prepare healthy plant-based meals according to established nutrition knowledge. Therefore, successful adoption of the PBCNM can be seen as a result of good understanding of the factors influencing the rate of adoption. Since DOI is a social process, the model is likely to be adopted by Sports Science students if it is communicated through the right network, such as Sports Nutrition courses at higher learning institutions. As commonly acknowledged, theories are an integral part of health care as they can be applied to clarify certain healthcare issues (Parker *et al.*, 2022). The PBCNM in this study was conceptualised based on the data from in-depth interviews and quantitative survey supported by SCT, HBM, and DOI theories.

Based on the study findings, the PBCNM for Sports Science students combined the principles of nutrition and culinary science to provide a foundation for Sports and Exercise Science curricula, courses, programmes, and others. While the PBCNM was developed primarily for Sports Science students and athletes, anyone can adapt it to their personal needs or for those unfamiliar with the diet (PCRM, 2014). Due to the lack of research in plant-based culinary nutrition locally, this study can be a steppingstone to expanding research in this field. Future research can apply intervention design and randomised controlled trials, both of which are common in health promotion studies. Researchers can also use the ADDIE model to systematically evaluate the implementation of PBCNM, especially its impact on Sports Science students and athletes.

CONCLUSION

Diet and exercise are considered "medicine" for healthy lifestyle. Despite certain prejudices against plant-based diets and their associated benefits for health, fitness, and athletic performance, convincing scientific evidence related to this diet has surfaced. The PBCNM in this study can be a reference for athletes who wish to practise culinary skills built on plant-based nutrition knowledge for fitness management. However, it will only be effective if athletes adopt and adapt the PBCNM into their lifestyle, regardless of their background and religious beliefs.

In summary, athletes who would like to try plant-based diet must be equipped with the necessary scientific knowledge to prepare balanced meals to meet their nutritional needs. Comprehensive information about plant-based diet is critical in promoting plant-based food consumption to reduce the negative

impacts of global health. Scientific knowledge can come from trainers, reading materials, videos, seminars, and even "culinary medicine", the latest booming trend in the medical world. This study highlighted the importance of plant-based culinary nutrition to maintain fitness among Sports Science students and athletes. To further support the beneficial effects of plant-based nutrition on athletic performance, more research on the effects of vegetarian diets on the fitness of athletes at various levels of performance in sports is recommended.

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Authors' contributions

Baboo Morji B, conducted the study, data collection, prepared the draft of the manuscript; Sareena Hanim H, principal investigator, conceptualised and designed the study, reviewed the manuscript; Ahmad Zabidi AZ, advised on data analysis and interpretation, and reviewed the study design and model development.

Conflict of interest

The authors declare no conflict of interest.

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Dietary pattern, nutrient intake, and oral symptoms in a sample of pregnant women in Malaysia

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ABSTRACT

Introduction: Nutritional requirements of pregnant women differ considerably from those of non-pregnant women. Nutritional status during pregnancy is critical to maintain health, including oral health, and to promote growth and development of the baby. This study assessed dietary pattern, nutrient intake, and oral symptoms among pregnant women. **Methods:** Seventy-one women with singleton pregnancy attending a public healthcare centre in Malaysia participated in this cross-sectional study. Dietary pattern and nutrient intake of the women were assessed using food frequency questionnaire and 3-day 24-hour diet recall, respectively. Information on sociodemographic and obstetric profiles, and oral symptoms were obtained from self-administered questionnaire. **Results:** White rice, chicken, green leafy vegetables, granulated sugar, and salt were foods most consumed. Intakes of iron, folate, vitamin C, vitamin D, calcium, iodine, zinc, and fluoride for most respondents were below Recommended Nutrient Intake (RNI). Cavitated tooth (46.5%), bleeding gums (35.2%), pain upon eating and drinking (23.9%), and bad breath (23.9%) were common oral symptoms reported. Significant association was found between complaint of brown, yellow, and white spots on tooth surface with vitamin A intake, and between complaint of bleeding gums with zinc intake. **Conclusion:** Intake of most nutrients by most respondents in this study was below the RNI. Oral symptoms were common and a few were associated with nutrient intakes.

Keywords: dietary intake, nutrients, oral health, pregnancy, pregnant women

INTRODUCTION

Pregnant women are vulnerable to nutritional inadequacy due to metabolic changes and increased nutritional requirements of the growing foetus (Marshall *et al.*, 2022). Inadequate nutrient intake in pregnant women has been shown to be associated with poor dietary pattern as they tend to continue their pre-pregnancy dietary intake instead of adjusting their diet to meet the changing nutrient requirements during pregnancy (Savard *et al.*, 2018). Additionally, women living in low- and middle-income countries are often unable to meet the high nutrient demands of pregnancy due to social-related issues, leading to a chronically poor diet (Lee *et al.*, 2013). Iron deficiency is one of most prevalent forms of malnutrition in pregnant women (Madanijah *et al.*, 2016; Savard *et al.*, 2018). Folate and calcium are other nutrients commonly reported to be deficient among pregnant women (Madanijah *et al.*, 2016; Savard *et al.*, 2018). Energy intake has also been

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shown to be insufficient among pregnant women in developing countries, mainly due to low dietary diversity and having a starch-based diet (Madanijah *et al.*, 2016).

Inadequate intake of essential nutrients may affect not only the health of the foetus, but also the health of the mother (Marshall *et al.*, 2022), including her oral health (Rahman & Walls, 2020). The relationship between nutrition and oral health is bi-directional. Nutrients play an important role in oral health maintenance and oral disease prevention, and the health of the oral cavity influences the type of foods that can be eaten and subsequent supply of nutrients (Rahman & Walls, 2020). Additionally, the early symptoms of nutrient deficiencies often manifest in the mouth (Rahman & Walls, 2020).

The prevalences of periodontal disease and dental caries have been shown to be high in pregnant women, and are significantly associated with the corresponding oral symptoms (Yunita Sari, Saddki & Yusoff, 2020). While the increased risk for these oral diseases during pregnancy are mainly attributed to the elevated levels of sex hormones oestrogen and progesterone (Silva de Araujo Figueiredo *et al.*, 2017), a diet lacking in certain nutrients can also lead to the progression of oral diseases through decreased tissue homeostasis, lowered resistance to microbial biofilm, and impaired tissue healing (Rahman & Walls, 2020). The important link between nutrient and oral health underlines the need to investigate this association in pregnant women.

