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Factors associated with food choices among elderly: a scoping review

Fatin Izzaty Mohd Shahrin[1], Noraida Omar[1,2*], Zulfitri 'Azuan Mat Daud[1] & Nor Fadhlina Zakaria[3]

[1]*Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia;* [2]*Malaysian Research Institute on Ageing (MyAgeing), Universiti Putra Malaysia, Selangor, Malaysia;* [3]*Department of Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia*

ABSTRACT

Introduction: The aging population is a matter of global concern. Age-related physiological, pathological, psychosocial, economic, cultural and environmental changes are common and may greatly influence the quality of life of the elderly. The aim of this review was to explore the determinants and motivations that drive the elderly in making food choices. **Methods:** The search strategy of this literature review used the PRISMA protocol. Potential literature that was related to food choices was identified using two different combinations of keywords and two major electronic search engines, namely Pubmed and Science Direct. The articles that were selected for this review had to be in the English language, open-accessed and published between January 2007 and December 2017. **Results:** From a search of 1398 articles, 15 articles (seven quantitative and eight qualitative) were identified that were related to food choices among the elderly. The key factor that determined food choices among the elderly population was identified to be health. Others included convenience, sensory appeal, price, early food experience and more. The limitations of these studies that were reported were the small sample size and the reliance on self-reporting. The conclusions that were drawn were for specific groups that were studied in this review should be extrapolated or generalised with caution. **Conclusion:** Strategies for intervention programmes should be undertaken in collaboration with health professionals, researchers, policymakers, and the food industry. Future research is needed in the elderly who have chronic diseases, are dependent or who have disabilities.

Keywords: Food choice, food intake, elderly, nutrition, scoping review

INTRODUCTION

The aging population is a matter of global concern. The elderly, by definition, are those aged ≥ 60 years. In 2017, 13% of the global population aged ≥ 60 years was approximately 962 million people, with the highest percentage (25%) to be found in Europe. The world population of the elderly is forecast to be 1.4 billion in 2030, increasing to 2.1 billion in 2050 and to 3.1 billion in 2100 (UN DESA, 2017).

Age-related physiological, pathological, psychosocial, economic and cultural

*Corresponding author: Noraida Omar, PhD
Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
Tel: +6089472463; Fax: 03-89426769; E-mail: noraidaomar@upm.edu.my
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environmental changes (Rozin, 2006; Sharpe, Huston & Finke, 2003) are common and may greatly influence the quality of life of the elderly. This complex phenomenon, interlaced with several socio-demographic factors such as gender (Wardle *et al.*, 2004) and religion (Asma *et al.*, 2010) can influence food choices and preferences (Rolls, 1999). Changes in food choices may have adverse effects on the energy and macronutrient intake and which in turn may eventually impact or aggravate nutrition-related illness, body weight, body composition, disability and the quality of life (Rolls, 1999).

The determinants of food choice in the elderly, as shown by previous studies, include the decline in chemosensory system sensitivity (i.e. in taste and smell) (Rolls, 1999), loss of appetite (Shatenstein *et al.*, 2013) and life course events such as parental influence, early adult events and new health diagnoses during aging (Pucciarelli & Thomas, 2011). The other factors that contribute to food choices are, *inter alia*, taste, convenience, cost, nutritional knowledge, health status and food accessibility (Ree, Riediger & Moghadasian, 2008; Gunsam & Murden, 2007). The understanding of these factors in influencing food choices is still unclear, limited and requires investigation (Gunsam & Murden, 2007). Brownie & Coutts (2013) stated that knowledge on how available dietary guidelines of food intake recommendations can be applied to food choices and daily life for the elderly is limited. Hence, the aim of this review is to explore the determinants and motivations of the elderly in their healthy food choices.

METHODS

The search strategy was undertaken according to the Preferred Reporting Items for Systematic and Meta-Analyses (PRISMA) Statement Protocol (Moher *et al.*, 2009), as shown in Figure 1. The electronic search engines namely Pubmed and Science Direct were used to identify potential literatures that were related to food choices. Two different combinations of keywords that were used in order to locate studies that were related to food choices were "food choice and elderly" and "food choice or elderly". The articles that were obtained in the identification step were then subjected to screening where duplicated articles were discarded and abstracts were examined to identify articles that were relevant to the research question of this review. The eligibility criteria that were adopted to accept the articles for this review, were as follows: (1) the article was written in the English language and was open-accessed; (2) publications for the period January 2007 to December 2017; (3) the definition of the elderly population by age was according to the country of origin and (4) the articles had explored factors related to food choices among older people. The exclusion criteria were articles with other age ranges instead of elderly subjects and informal narrative or reviews on food choices.