Evidence suggest that the intakes of micronutrients, such as calcium, iron, vitamin D, folic acid, and niacin, among pregnant women in Malaysia are lower than the national recommendations (Mohamed *et al.*, 2022). These micronutrients are not only important for general growth, development, wellbeing, and disease prevention, they are also necessary in maintaining the health of the oral cavity structures (Rahman & Walls, 2020). In addition, vitamin D deficiency during pregnancy may affect calcification of the primary teeth that begins during the fourth month of intrauterine life, leading to enamel defects that may increase the subsequent risk of early childhood caries (Rahman & Walls, 2020). Therefore, in this study, dietary pattern, nutrient intake, and oral symptoms of pregnant women were determined. The association between nutrient intake and oral symptoms of the women was also examined. The findings can provide baseline information that can help restrategise nutrition and oral health programmes for pregnant women.

MATERIALS AND METHODS

Study design, population, and sample

This cross-sectional study was conducted from February 20, 2020 to March 15, 2020 among pregnant women attending a Maternal and Child Healthcare Clinic (MCHC) in Kota Bharu, Kelantan. Ethical approval for this study was obtained from the Universiti Sains Malaysia Human Research and Ethics Committee (USM/JEPeM/17120729) and the Ministry of Health Malaysia Medical Research Ethics Committee (NMRR-19-3398-51678). Women aged 19-50 years with singleton pregnancy were eligible to participate, while those with diabetes mellitus, hypertension, and hyperemesis gravidarum were excluded.

The sample sizes for all specific objectives of this study were calculated and the largest affordable sample size was obtained from the objective to determine oral symptoms of pregnant women using the formula to estimate a single proportion with a 95% confidence interval (CI). The proportion of pregnant women who complained of having cavitated tooth was estimated at 62.0% (Yunita Sari *et al.*, 2020). At a precision

of 0.1, a sample size of 91 was obtained. Anticipating a 10% non-response rate, a sample size of 100 was selected.

Research tools and variables

Dietary pattern was assessed using a validated food frequency questionnaire (FFQ) by Loy *et al.* (2011), designed specifically for pregnant women in Malaysia. The FFQ comprised of 82 food items, organised into 10 food groups, as follows: 1) Cereal and cereal products, 2) Meat and eggs, 3) Milk and milk products, 4) Nuts, 5) Vegetables, 6) Fruits and fruit juices, 7) Beverages, 8) Fats, 9) Sweet and baked goods, and 10) Condiments. The frequency of food intake was assessed based on habitual intake over the past six months using a 7-point scale as follows: 1 = never or rarely, 2 = once a month, 3 = 2 to 3 times a month, 4 = once a week, 5 = 2 to 3 times a week, 6 = once daily, and 7 = 2 to 3 times daily. The serving or portion size of each food item taken was also captured. Samples of household utensils and measuring instruments were provided to increase the accuracy of serving and portion sizes estimation.

Dietary pattern was determined as food consumption frequency for each food item using a formula by Reaburn, Krondl, & Lau (1979) as follows: Food consumption frequency score = (R1S1 + R2S2 +R3S3 + R4S4 + R5S5 + R6S6 + R7S7) / 7, where S1-S7 referred to the rating scale, while R1-R7 referred to the percentage of respondents selecting the respective rating, with 7 as the maximum rating. The food items were categorised into mostly consumed foods (score of 80.0-100.0), moderately consumed foods (score of 30.0-79.9), and less consumed foods (score of 10.0-29.9) as recommended by Zainal Badari *et al.* (2012). Additionally, the amount of food intake (g) for each food group was calculated using the following formula: Amount of food (g) per day = frequency of intake (conversion factor) x serving size x total number of servings x weight of food in one serving (Norimah *et al.*, 2008).

A 3-day 24-hour diet recall was used to measure nutrient intake. Detailed information about all foods and beverages consumed by the respondents in 2 weekdays and 1 weekend were analysed using the Nutritionist Pro™ software to get the nutrient values. The nutrient values obtained were compared to the Recommended Nutrient Intake (RNI) for Malaysian pregnant women (Ministry of Health Malaysia, 2017) and were categorised as follows: 1) Inadequate, for values below the estimated average requirement, 2) Adequate, for values between the estimated average requirement and tolerable upper intake level, and 3) Excessive, for values exceeding the tolerable upper intake level.

A structured self-administered questionnaire was used to capture the respondents' current experience of symptoms associated with periodontal disease (bleeding gums, swollen gums, gum pain, red gums, loose tooth, bad breath, gum abscess, receding gums, and longer appearing tooth) and dental caries (spontaneous pain, pain upon eating/drinking sweet and hot or cold food/beverages, pain upon biting, cavitated tooth, brown, black, or white spots on tooth surface). Additionally, sociodemographic profile (maternal age, ethnicity, education level, employment status, monthly household income) and obstetric profile (stage of gestation and parity) of the respondents were obtained.

Data collection

A non-proportionate stratified random sampling method was used to capture equal number of samples from the first, second, and third trimesters of pregnancy. Following the establishment of eligibility, simple random sampling was used to select respondents from each stratum. Potential respondents who came for antenatal care follow-up at

the MCHC were individually approached by the main author. The women were informed of the importance, objectives, procedures, and other essential information regarding this study. Written informed consent was obtained from all women who agreed to participate.

Further instructions about the study procedures were provided prior to data collection. Administration of the FFQ, structured questionnaire, and interview for Day 1 of the 3-day 24-hour diet recall were done immediately at the MCHC, while interviews for Day 2 and Day 3 of the diet recall were conducted via telephone calls. Data collection was conducted fully by the main author.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics for Windows software, version 24.0 (Armonk, New York, USA). Descriptive statistics for dietary pattern, nutrient intake, oral symptoms, and other variables were determined; mean and standard deviation (*SD*) for continuous variables, and frequency and percentage for categorical variables. Spearman's correlation analysis was used to determine the association between amount of food intake and nutrient intake values. Chi-square test was used to determine the associations between selected nutrients (vitamin A, calcium, and zinc) and dental caries symptoms, and the associations between selected nutrients (vitamin C, iron, and zinc) and periodontal disease symptoms. A Fisher's Exact test was used when the conditions for chi-square test were not met. The level of significance was set at 0.05.