RESULTS

The identification step retrieved 1,398 publications by using the search engines mentioned and the two different combinations of keywords. The unrelated studies and duplicates were then removed and 1,148 articles were examined thoroughly for relevant abstracts. This procedure resulted in 41 full-text articles that were reviewed for eligibility for inclusion in the final review. Finally, 15 articles that met the inclusion criteria were summarised and tabulated by using the key concepts and themes of the studies. The outcomes were categorised into two research designs:

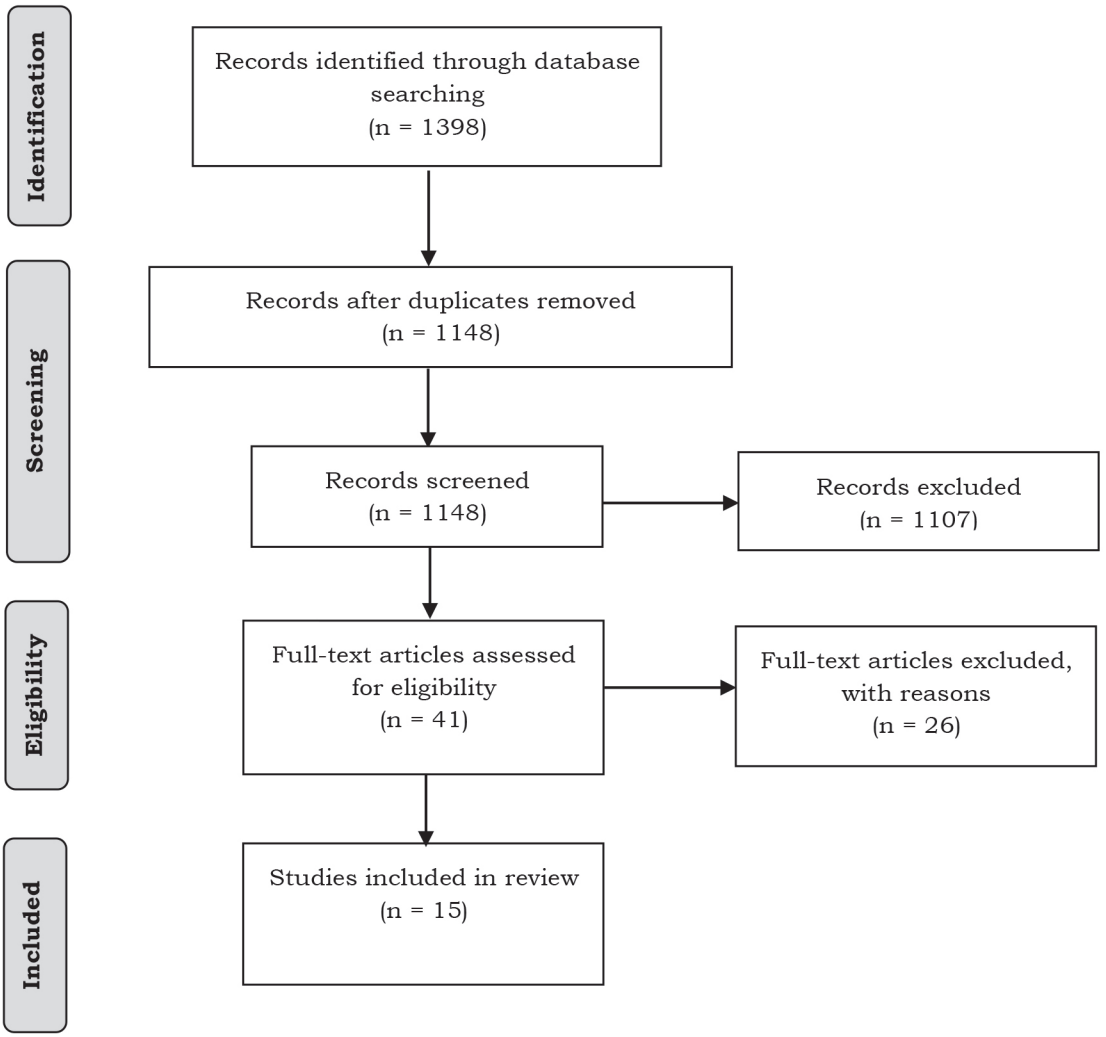

Figure 1. PRISMA flow chart illustrating the selection process of articles for this scoping review.

seven quantitative and eight qualitative types of research as presented in Table 1 and Table 2, respectively.

Table 1 indicates the summary of factors that are associated with food choices among elderly. Similar food choice factors were found repeatedly in various studies. These are health, taste, food access, body weight concern, nutritional knowledge, price, sensory appeal, and convenience. The themes that emerged from the qualitative studies are reported in Table 2 and they are food habits, social and economic circumstances, healthiness of the food and food access.

DISCUSSION

To the best of our knowledge, this is the first scoping review that has investigated the various factors that influence the food choices of the elderly, after a supporting research article that was published about 24 years ago by Herne

Table 1. Summary of factors associated with food choices among elderly (quantitative studies)

Author, Year, Origin	*Purpose of the study*	*Study characteristics*	*Outcome measurements*	*Main results*
Gunsam & Murden (2007) Mauritius	To investigate the several possible factors and their respective significance in influencing food choices and thus food intake of the elderly people in Mauritius	Design: Cross-sectional Setting: Rural and urban region around the island of Mauritius Subjects: 60 elderly from an average socioeconomic level Age: ≥58 years	Interviewer-administered food-frequency questionnaire (FFQ)	According to the order of importance: culture (93.3%) followed by taste (90.0%), routine (85.1%), nutritional knowledge (75.0%), ease of food availability (71.7%), company or peer at meal times (31.7%) and media (11.6 %) influenced food choice among elderly. Only the factor of taste influencing food choice was significantly different between the two sexes (significant value=0.02, $p<0.05$).
Ree, Riediger & Moghadasian (2008) Canada	To investigate education, income, gender, ethnicity and age affecting Canadian food choices for health-related reasons	Design: Cross-sectional Setting: Canadian community Subjects: 98733 Canadians (25338 older adults; 9580 elderly) Age range: Older adult (55-74 years) Elderly (>75 years)	Questionnaire: (1) Choose or avoid foods based on health concerns (2) Choose foods for their nutrient content (3) Avoid foods for their nutrient content Demographic characteristics	48% of older adults and 31% of the elderly had concerns with body weight. 47% of older adults and 31% of the elderly had heart disease as concern. Response of elderly was lower than older adults in choosing low fat as influencing choice on food content. 67% and 60% of older adults and elderly choose foods based on their fibre content, respectively. 70% of older adults and 59% of the elderly reported avoiding foods because of ~~for~~ their fat content.

Author, Year, Origin	*Purpose of the study*	*Study characteristics*	*Outcome measurements*	*Main results*
Dean *et al.* (2009) 8 European countries	To investigate the effects of resources and food-related goals on the variety of food choice among elderly	Design: Cross-sectional Setting: Poland, Portugal, United Kingdom, Germany, Sweden, Denmark, Italy and Spain Subjects: 3200 participants (400 from each country) and living in their own homes. Age: 65 years of age	Questionnaire: (1) 11 goals (5-point scale) (2) 22 resources (5-point scale) Weekly Food Variety Score Demographic Characteristics	There were 8 significant perceived resources that influenced variety of diet: good appetite, food knowledge, access to convenient food products, access to a good food service provider, kitchen appliances, short distance to the shops, access to high-quality products and support from friends and neighbours. There were 3 significant goal predictors on variety of diet: controlling weight, having a variety of foods on the menu, and cooking for others.
Locher *et al.* (2009) Birmingham	To investigate the motivations and perceived barriers related to food choices made by homebound older adults	Design: Observational Setting: Home health, a university-affiliated geriatric medicine outpatient clinic, a university-affiliated inpatient rehabilitation facility, area churches. Subjects: 185 homebound older adults. Mean age: 78.9 years old	The Food Choice Questionnaire Vailas Food Enjoyment Questionnaire Social demographic characteristics 24-hour dietary recalls	Key motivations to food choice: (1) Convenience (58.9%) (2) Sensory appeal (55.7%) (3) Price (47.6%) Key barriers to food choice: (1) Health (25.5%) (2) A special diet (22.2%) (3) Unable to shop for self (16.8%) Majority of subjects had insufficient total calorie intake and vitamin D

Author, Year, Origin	*Purpose of the study*	*Study characteristics*	*Outcome measurements*	*Main results*
Kim *et al.* (2013) South Korea	To determine the structural association between LOHAS, healthy food choices, trust, and emotional loyalty and the moderating role of age among elderly and non-elderly in restaurants	Design: Cross sectional. Setting: Restaurant chain that specializes in vegetable- and soybean-based dishes. Subjects: 413 diners. Age: Seniors (≥50 years of age) Non-senior (< 50 years)	All four constructs were rated on five point-Likert scale: (1) Lifestyle of health and sustainability (LOHAS) (nine items) (2) Healthy Food Choice scale (six items) (3) Trust scale (four items) (4) Emotional Loyalty (three items)	LOHAS had a significantly positive effect on the perception of healthy food choices in restaurants (β=0.320, t=5.877, p<0.001). Healthy food choices had a significant effect on trust (β=0.623, t-value=17.421, p<0.001) and emotional loyalty (β=0.220, t=3.515, p<0.001). Greater variance in healthy food choices (4.9 %) and trust (20.8 %) among the senior group than the non-senior group.
Kim (2016) South Korea	To investigate the relationship between social network type, food choice value, and diet quality in frail older adults with low socioeconomic status	Design: Cross-sectional. Setting: National Home Healthcare Services in Seoul, South Korea Subjects: 87 frail older adults Age: ≥ 65 years	The Practitioner Assessment of Network Type Instrument (PANT) The Food Choice Questionnaire (FCQ) Mean adequacy ratio (MAR)	According to the order of importance: price (3.22±0.88), sensory appeal (3.02±0.93), healthiness of food (2.90±0.95), and convenience (2.89±0.87). The private restricted and local self-contained network types were more likely to be affected by price (OR 4.28, 95% CI 1.36-13.42, p=0.013) and healthiness of food (OR 10.79, 95% CI 2.58-45.13, p<0.001) respectively.

Author, Year, Origin	*Purpose of the study*	*Study characteristics*	*Outcome measurements*	*Main results*
Appleton *et al.* (2017) France, Italy and UK	To investigate factors associated with the quantity and variety of vegetables predicted by different food choice motives consumed by older adults.	Design: Cross sectional. Setting: France, Italy and UK Subjects: 497 older adults Age: ≥ 65 years	Questionnaire: (1) Demographic characteristics. (2) Quantity of vegetable consumption (3) Regular consumption of various vegetables (4) Liking for various vegetables (5) Attitudes to food consumption.	Higher quantities of vegetables consumption was significantly associated with a higher age (β=0.16, p<0.01). Greater variety of vegetable intake was significantly associated with a higher importance in consumption given to health benefits (β=0.13, p= 0.02).