RESULTS

Characteristics of respondents

Owing to the COVID-19 lockdown, data collection period had to be shortened. As of March 15, 2020, we only managed to recruit 71 respondents. Mean age of the respondents was 29.4±4.4 years. Most respondents were from the Malay ethnicity (95.8%), received at least post-secondary education (97.2%), and unemployed (62.0%). Median monthly household income was MYR 2000 (*IQR* 1800). Most respondents were in the third trimester (43.7%), followed by second (32.4%) and first (23.9%) trimesters of pregnancy. More than half of the respondents (74.6%) had given birth at least once previously.

Food intake pattern of respondents

Food consumption frequency scores for all food items based on pregnancy trimester are shown in Table 1. White rice (from the cereal and cereal products group) was the most consumed food by respondents in all trimesters, while chicken (from the meat and eggs group) was the most consumed food by respondents in the second and third trimesters. Other most consumed food items by respondents included mustard green, water spinach, Chinese broccoli, spinach, cassava leaves, and fern (cooked) from the vegetable food group in the third trimester, granulated sugar from the sweet and baked goods food group in the second trimester, and salt from the condiment food group in the second and third trimesters. Most food items from the milk and milk products, as well as from other food groups (nuts, vegetables, fruits and fruit juices, beverages, fats, sweet and baked goods, and condiments) were only moderately consumed by respondents in all trimesters.

Nutrient intakes of respondents

Energy and nutrient intakes of the respondents are shown in Table 2. A total of 13 nutrients were extracted from the respondents' diet. The analysis of mean nutrient intake was done according to the availability of Recommended Nutrient Intake (RNI) values. Analysis for energy and zinc were done according to the trimester of pregnancy as there were differences in RNI between different

Table 1. Food consumption frequency score based on pregnancy trimester

Food consumption	*Trimester 1*		*Trimester 2*		*Trimester 3*	
	Food item (food group)	*Score*	*Food item (food group)*	*Score*	*Food item (food group)*	*Score*
Mostly consumed foods (80.0-100.0)	White rice (G1)	86.55	White rice (G1)	98.14	White rice (G1)	97.70
			Chicken (G2)	80.75	Chicken (G2)	83.41
			Salt (G10)	82.51	Mustard green, water spinach, Chinese broccoli, spinach, cassava leaves, fern (cooked) (G5)	81.16
			Granulated sugar (G9)	80.00	Salt (G10)	80.61
Moderately consumed foods (30.0-79.9)	Biscuit (G9)	78.20	Biscuit (G9)	69.53	Cabbage, broccoli (cooked) (G5)	76.10
	Granulated sugar (G9)	78.13	Garlic, shallot (G5)	68.83	Biscuit (G9)	75.59
	Indian mackerel, yellowstripe scad, sardine, skipjack tuna, Spanish mackerel, threadfin bream (G2)	77.31	White bread, wholemeal, bun (G1)	67.70	Granulated sugar (G9)	73.27
	Salt (G10)	77.29	Mustard green, water spinach, Chinese broccoli, spinach, cassava leaves, fern (cooked) (G5)	67.60	White bread, wholemeal, bun (G1)	72.81
	Chicken (G2)	74.79	Cabbage, broccoli (cooked) (G5)	67.06	Garlic, shallot (G5)	72.43
	Malt drink (Milo®, Horlick®) (G7)	74.01	Malt drink (Milo®, Horlick®) (G7)	66.97	Indian mackerel, yellowstripe scad, sardine, skipjack tuna, Spanish mackerel, threadfin bream (G2	72.35

Table 1. Food consumption frequency score based on pregnancy trimester *(continued)*

Food consumption	*Trimester 1*		*Trimester 2*		*Trimester 3*	
	Food item (food group)	*Score*	*Food item (food group)*	*Score*	*Food item (food group)*	*Score*
	White bread, wholemeal, bun (G1)	73.95	Indian mackerel, yellowstripe scad, sardine, skipjack tuna, Spanish mackerel, threadfin bream (G2)	66.46	Turmeric (G10)	70.99
	Local dishes (G9)	69.74	Soy sauce, dark soy sauce (G10)	65.84	Malt drink (Milo®, Horlick®) (G7)	70.13
	Mustard green, water spinach, Chinese broccoli, spinach, cassava leaves, fern (cooked) (G5)	67.16	Chili sauce, tomato ketchup (G10)	65.19	Egg (G2)	68.74
	Fried rice, *nasi lemak, nasi dagang, nasi kerabu, nasi minyak* (G1)	66.39	Tea (G7)	62.04	Fried rice, *nasi lemak, nasi dagang, nasi kerabu, nasi minyak* (G1)	68.66
	Tea (G7)	65.60	Fried rice, *nasi lemak, nasi dagang, nasi kerabu, nasi minyak* (G1)	60.87	Local dishes (G9)	67.27
	Meat (G2)	65.55	Turmeric (G10)	59.64	Chili sauce, tomato ketchup (G10)	63.61
	Soy sauce, dark soy sauce (G10)	65.54	Local dishes (G9)	59.50	Tea (G7)	61.20
	Turmeric (G10)	65.54	Egg (G2)	59.00	Coconut water, young coconut flesh (G7)	59.93
	Banana, banana fritter (G6)	63.89	Chili paste, *sambal belacan* (G10)	58.34	Soy sauce, dark soy sauce (G10)	59.59
	Cabbage, broccoli (cooked) (G5)	63.80	UHT cow's milk, goat's milk (full cream, low fat) (G3)	56.47	Banana, banana fritter (G6)	58.99
	Condensed milk (G9)	62.21	Apple (G6)	54.54	Potato, sweet potato, carrot (G5)	57.63

Table 1. Food consumption frequency score based on pregnancy trimester *(continued)*