Table 2. Summary of factors associated with food choices among elderly (qualitative studies)

Author, Year, Origin	*Purpose of the study*	*Study characteristics*	*Outcome measurements*	*Main results*
Pucciarelli & Thomas (2011) Indiana	To record Muncie, Indiana residents' change in eating habits over time To investigate factors shaping the food choices made by elderly	Design: Cross-sectional. Setting: Mid-western town, Muncie, Indiana Subjects: 25 elderly who were born and lived all but 8 years in Muncie, Indiana Age: 65-100 year old	A semi-structured, questionnaire/interview script where subjects need to recall what foods/meals they consumed while: (1) living with a parent (2) after they transitioned to be the primary food processor (3) after leaving work and/or > 65 years.	Two broad factors shaping the food choices made by elderly: (1) External loci of control (economics, market availability, technology, social norms) (2) Internal loci of control (convenience, health status, ideals, life course)

Author, Year, Origin	*Purpose of the study*	*Study characteristics*	*Outcome measurements*	*Main results*
Delaney & McCarthy (2011) Ireland	To describe the crucial contextual influences on food choice patterns in older Irish adults	Design: Qualitative Setting: Southwest of Ireland Subjects: 32 older adults who participated in health screening session. Age: 61-79 years old	Semi-structured interview includes: (1) Current eating habits, attitudes, and beliefs about food. (2) Memories and perceptions about food and dietary change at different life stages.	Three main factors that influenced present food choice pattern: (1) Early food experiences (2) Changing political, economic, social, and cultural circumstances - Economic development - Food system - Knowledge and awareness (3) Changes in individual life circumstances - School, work, family, etc. - Health - Ageing
Edfors & Westergren (2012) Sweden	To explore home-living elderly people's perceptions on essential circumstances regarding food and meals	Design: Qualitative Setting: Small community in southern Sweden Subjects: 12 elderly living in their own home Age: ≥ 65 years	Semi-structured interview includes: (1) Food and meals on ordinary day (2) Food preferences and intake (3) Physiological difficulties (4) Functional difficulties (5) Social dimensions of eating	Three major categories related to views on food and meals: (1) Habits founded in past life affected present life - Food and meals - Gender roles (2) Getting help from others with food and meals - The breaking point - Transition from independence to dependence (3) Food and meals in present life - Meals during the day - Quality of food - Buying and transporting food - Cooking - Eating

Author, Year, Origin	*Purpose of the study*	*Study characteristics*	*Outcome measurements*	*Main results*
Brownie & Coutts (2013) Australia	To explore views and practices about what composes a healthy diet for Australians' older people	Design: Qualitative (Focus group) Setting: Northern NSW, Australia Subjects: 29 independently- living retirees Age: 60-93 years old	Focus group questions: (1) What are you doing to achieve a healthy diet? (2) Do you think that as people get older their dietary requirements change?	Four themes, viz.: (1) Healthy foods - Fruit and vegetables important factors of healthy diet. (2) Quantity - Eating less and making different food choices were favourable to health in elderly. (3) Personal circumstances - Social situation may have constrained elderly to adopt a healthy diet and food choices. (4) Good intention - Desire of elderly to preserve wellbeing and health was a significant determinant of food choices.
Kamphuis, de Bekker-Grob & Van Lenthe (2015) Netherlands	To investigate the relative importance of health considerations for food choices compared with other motives To investigate differences in preference structures between low and high socioeconomic groups	Design: Cohort Setting: Southeastern region of The Netherlands Subjects: 399 older adults (a subsample from GLOBE study) Mean age: 63.3 years old	A discrete choice experiment (DCE) is used to develop 24 choice sets about a usual dinner at home and subjects need to choose the meals alternative that appealed most to them Background characteristics (age, sex, educational level and household income)	In order of importance: healthiness followed by taste, price and travel time to the grocery store all significantly influenced older adults' meal decisions. There were significant interactions among education and healthiness (+) and education with price (-). There was positive association between income with both healthiness and very good taste.

Author, Year, Origin	*Purpose of the study*	*Study characteristics*	*Outcome measurements*	*Main results*
Host *et al.* (2016) Australia	To identify major factors that influences the food choice and dietary behaviours amongst healthy, independent-living older Australians	Design: Qualitative (focus group) Setting: Three low-care Illawarra Retirement Trust (IRT) lifestyle residential facilities Subjects: 18 independently living residents and in good health Age: ≥ 60 years	A semi-structured focus group was required to discuss a set of 12 questions that includes Components of Bandura's Social Cognitive Theory	Three broad themes that influenced the food choice and dietary behaviours: (1) Adaptation - Variability of life circumstances - Management of physiological change (2) Psychosocial parameters - Maintenance of independence - Sense of community - Interest in and understanding of health and nutrition - Preferences, aversions and beliefs (3) Food landscape - Price - Quality - Country of origin - Store attributes (accessibility and service)
Shanks *et al.* (2016) Montana	To investigate how the rural food environment affects food choices of older adults	Design: Qualitative (focus group) Setting: Rural Montana communities with several senior centres Subjects: 33 older adults residing in rural Montana community Age: 50 years or older	Brief socio demographic survey. Semi-structured focus group questions include elements of food choices: (1) community (2) food preferences (3) budgeting (4) food availability (5) food community public programmes	Four major themes with 12 sub-themes influenced food choices among rural older adults: (1) Perception of the rural community environment (2) Community support (3) Personal food access (4) Dietary factors influencing food consumption

Author, Year, Origin	*Purpose of the study*	*Study characteristics*	*Outcome measurements*	*Main results*
Oemichen & Smith (2016) Minnesota	To investigate food choice, food access, and food insecurity among elderly	Design: Qualitative (focus group) Setting: Minnesota community centre Subjects: 62 elderly who had the ability to shop Age: ≥ 60 years old	Open-ended questions focus group: (1) food choices (2) shopping strategies (3) food access points (4) food security issues Socio-demographic characteristics Anthropometric measurements (weight, height and BMI)	Five major themes identified: (1) Eating behaviour affected by former experiences (2) Financial and food security driving use of food assistance programmes (3) Food access strategies: restaurants, retail markets and alternative sources (4) Food access and intake influenced by physical changes associated with aging (5) Social impact as an aspect in decision making

(1995). A small number of research studies were conducted on elements affecting food choices in the elderly, and only 15 relevant studies that were published between 2007 and 2017 were retrieved. Therefore, this review is a key step in conveying to healthcare professionals, future researchers, policymakers and the food industry itself, the impact of food choices among the elderly in different settings.

This scoping review should contribute to the small but growing body of literature predominantly from Europe followed by North America, Asia, Australia and Africa, that examines food choices among the elderly. The majority of the studies (9 of 15) identified health as the most important factor that determined the food choices among the elderly. Ree *et al.* (2008) revealed that the health-conscious group was mainly middle-aged and older adults who tended to select food wisely for different reasons related to health such as disease prevention, disease management, or maintaining physical independence. It has been observed that the elderly readily modify their food habits and are willing to fight the urge of eating their favourite foods as instructed by their doctors (Shanks *et al.*, 2016). This has been categorised as internal indicators that shape the food choice of older people (Pucciarelli & Thomas, 2011). The lowering of fat and cholesterol intake with healthier food choices in order to meet health goals were among the changes specifically made by the elderly (Delaney & McCarthy, 2011; Pucciarelli & Thomas, 2011; Host *et al.,* 2016). A significant association was found between vegetable intake of different varieties and the higher importance given to health benefits (Appleton *et al.*, 2017) as the elderly assumed that fruits and vegetables were important parts of a healthy diet (Brownie & Coutts, 2013).

A study by Kim (2016) showed that persons who did not have local kinfolk, few nearby friends, low levels of community contacts and infrequent contact with at least one relative, and the frail elderly were, surprisingly, concerned about the healthiness of food. An earlier study conducted by Kim *et al.* (2013) showed the strong impact of the lifestyle of health and sustainability (LOHAS) on healthy food choices within a group of seniors. This should trigger health-oriented marketers to treat the elderly as a separate market segment.

In contrast, homebound elderly adults chose health as the major perceived barrier to food intake as per participants' meal preference (Locher *et al.,* 2009). Gender also played a role as women were seen to be more likely to select or discard foods due to health reasons and nutrient content (Ree *et al.,* 2008). Highly educated and higher income older adults rated a healthy meal to be a more important consideration in making meal decisions (Kamphuis, de Bekker-Grob & Van Lenthe, 2015).

Convenience was the second most common consideration that determined food choices for older people. Locher *et al.* (2009) reported that convenience was the most important perceived motivation related to food selection whilst it was the fourth consideration in the report by Kim (2016) after the price, sensory appeal, and healthiness of food. Convenience meant the ease of food preparation (Pucciarelli & Thomas, 2011), and convenient transport. A short distance to go to the shops provided more varied dietary choices (Dean *et al.*, 2009).

Most of the studies that we examined reported similar limitations. The first limitation was the small sample size (Locher *et al.,* 2009; Kim, 2016; Appleton *et al.*, 2017; Delaney & McCarthy, 2011; Edfors & Westergren, 2012; Host *et al.*, 2016; Shanks *et al.*, 2016). Thus, future studies with a larger sample sizes will benefit the complexity of food choices

in the elderly population. Secondly, the dependence on self-reporting inevitably results in inaccuracies and biases (Locher *et al.,* 2009; Kim, 2016; Appleton *et al.*, 2017; Pucciarelli & Thomas, 2011; Host *et al.*, 2016). There were also unmeasured confounding factors that might have had an effect on the study results of Kim (2016) and Kamphuis *et al.* (2015). Lastly, as the studies in this review were focused only on homebound older adults (Locher *et al.,* 2009), Korean seniors in restaurants (Kim *et al.*, 2013), frail older adults (Kim, 2016), seniors from Midwestern town (Pucciarelli & Thomas, 2011), elderly in rural and urban environment (Delaney & McCarthy, 2011) and older adults from a senior centre (Host *et al.*, 2016), extrapolation of findings to other ethnic groups and geographical areas may not always be valid. Further cross-cultural studies are necessary to apply findings in groups across geographical borders.

Nevertheless, the findings and conclusions of this review have vital relevance to clinical practice and implementation in the area of nutrition. Health promotion interventions and policies with a multifactorial approach that are aimed to promote a healthy diet, food modifications and eating behaviour (Locher *et al.,* 2009) of the elderly should take into account the potential influence of health, interpersonal and social issues to them (Gunsam & Murden, 2007). Explicit strategies on awareness, health messages (Delaney & McCarthy, 2011), affordable and easy meal preparation will have a greater chance to be put into practice by the elderly (Pucciarelli & Thomas, 2011) to prevent and manage chronic diseases. Furthermore, the involvement of caregivers in medical nutrition therapy for the elderly (Locher *et al.,* 2009) and raising the awareness of age-adjusted nutrient targets through media campaigns (Brownie & Coutts, 2013) may help to change the behaviour of the elderly to make them realise the importance of food choices. Health professionals need to be aware of existing information regarding social network of elderly with local family and/or friends and neighbours. This may help in deciding suitable interventions to develop healthy food choice values especially among the elderly with few community contacts (Kim, 2016).