Food consumption	*Trimester 1*		*Trimester 2*		*Trimester 3*	
	Food item (food group)	*Score*	*Food item (food group)*	*Score*	*Food item (food group)*	*Score*
	Egg (G2)	61.36	Oyster sauce (G10)	53.99	Cucumber, luffa (boiled), tomato (G5)	57.60
	Garlic, shallot (G5)	61.36	Noodles, rice vermicelli, flat rice noodles, *laksa* (G1)	52.80	Dates (G6)	56.73
	Chili sauce, tomato ketchup (G10)	61.33	Coconut milk (G8)	52.16	Oyster sauce (G10)	56.67
	Orange (G6)	58.83	Anchovy sauce (*budu*) (G10)	52.11	Watermelon (G6)	55.84
	Apple (G6)	56.33	Coconut water, young coconut flesh (G7)	51.49	Meat (G2)	55.76
	Sweets (G9)	56.33	Condensed milk (G9)	51.41	Apple (G6)	55.36
	Oyster sauce (G10	56.30	Skimmed milk (vanilla, chocolate) (G3)	49.07	Noodles, rice vermicelli, flat rice noodles, *laksa* (G1)	54.84
	Chocolate (G9)	56.29	Raisin (G6)	47.81	Chili paste, *sambal belacan* (G10)	53.51
	Watermelon (G6)	56.27	Cucumber, luffa (boiled), tomato (G5)	47.17	Skimmed milk (vanilla, chocolate) (G3)	52.93
	UHT cow's milk, goat's milk (full cream, low fat) (G3)	55.51	Powdered milk (full cream) (G3)	45.87	Raisin (G6)	50.64
	Noodles, rice vermicelli, flat rice noodles, *laksa* (G1)	55.46	Meat (G2)	45.34	Condensed milk (G9)	50.24
	Mango (G6)	55.43	Mango (G6)	45.23	Coconut milk (G8)	49.90
	Cucumber, luffa (boiled), tomato (G5)	54.61	Orange (G6)	44.67	Guava (G6)	49.37

Table 1. Food consumption frequency score based on pregnancy trimester *(continued)*

Food consumption	*Trimester 1*		*Trimester 2*		*Trimester 3*	
	Food item (food group)	*Score*	*Food item (food group)*	*Score*	*Food item (food group)*	*Score*
	Keropok ikan & keropok lekor (G2)	53.78	Rose syrup drink (G7)	44.00	UHT cow's milk, goat's milk (full cream, low fat) (G3)	49.34
	Papaya (G6)	53.00	Guava (G6)	42.20	Anchovies (G2)	48.85
	Malaysian flatbread (*roti canai), dhal*/curry gravy (G1)	52.94	Dates (G6)	42.19	Mango (G6)	47.99
	Coconut milk (G8)	52.93	Chocolate (G9)	42.17	Chocolate (G9)	47.96
	Potato, sweet potato, carrot (G5)	52.11	Watermelon (G6)	41.60	Orange (G6)	47.13
	Chili paste, *sambal belacan* (G10)	52.09	Banana, banana fritter (G6)	40.30	Long bean, winged bean (G5)	46.63
	Rose syrup drink (G7)	51.31	Prawn (G2)	39.13	*Keropok ikan & keropok lekor* (G2)	46.54
	Yellow pear (G6)	51.27	*Keropok ikan & keropok lekor* (G2)	38.51	Anchovy sauce (*budu*) (G10)	44.70
	Honeydew (G6)	51.23	Malaysian flatbread (*roti canai*), dhal/curry gravy (G1)	38.51	Prawn (G2)	43.78
	Dates (G6)	49.60	Anchovies (G2)	38.51	Malaysian flatbread (*roti canai*), dhal/curry gravy (G1)	43.78
	Anchovies (G2)	49.58	Papaya (G6)	38.49	Rose syrup drink (G7)	42.87
	Anchovy sauce (*budu*) (G10)	48.80	Margarine (G8)	37.87	Squid (G2)	42.86
	Prawn (G2)	48.74	Potato, sweet potato, carrot (G5	37.74	Papaya (G6)	42.40
	Raisin (G6)	47.83	Sweets (G9)	37.73	Soy drink (G4)	40.63

Table 1. Food consumption frequency score based on pregnancy trimester *(continued)*

Food consumption	*Trimester 1*		*Trimester 2*		*Trimester 3*	
	Food item (food group)	*Score*	*Food item (food group)*	*Score*	*Food item (food group)*	*Score*
	Coconut water, young coconut flesh (G7)	47.06	Soy drink (G4)	36.00	Grape (G6)	39.57
	Grape (G6)	46.29	Long bean, winged bean (G5)	35.33	Margarine (G8)	36.97
	Guava (G6)	45.46	Squid (G2)	34.78	Sweets (G9)	36.47
	Skimmed milk (vanilla, chocolate) (G3)	43.71	Yellow pear (G6)	34.73	Honeydew (G6)	36.13
	Groundnut, horse bean, mung bean, dhal, cashew nut (G4)	43.69	Honeydew (G6)	34.16	Salted egg (G2)	35.51
	Ready-made cereal (G1)	42.86	Grape (G6)	32.84	Yellow pear (G6)	35.09
	Peanut butter (G8)	42.84	Water guava (G6)	32.29	Chicken liver, cow liver (G2)	34.56
	Water guava (G6)	42.03	Fish ball (G2)	31.68	Groundnut, horse bean, mung bean, dhal, cashew nut (G4)	34.16
	Squid (G2)	42.02	Groundnut, horse bean, mung bean, dhal, cashew nut (G4)	31.03	Yoghurt (G3)	32.21
	Salted egg (G2)	41.26	Chicken liver, cow liver (G2	30.43	Coffee (G7)	31.27
	Long bean, winged bean (G5)	41.23	Catfish, silver catfish (G2)	30.43	Blood clam (G2)	30.90
	Soy drink (G4)	41.20			Fish ball (G2)	30.88
	Powdered milk (full cream) (G3)	41.17			Corn (G1)	30.41
	Pegaga, ulam raja (G5)	40.39				
	Margarine (G8)	40.37				

Table 1. Food consumption frequency score based on pregnancy trimester *(continued)*

Food consumption	*Trimester 1*		*Trimester 2*		*Trimester 3*	
	Food item (food group)	*Score*	*Food item (food group)*	*Score*	*Food item (food group)*	*Score*
	Ais batu kacang (ABC) (G9)	40.36				
	Catfish, silver catfish (G2)	38.66				
	Corn (G1)	37.82				
	Yoghurt (G3)	35.36				
	Cheese (G3)	35.31				
	Blood clam (G2)	33.67				
	Pineapple (G6)	33.64				
	Coffee (G7)	33.63				
	Stuffed green chili peppers (*solok lada*) (G2)	32.81				
	Pickles (G6)	32.79				
	Fish ball (G2	32.77				
	Chicken liver, cow liver	31.93				
	Rambutan (G6)	31.14				
	Canned sardine & tuna (G2)	30.25				
Less consumed foods (10.0-29.9)	*Langsat, duku, dokong* (G6)	29.47	White glutinous, fermented rice, grated coconut (G1)	29.81	Salted fish (G2)	29.95
	Salted fish (G2)	29.41	*Ais batu kacang (ABC)* (G9)	29.74	*Pegaga, ulam raja* (G5)	29.54
	Jackfruit (G6)	28.60	Peanut butter (G8)	27.91	*Ais batu kacang (ABC)* (G9)	28.94
	Cempedak, cempedak fritter (G6)	28.59	Blood clam (G2)	27.90	Canned sardine & tuna (G2)	28.57

Table 1. Food consumption frequency score based on pregnancy trimester *(continued)*

Food consumption	*Trimester 1*		*Trimester 2*		*Trimester 3*	
	Food item (food group)	*Score*	*Food item (food group)*	*Score*	*Food item (food group)*	*Score*
	White glutinous, fermented rice, grated coconut (G1)	27.73	*Pegaga, ulam raja* (G5)	27.24	White glutinous, *tapai*, grated coconut (G1)	28.11
	Mangosteen (G6)	25.26	Corn (G1)	26.71	Stuffed green chili peppers *(solok lada)* (G2)	25.81
	Durian (G6)	21.00	Ready-made cereal (G1)	24.22	Catfish, silver catfish (G2)	25.81
			Coffee (G7)	24.19	Ready-made cereal (G1)	25.35
					Water guava (G6)	25.29
			Salted fish (G2)	22.36	Pineapples (G6)	24.43
			Canned sardine & tuna (G2)	22.98	Jackfruit (G6)	23.99
			Stuffed green chili peppers *(solok lada)* (G2)	23.54	Peanut butter (G8)	22.16
			Rambutan (G6)	22.29	*Cempedak, cempedak* fritter (G6)	21.20
			Salted egg (G2)	21.70	Powdered milk (full cream) (G3)	20.49
			Cempedak, cempedak fritter (G6)	21.11	Cheese (G3)	20.50
			Mangosteen (G6)	21.09	Rambutan (G6)	20.30
			Cheese (G3)	20.50	*Langsat, duku, dokong* (G6)	18.93
			Yoghurt (G3)	20.49	Mangosteen (G6)	18.93
			Pickles (G6)	20.46	*Durian* (G6)	18.90
			Jackfruit (G6)	19.24	Pickles (G6)	18.41
			Pineapple (G6)	18.61		
			Durian (G6)	17.36		
			Langsat, duku, dokong (G6)	16.14		

Food groups: G1=Cereal and cereal products, G2= Meat and eggs, G3= Milk and milk products, G4= Nuts, G5= Vegetables, G6=Fruits and fruit juices, G7= Beverages, G8= Fats, G9= Sweet and baked goods, G10=Condiments

trimesters. For other nutrients, the RNI values were similar for all trimesters and thus, only one mean was derived for all trimesters.

The frequency of respondents with adequate energy intake reduced as the trimester increased. At the first trimester, the intake of energy was adequate for most respondents (82.4%). At the second trimester, only about half had adequate energy intake (52.2%) and at the third trimester, the energy intake was inadequate for most respondents (71.0%). Only slightly more than half of the respondents had adequate carbohydrate intake (54.9%) and vitamin A intake (52.1%). While most respondents had adequate protein intake (85.9%), the intakes of iron, folate, vitamin C, vitamin D, calcium, iodine, zinc, and fluoride were below the RNI values. The intake of zinc was deficient at all trimesters.

Associations between food and nutrient intakes

The associations between food and nutrient intakes are shown in Table 3. The amount of milk and milk products consumed had a significant, positive association with the levels of folate (p=0.003) and vitamin C (p=0.020). The level of folate intake was also positively associated with the amount of vegetables consumed (p=0.014). A significant, positive association was found between the level of vitamin D with the amount of beverage (p=0.040) and confections

Table 2. Energy and nutrient intakes of pregnant women (n=71)

Nutrient	*RNI*	*Nutrient Intake Mean±SD*	*Frequency (%)*		
			Inadequate	*Adequate*	*Excessive*
Energy (kcal)	1690†	1916±282	3 (17.6)	14 (82.4)	0 (0.0)
	1890‡	2040±344	11 (47.8)	12 (52.2)	0 (0.0)
	2080§	1972±226	22 (71.0)	9 (29.0)	0 (0.0)
Carbohydrate (%)	50-65 a¶	50.4±6.7	32 (45.1)	39 (54.9)	0 (0.0)
Fat (%)	25-30 a¶	31.7±5.1	0 (0.0)	0 (0.0)	71 (100.0)
Protein (%)	10-20 a¶	17.9±2.8	0 (0.0)	61 (85.9)	10 (14.1)
Iron (mg)	29¶	19.1 (14.2, 22.6)b	66 (93.0)	5 (7.0)	0 (0.0)
Folate (µg)	600¶	74.9 (46.0, 104.9)b	71 (100.0)	0 (0.0)	0 (0.0)
Vitamin A (µg)	800¶	805.5 (560.2,1095.0)b	34 (47.9)	37 (52.1)	0 (0.0)
Vitamin C (mg)	80¶	44.5 (27.6, 73.2)b	60 (84.5)	11 (15.5)	0 (0.0)
Vitamin D (µg)	15¶	1.7±1.2	71 (100.0)	0 (0.0)	0 (0.0)
Calcium (mg)	1000¶	589.2±189.9	70 (98.6)	1 (1.4)	0 (0.0)
Iodine (µg)	200¶	0.0 (0.0, 13.0)b	71 (100.0)	0 (0.0)	0 (0.0)
Zinc (mg)	5.5†	4.3±1.8	13 (76.5)	4 (23.5)	0 (0.0)
	7‡	4.5±2.0	20 (87.0)	3 (13.0)	0 (0.0)
	10§	4.1±1.7	31 (100.0)	0 (0.0)	0 (0.0)
Fluoride (mg)	3¶	0.8 (0.0, 12.7)b	71 (100.0)	0 (0.0)	0 (0.0)

a% based on total energy intake (TEI)
bMedian (25th, 75th), Kolmogorov-Smirnov p<0.050
†RNI for first trimester
‡RNI for second trimester
§RNI for third trimester
¶Similar RNI for all trimesters

consumed (p=0.007).