CONCLUSION

This review of seven quantitative and eight qualitative studies conducted in different continents has given new insights on food choices among the elderly. The health domain was the most commonly reported factor that influenced food choices besides convenience, price, sensory appeal, among others. Small sample size, reliance on self-reporting and the inability to make generalisations, were the most important limitations of this review. New strategies for intervention programs should be undertaken by a joint force of health professionals, researchers, policymakers and the food industry. Future research in the elderly who have developed a particular chronic disease, and those who are independent or with a disability is needed.

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Authors' contributions

FIMS, designed and conceptualised the study and search strategy, undertook the analyses and drafted and edited the manuscript; NO, advised on the analysis, description and classifying the study and reviewed the manuscript; ZAMD, and co-supervisors reviewed the manuscript; NFZ and co-supervisors reviewed the manuscript.

Conflict of interest

The authors declare that they have no competing interests.

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Overweight and obesity among Orang Asli adults in Krau Wildlife Reserve, Pahang: a four-year follow-up study

Chua Ee Yin, Zalilah Mohd Shariff*, Norhasmah Sulaiman & Geeta Appannah

Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia

ABSTRACT

Introduction: Obesity and excess weight gain in adults are linked to an increased risk of cardiometabolic abnormalities. The changing lifestyle experienced by the Orang Asli predisposes the population to the risk of obesity and non-communicable diseases. This study aimed to describe the prevalence of overweight and obesity as well as body-weight change over a period of four years among Orang Asli adults. **Methods:** Data were collected from Orang Asli adults aged ≥18 years, who were enrolled in the 2011-2012 and 2015-2016 surveys, and who were residing within the Krau Wildlife Reserve. Weight and height of the adults ($N_{2011\text{-}2012}$=828; $N_{2015\text{-}2016}$=662) were measured at both time points. Follow-up data were available for 378 adults (male:113; female:265). **Results:** The prevalence of overweight and obesity were 18.8% and 7.4% in 2011-2012 and 26.1% and 9.5% in 2015-2016. In the follow-up group, significant differences in body weight and body mass index (BMI) were observed in men and women, respectively. More than one-third (35.5%) of the adults had weight gain of more than 5.0%. The increasing percentage of body weight change was associated with being female, younger age, more years of schooling and reduced household income. **Conclusion:** Obesity is a growing health problem in the Orang Asli adult population. Weight gain was associated with socioeconomic indicators and it was more prominent in women. Effective strategies are needed to address the increasing prevalence of overweight and obesity in this population to further reduce adverse health outcomes.

Keywords: Overweight and obesity, weight gain, Orang Asli adults

INTRODUCTION

The global age-standardised mean body mass index (BMI) increased between 1975 and 2014 in both men (from 21.7 to 24.2) and women (from 22.1 to 24.4) (NCD-RisC, 2016). Over these four decades, the age-standardised prevalence of obesity increased from 3.2% to 10.8% in men and from 6.4% to 14.9% in women. Meanwhile, age-standardised global prevalence of underweight decreased from 13.8% to 8.8% in men and from 14.6% to 9.7% in women. Obesity is a global health problem. Likewise, the 2015 Global Burden of Disease study (GBD 2015 Obesity Collaborators, 2017) reported a rapid rise in the prevalence and disease burden of elevated BMI.

*Corresponding author: Prof. Dr. Zalilah Mohd Shariff
Professor (Community Nutrition), Department of Nutrition and Dietetics
Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang Selangor, Malaysia
Tel: 603 89472472; Fax: 603 89426769; Email: zalilahms@upm.edu.my
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Weight gain had a negative impact on cardiometabolic risk factors in metabolically healthy (MH) adults, irrespective of their weight status (Cui *et al.*, 2015). It is associated with increased systolic and diastolic blood pressure, triglycerides and blood glucose in both obese and normal weight adults. A cohort study showed that MH obese and normal weight adults with weight gain of ≥10.0% and ≥15.0% of body weight, respectively, were more likely to have developed metabolic complications compared to those who did not gain weight (Espinosa De Ycaza, Donegan & Jensen, 2018).

Previous studies have highlighted the double burden of malnutrition in Orang Asli population (Wong *et al.*, 2015; Geik, Sedek & Awang, 2016; Siti Fatihah *et al.*, 2018). Although undernutrition is still prevalent in children, overweight and obesity are increasing in adults. Similar to the non-indigenous population in Malaysia, the changing disease patterns are towards non-communicable diseases in the adult Orang Asli population (Phipps *et al.*, 2015; Chua *et al.*, 2017). This may reflect the changes in body weight status driven by changes in their dietary and lifestyle behaviours.

Monitoring the change in body weight of indigenous peoples, particularly adults, is a challenge as the population is often mobile and difficult-to-reach. This study aimed to assess body weight status of Orang Asli adults, specifically their BMI and change in body weight, over a period of four years. The associations between percentage change in body weight and socioeconomic variables were also examined.