Oral symptoms of pregnant women

The most common oral symptom reported was cavitated tooth (46.5%), followed by bleeding gums (35.2%), pain upon eating/drinking sweet, hot or cold food/beverage (23.9%), bad breath (23.9%), brown, black, or white spots on tooth surface (19.7%), spontaneous pain (15.5%), swollen gums (15.5%), gum pain (12.7%), and pain upon biting (11.3%). Other less common problems were red gums (5.6%), receding gums (4.2%), loose tooth (1.4%), and longer appearing tooth (1.4%). None of the respondents reported having gum abscess.

Associations between nutrient intakes and oral symptoms

The associations between selected nutrients (vitamin A, calcium, and zinc) and dental caries symptoms, and the associations between selected nutrients (vitamin C, iron, and zinc) and periodontal disease symptoms, except gum abscess, are presented in Table 4 and Table 5, respectively. Significant associations were found between vitamin A and the presence of brown, yellow and white spots on tooth surface (χ^2=4.89, p=0.030), and between zinc and bleeding gums (χ^2=6.49, p=0.047).

DISCUSSION

Findings of our study provided further evidence that most pregnant women are not getting the recommended amount of essential nutrients. Intakes of iron, folate, vitamin C, vitamin D, calcium, iodine, zinc, and fluoride were deficient in most respondents, in agreement with previous studies (Madanijah *et al.*, 2016; Saunders *et al.*, 2019; Savard *et al.*, 2018). Despite folate being

Table 3. Associations between food and nutrient intakes

Variable	*Correlation coefficient*†									
	Cereal and cereal products	*Meat and eggs*	*Milk and milk prod-ucts*	*Nuts*	*Vegetables*	*Fruits and juices*	*Beverages*	*Fats*	*Sweet and baked goods*	*Condiments*
Protein	0.11	-0.07	0.14	-0.03	0.14	-0.01	-0.18	-0.04	0.30	0.01
Carbohydrate	0.12	0.19	0.37	0.17	0.26	0.12	0.21	0.09	0.19	0.16
Fat	0.07	-0.11	-0.04	-0.03	-0.06	-0.01	-0.19	-0.06	-0.05	-0.18
Iron	0.07	0.30	-0.06	-0.04	0.15	-0.08	-0.02	-0.10	0.03	-0.05
Folate	0.20	0.15	0.34*	-0.06	0.29*	0.24	-0.20	-0.12	0.12	0.19
Vitamin A	0.04	0.05	0.05	-0.12	0.20	0.10	-0.26	-0.00	-0.09	0.11
Vitamin C	0.01	-0.04	0.28*	0.10	0.18	0.10	-0.05	0.02	0.19	0.23
Vitamin D	0.16	0.05	-0.28	-0.03	0.04	0.00	0.24*	0.16	0.32*	-0.01
Calcium	0.16	-0.02	0.16	-0.15	-0.02	0.01	-0.15	-0.05	-0.02	0.13
Iodine	0.04	-0.05	-0.17	-0.27	-0.18	0.08	-0.05	0.09	-0.00	-0.13
Zinc	0.16	0.04	0.21	-0.23	0.15	0.03	-0.25	-0.12	-0.03	0.09
Fluoride	0.17	-0.01	-0.11	-0.14	-0.00	0.02	-0.14	-0.01	-0.04	-0.07

†Spearman's rho
*p<0.05

naturally present in a wide variety of foods, including beef liver, fruits and fruit juices, nuts, beans, peas, and vegetables, that were highly consumed by respondents in the third trimester, all women in our study did not receive adequate amount of folate. Consumption of margarine, the only food mandated to be fortified with vitamin D in Malaysia (Ministry of Health Malaysia, 2018), was moderate, and all our respondents did not meet the RNI of 15 µg per day, compared to 67.7% reported in a previous local study by Lee *et al.* (2021). In addition, the consumption of foods rich in iron, vitamin C, calcium, iodine, zinc, and fluoride, such as animal liver, fish, dairy products, eggs, and yellow and orange fruits, was only low to moderate, consistent with the inadequacy of these respective nutrients. Although the respondents' mean daily intake of vitamin A met the RNI value, probably attributable to the high consumption of green leafy vegetables that are rich in vitamin A among respondents in the third trimester, almost half of them (47.9%) had inadequate vitamin A intake.

Energy requirements of pregnant women increase progressively during pregnancy to support growth of the foetus, while maintaining health of the mother (Marshall *et al.*, 2022). However, most

Table 4. Associations between nutrient intakes and symptoms of dental caries

Variable	*Vitamin A*			*Calcium*			*Zinc*		
	Frequency (%)		$\chi^2(df)$	*Frequency (%)*		$\chi^2(df)$	*Frequency (%)*		$\chi^2(df)$
	Adequate	*Inadequate*		*Adequate*	*Inadequate*		*Adequate*	*Inadequate*	
Spontaneous pain									
Yes	6 (54.5)	5 (45.5)	0.03(1)†	0 (0.0)	11 (100.0)	0.34(1)‡	1(9.1)	10 (90.9)	0.01(1)†
No	31 (51.7)	29 (48.3)		1 (1.7)	59 (98.3)		6 (10.0)	54 (90.0)	
Pain upon eating/drinking sweet and hot or cold food/beverages									
Yes	11 (64.7)	6 (35.3)	1.42(1)†	1 (5.9)	16 (94.1)	2.91(1)‡	3 (17.6)	14 (82.4)	1.53(1)†
No	26 (48.1)	28 (51.9)		0 (0.0)	54 (100.0)		4 (7.4)	50 (92.6)	
Pain upon biting									
Yes	5 (62.5)	3 (37.5)	0.39(1)‡	7 (87.5)	1 (12.5)	4.48(1)‡	0 (0.0)	8 (100.0)	0.99(1)†
No	32 (50.8)	31 (49.2)		63 (100.0)	0 (0.0)		7 (11.1)	56 (88.9)	
Cavitated tooth									
Yes	18 (54.5)	15 (45.5)	0.15(1)†	1 (3.0)	32 (97.0)	1.55(1)‡	2 (6.1)	31 (93.9)	1.04(1)‡
No	19 (50.0)	19 (50.0)		0 (0.0)	38 (100.0)		5 (13.2)	33 (86.8)	
Brown, black, or white spots on tooth surface									
Yes	11 (78.6)	3 (21.4)	4.89(1)†*	0 (0.0)	14 (100.0)	0.44(1)‡	2 (14.3)	12 (85.7)	0.34(1)†
No	26 (45.6)	31(54.4)		1 (1.8)	56 (98.2)		5 (8.8)	52 (91.2)	

†Chi-square test, ‡Fisher's Exact test
*$p<0.05$

Table 5. Associations between nutrient intakes and symptoms of periodontal disease

Variable	*Vitamin C*			*Iron*			*Zinc*		
	Frequency (%)		$\chi^2(df)$	*Frequency (%)*		$\chi^2(df)$	*Frequency (%)*		$\chi^2(df)$
	Adequate	*Inadequate*		*Adequate*	*Inadequate*		*Adequate*	*Inadequate*	
Bleeding gums									
Yes	2 (8.0)	23 (92.0)	1.66(1)†	2 (8.0)	23 (92.0)	0.53(1)‡	0 (0.0)	25 (100.0)	6.49(1)‡*
No	9 (19.6)	37 (80.4)		3 (6.5)	43 (93.5)		7 (15.2)	39 (84.8)	
Swollen gums									
Yes	2 (18.2)	9 (81.8)	0.72(1)†	2 (18.2)	9 (81.8)	1.92(1)‡	0 (0.0)	7 (11.7)	1.42(1)†
No	9 (15.0)	51 (85.0)		3 (5.0)	57 (95.0)		11(100.0)	53 (88.3)	
Gum pain									
Yes	3 (33.3)	6 (66.7)	2.51(1)†	1 (11.1)	8 (88.9)	0.23(1)‡	0 (0.0)	9 (100.0)	1.13(1)†
No	8 (12.9)	54 (87.1)		4 (6.5)	58 (93.5)		7 (11.3)	55 (88.7)	
Red gums									
Yes	0 (0.0)	4 (100.0)	1.39(1)‡	0 (0.0)	4 (100.0)	0.60(1)‡	0 (0.0)	4 (100)	0.86(1)‡
No	11 (16.4)	56 (83.6)		5 (7.5)	62 (92.5)		7 (10.4)	60 (89.6)	
Loose tooth									
Yes	1 (100.0)	0 (0.0)	3.81(1)‡	0 (0.0)	1 (100.0)	0.15(1)‡	0 (0.0)	1 (100.0)	0.21(1)‡
No	10 (14.3)	60 (85.7)		5 (7.1)	65 (92.9)		7 (10.0)	63 (90.0)	
Bad breath									
Yes	3 (17.6)	14 (82.4)	0.79(1)†	1 (5.9)	16 (94.1)	0.48(1)‡	3 (17.6)	14 (82.4)	1.53(1)†
No	8 (14.8)	46 (85.2)		4 (7.4)	50 (92.6)		4 (7.4)	50 (92.6)	
Receding gums									
Yes	1 (33.3)	2 (66.7)	0.62(1)‡	0 (0.0)	3 (100.0)	0.45(1)‡	1 (33.3)	2 (66.7)	1.31(1)‡
No	10 (14.7)	58 (85.3)		5 (7.4)	63 (92.6)		6 (8.8)	62 (91.2)	
Longer appearing tooth									
Yes	0 (0.0)	1 (100.0)	0.34(1)‡	0 (0.0)	1 (100.0)	0.15(1)‡	1 (100.0)	0 (0.0)	4.77(1)‡
No	11 (15.7)	59 (84.3)		5 (7.1)	65 (92.9)		6 (8.6)	64 (91.4)	

†Chi-square test, ‡Fisher's Exact test
*p<0.05

pregnant women did not change their food intake as recommended and continued to consume a low nutrient density pre-pregnancy diet (Savard *et al.*, 2018). In this study, the number of women who met their energy requirements reduced as pregnancy progressed. The mean daily intake of energy was highest in the second trimester and decreased to below the RNI level in the third trimester as energy intake was deficient for most respondents. Similar findings were reported by Savard *et al.* (2018) among pregnant women in Canada.

The westernisation of Asian diet has resulted in rapid changes in the pattern of food consumption from the traditional carbohydrate-rich staple foods to high protein and energy-dense diets consisting mainly of livestock and dairy products, vegetables and fruits, and fats and oils (Drewnowski & Poulain, 2018). Only about half (54.9%) of the pregnant women in this study had adequate carbohydrate intake and this finding is comparable to the result of Saunders *et al.* (2019) among pregnant women in Norway at 56.1%. The source of carbohydrate for our respondents was mainly from the highly consumed white rice. The food intake pattern of our respondents seemed to concur with the reported shift in the Malaysian dietary patterns characterised by increasing intakes of refined grains, animal-based foods, added fat, and sugar-sweetened beverages, as well as fast food (Goh *et al.*, 2020). Our findings showed that animal products, fats, sugar-sweetened beverages such as rose syrup drink, and refined grain products such as white bread, white rice, and foods made with white flour including ready-made cereals and flatbreads, as well as sweet and baked goods were mostly or moderately consumed by the women.

Despite changes in food sources, the changing landscape of nutrition from dietary plant proteins to proteins of animal origin has not affected the adequacy of protein in the diet (Drewnowski & Poulain, 2018), and this was reflected from our findings that protein intake was adequate for most respondents and excessive for some. The increase of protein in everyday diet, particularly from animal origin, and the increase of fat, with decreasing carbohydrate energy, are a major concern worldwide due to their contribution to the increased prevalence of chronic diseases such as obesity, diabetes, cancer, and cardiovascular diseases (Billingsley, Carbone, & Lavie, 2018).

The high intake of salt by our respondents in the second and third trimesters, most likely in the cooking to make dishes more palatable, should therefore also be a matter of concern. While consumption of a moderate amount of salt during pregnancy can help maintain a normal balance of fluids and minerals in the body, excessive salt intake in the diet may increase the risk of hypertension, which is a major risk factor for cardiovascular diseases and kidney diseases (Asayama & Imai, 2018). In addition, high consumption of granulated sugar in the second trimester, in addition to sugar-sweetened beverages, sweet and baked goods, and high fat products may put respondents and their foetus at risk of metabolic impairments with subsequent poor health (Zambrano & Nathanielsz, 2017).