MATERIALS AND METHODS

During the period 2011-2012, a survey was conducted among Orang Asli of the Jah Hut sub-tribe who were living seven villages within the Krau Wildlife Reserve in Peninsular Malaysia. Out of 467 households within the study location, 465 households were visited and 1368 adults aged ≥18 years were identified based on interviews with household heads. Among these adults, 34 pregnant women and 12 bedridden adults were excluded from the study. A total of 914 adults were located and approached but only 828 adults gave consent to participate. All the eligible adults were measured for weight and height and interviewed on a range of sociodemographic characteristics.

In 2015-2016, another survey was conducted in the same seven Orang Asli villages and 446 out of 458 households were visited. Based on household interviews, there were 1,596 adults aged ≥18 years who were available within the study location. A total of 32 adults, comprising 18 pregnant women and 14 bedridden adults, were excluded from the study. Out of 1,564 adults, 986 were identified and invited to participate in the study. Of the 662 adults who agreed to participate and completed the interview and anthropometric measurements, 378 adults were identified as the cohort of 2011-2012 survey and 284 adults were newly recruited participants. Reasons for attrition included refusal, death of respondents and households that had moved from the location.

The BMI of each participant was derived from weight and height measurements. In the follow-up group, the change in body weight (kg) and percentage (%) body weight change were calculated. The latter was categorised into five groups: (i) weight loss (weight loss >5.0%), (ii) stable weight (weight loss or gain ≤5.0%), (iii) slight weight gain (weight gain >5.0% and up to 10.0%), (iv) moderate weight gain (weight gain >10.0% and up to 20.0%) and (v) excess weight gain (weight gain >20.0%) (Yiengprugsawan *et al.*, 2017).

Statistical analyses were performed using IBM SPSS Statistics 21.0 (IBM

Corporation, New York, USA). All variables are presented as mean, standard deviation, and frequency. Paired-*t* test assessed the differences in anthropometric measures of the follow-up group between the year 2011-2012 and 2015-2016. Pearson's correlation and Chi-square tests were used to assess the associations of continuous and categorical variables, respectively. The level of significance was set at $p<0.05$.

Ethical approval

The Medical Research Ethics Committee of the Faculty of Medicine and Health Sciences, Universiti Putra Malaysia approved the study protocol. Permission to conduct this study was obtained from the Malaysia Department of Orang Asli Development (JAKOA) and the Department of Wildlife and National Parks of Peninsular Malaysia (PERHILITAN).

RESULTS

More than one-fourth (26.2%) and one-third (35.6%) of Orang Asli adults were either overweight or obese, in 2011-2012 and 2015-2016, respectively (Table 1). The proportion of overweight and obesity was higher in women than men at each time point. About 10.0% of the study population (11.1% in 2011-2012 and 9.4% in 2015-2016) were underweight. Between 2011-2012 and 2015-2016, the mean body weight (kg) increased slightly for men (from 59.3 to 61.1) and women (from 51.4 to 52.8). Corresponding to these changes, the mean BMI increased from 23.2 to 23.9 in men and from 23.3 to 23.8 in women. The changes in body weight and BMI in both men and women were statistically significant.

All the obese men were found to remain in the same BMI category over the period of four years. Meanwhile, changes across the BMI distribution were revealed among 25.7% of the follow-up group, where 18% of them had an increase in BMI that shifted them to a higher BMI category and the other 7.7% adults moved to a lower BMI category due to weight loss. There were 10.1% and 4.7% adults who developed overweight and obesity between the 2011-2012 and 2015-2016 surveys, respectively. The 4-year-incidence of overweight was higher in men, whereas women had a higher incidence of obesity.

About 50.0% of the adults in the follow-up group maintained a stable weight (weight loss or gain ≤5.0%), while 14.5% of adults lost >5.0% of their baseline weight, and 35.5% had a weight gain of >5.0% (Table 2). Weight gain was more apparent in women, adults in younger age group, those with higher educational attainment and reduced household income. The proportion of individuals with weight gain of >5.0% in women, adults aged 18-28 years old and adults with secondary/tertiary education were 38.1%, 48.8% and 45.2%, respectively. Across the baseline BMI categories, the proportion of adults with weight gain of >5.0% was highest in the category of normal weight, followed by those who were overweight.

DISCUSSION

The present study found that overweight and obesity are important health problems prevalent among the Orang Asli adults living in this forest reserve area, as more than one-third were either overweight or obese. The study also showed that over the period of the follow-up, about 15.0% of these Orang Asli adults developed overweight and obesity. An earlier study of 16-35-year-old multi-ethnic persons in Malaysia showed that the Orang Asli had the highest prevalence of overweight and obesity (Pell *et al.*, 2016). Our study has shown that the problem of overweight

Table 1. Anthropometric measurements and body weight status of respondents in 2011-2012 and 2015-2016