In this study, the amount of milk and milk products consumed by the respondents had a significant, positive association with their mean daily intake of folate. Milk and milk products, such as cheese and yoghurt, are not only excellent sources of protein, carbohydrates, fat, vitamins A, B2 and B12, and minerals, such as calcium, magnesium, phosphorus, and potassium, but also good dietary sources of folate. The level of folate intake among our respondents was also positively associated with the amount of vegetables they consumed.

Vegetables commonly consumed by the Malaysian population, such as cabbage, cauliflowers, carrot, cucumber, cabbage, chilli, lady's finger, spinach, tomato, tapioca shoot, and pumpkin, contain folate in the range of between 2-8 μg per 100 g (Chew, Khor & Loh, 2012).

Besides folate, the amount of milk and milk products consumed by our respondents had a significant, positive association with their mean daily intake of vitamin C. The content of vitamin C in milk varies depending on the source and may also change with season. The content was found to be higher in goat's milk, with a mean of 5.48 mg per 100 ml and lower in cow's milk, ranging 1.65-2.75 mg per 100 g (Morrissey & Hill, 2009). Although milk and milk products are not important sources of vitamin C, consumption of vitamin C fortified dairy products could have contributed to the finding.

In this study, the level of vitamin D was positively associated with the consumption of sweet and baked goods, probably from the yeast used in baking (Kessi-Pérez *et al.*, 2022) and from cocoa or chocolate that are among the common flavouring ingredients used in baked goods (Benedik, 2022). In addition, vitamin D was found to be positively associated with the consumption of beverages, most likely from fortified beverages. Plant-based beverages like malt drinks and soy drinks are among the most common beverages fortified with vitamin D (Benedik, 2022) and these were moderately consumed by our respondents.

The most common symptom associated with dental caries reported by the women in this study was cavitated tooth (46.5%). This finding is consistent with the result of a previous local study among pregnant women by Yunita Sari *et al.* (2020), with a higher proportion of 62.0%. Although not all carious lesions progress to cavitation, the visible breakdown of tooth surface is the endpoint of the disease process when there is an extensive loss of minerals. It may take months or years for a carious lesion to become cavitated, if ever, as not all lesions progress at the same rate (Pitts *et al.*, 2017). During pregnancy, surges in oestrogen and progesterone levels make women more susceptible to developing gingivitis (Silva de Araujo Figueiredo *et al.*, 2017). Bleeding gums is a common symptom of gingivitis, the earliest stage of periodontal disease. Gum bleeding was the most common periodontal disease symptom reported by our respondents (35.2%), in agreement with findings among pregnant women in other population groups (Stelmakh, Slot & van der Weijden, 2017).

Vitamin A is crucial in the maintenance of healthy teeth, skeletal and soft tissue, mucous membranes, and skin by preserving the integrity of the epithelial tissues. Vitamin A also supports the immune function, foetal development, and vision. Although there is currently no evidence of the association between vitamin A and dental caries in human, vitamin A deficiency has been shown to cause salivary gland atrophy (Rahman & Walls, 2020), which can result in reduced salivary flow, leading to increased risk for caries (Llena-Puy, 2006). In this study, a significant association was found between vitamin A status and the presence of brown, yellow and white spot lesions on tooth surfaces, which are the early signs of caries.

Zinc is a trace element that plays a critical role in human health maintenance, particularly related to the immune system (Chasapis *et al.*, 2012). With its numerous roles in basic cellular functions, zinc deficiency has been associated with increased susceptibility to infection and delayed healing that can affect many organ systems of the body, including the oral cavity (Bhattacharya, Misra & Hussain, 2016). Zinc deficiency can therefore be a potential risk factor

for periodontal disease (Bhattacharya *et al.*, 2016), which begins with gingivitis, the localised inflammation of the gingiva, causing the gums to become swollen and red, and may also bleed. Zinc was found to have a significant association with gingival bleeding symptom among our respondents.

Our findings contributed to the body of evidence that showed that pregnant women were not getting the recommended amount of nutrients. However, in our study, the nutrient levels were obtained only from the respondents' food intake, not taking into consideration additional supplements that may be taken by the women. Nevertheless, the levels of important nutrients necessary to keep the mother and baby healthy, such as folate, vitamin D, and calcium, were very much below the RNI such that additional supplementation of vitamins and minerals may still be inadequate to meet their nutritional requirements during pregnancy.

Our study also added to the growing literature supporting the link between nutrients and oral health. However, caution should be exercised when interpreting findings of this study as the use of self-perceived oral health problems may be less accurate than clinical diagnosis by dental professionals. Additionally, dietary assessment was done only once in this cross-sectional study and may not reflect the participants' dietary pattern throughout the pregnancy. We were also unable to control the effects of potential confounders in the association between nutrient intakes and oral symptoms as some symptoms were reported only by a few, if any, creating substantial imbalance in the outcome variables and precludes the use of multivariable analysis.

Our study population was limited to pregnant women in Kelantan, and we managed to recruit only about 70% of the required sample size. This may compromise the extent to which the study findings can be generalised to pregnant women in Malaysia and even to the target population. Hence, we consider our study as foundational and hopefully will provide the basis for further work in this area. We recommend for larger studies that employ an appropriate experimental design to test the hypothesis of the association between nutrients and oral health of pregnant women.

CONCLUSION

Most pregnant women in this study did not consume adequate amount of recommended nutrients. Significant association was found between the intake of certain nutrients and the amount of food consumed. Oral symptoms were common among the women and the presence of certain symptoms was associated with adequacy of nutrient. Intake of a balanced healthy diet with selections of foods that contain vitamins and minerals essential for pregnancy is the best way to get the nutrients needed by the mother, as well as the baby. A more focused dietary counselling and intervention is therefore indicated to reduce the health risks of malnutrition during pregnancy, which can be lifelong and even life-threatening.

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Authors' contribution

Ibrahim Z, contributed to the conception and design of the study, data acquisition, management and analyses, data interpretation, preparation of the manuscript, revised and approved the final manuscript; Saddki N and Hasan R, contributed to the conception and design of the study, data interpretation, critically reviewed the manuscript, revised and approved the final manuscript.

Conflict of interest

The authors declare no conflict of interest.

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