	2011-2012[†]			*2015-2016*[‡]		
	Men	*Women*	*Overall*	*Men*	*Women*	*Overall*
Overall, n	*359*	*469*	*828*	*228*	*434*	*662*
Weight (kg), Mean±SD	59.0±11.8	50.8±12.1	54.4±12.6	60.8±11.9	53.1±12.1	55.8±12.6
Height (cm), Mean±SD	160.0±6.1	148.1±5.7	153.3±8.3	159.9±6.2	149.1±5.7	152.8±7.8
Body Mass Index (BMI), Mean±SD	23.0±4.1	23.1±4.9	23.0±4.6	23.7±3.9	23.8±4.7	23.7±4.4
Underweight (<18.5), n (%)	26 (7.2)	65 (13.9)	91 (11.0)	15 (6.6)	47 (10.8)	62 (9.4)
Normal (18.5-24.9), n (%)	246 (68.5)	274 (58.4)	520 (62.8)	137 (60.1)	227 (52.3)	364 (55.0)
Overweight (25.0-29.9), n (%)	64 (17.8)	92 (19.6)	156 (18.8)	54 (23.7)	119 (27.4)	173 (26.1)
Obese (≥30.0), n (%)	23 (6.4)	38 (8.1)	61 (7.4)	22 (9.6)	41 (9.4)	63 (9.5)
Follow-up group, n	*113*	*265*	*378*	*113*	*265*	*378*
Weight (kg), Mean±SD	59.3±11.6 [a]	51.4±12.0 [b]	53.8±12.4	61.1±12.3 [a]	52.8±12.5 [b]	55.2±13.0
Change in body weight (kg)	N/A	N/A	N/A	1.5±5.1	1.4±6.4	1.4±6.0
% Change in body weight	N/A	N/A	N/A	2.5±8.7	3.2±11.4	3.0±10.7
Height (cm), Mean±SD	159.7±6.3	148.3±5.8	151.6±8.0	159.8±6.2	148.5±5.3	151.8±7.5
Body Mass Index (BMI), Mean±SD	23.2±3.7 [a]	23.3±4.8 [b]	23.3±4.5	23.9±4.0 [a]	23.8±4.9 [b]	23.8±4.7
Underweight (<18.5), n (%)	7 (6.2)	29 (10.9)	36 (9.5)	7 (6.2)	35 (13.2)	42 (11.1)
Normal (18.5-24.9), n (%)	78 (69.0)	158 (59.6)	236 (62.4)	66 (58.4)	136 (51.3)	202 (53.4)
Overweight (25.0-29.9), n (%)	21 (18.6)	59 (22.3)	80 (21.2)	28 (24.8)	64 (24.2)	92 (24.3)
Obese (≥30.0), n (%)	7 (6.2)	19 (7.2)	26 (6.9)	12 (9.2)	30 (11.3)	42 (11.1)

	2011-2012†			2015-2016‡		
	Men	*Women*	*Overall*	*Men*	*Women*	*Overall*
Change in BMI category, n (%)						
Remained in the same state	N/A	N/A	N/A	84 (74.3)	197 (74.3)	281 (74.3)
underweight				3 (2.7)	21 (7.9)	24 (6.3)
normal				60 (53.1)	120 (45.3)	180 (47.7)
overweight				14 (12.3)	39 (14.7)	53 (14.0)
obesity				7 (6.2)	17 (6.4)	24 (6.3)
Shift to higher BMI category	N/A	N/A	N/A	22 (19.5)	46 (17.4)	68 (18.0)
underweight to normal				4 (3.5)	8 (3.0)	12 (3.2)
normal to overweight				14 (12.5)	24 (9.1)	38 (10.1)
normal to obesity				-	2 (0.8)	2 (0.5)
overweight to obesity				4(3.5)	12 (4.5)	16 (4.2)
Shift to lower BMI category	N/A	N/A	N/A	7 (6.2)	22 (8.3)	29 (7.7)
obesity to overweight				-	1 (0.4)	1 (0.3)
obesity to normal				-	1 (0.4)	1 (0.3)
overweight to normal				2 (1.8)	7 (2.6)	9 (2.4)
overweight to underweight				1 (0.9)	1 (0.4)	2 (0.5)
normal to underweight				4 (3.5)	12 (4.5)	16 (4.2)

† A total of 914 adults were surveyed and 828 of them gave consent to participate (90.6% participation rate).
‡ The follow-up adults were 378 (45.7% follow-up rate), an additional 284 adults agreed to be recruited. The reasons for attrition included refusal, non-contact, deceased respondents and moved households.
[a, b] Significant differences in body weight and BMI between 2011-2012 and 2015-2016 in men and women, respectively ($p<0.01$)
N/A: Not applicable

Table 2. Distribution of percent of body weight change in follow-up group

Characteristics	*Percent of body weight change, n (%)*					
	> -5.0 %	*- 5.0% to +5.0%*	*> +5.0% to +10.0%*	*> +10.0 % to +20.0%*	*> +20.0%*	*Correlation, r*[†]
Overall (*N*=378)	55 (14.5)	189 (50.0)	63 (16.7)	52 (13.8)	19 (5.0)	
Sex						
Men (*n*=113)	10 (8.8)	70 (61.9)	20 (17.7)	9 (8.0)	4 (3.5)	$\chi^{2‡}$=12.94*
Women (*n*=265)	45 (17.0)	119 (44.9)	43 (16.2)	43 (16.2)	15 (5.7)	
Baseline age group						
18-28 years (*n*=123)	12 (9.7)	51 (41.5)	30 (24.4)	22 (17.9)	8 (6.5)	-0.13*
29-39 years (*n*=127)	22 (17.3)	68 (53.5)	16 (12.6)	15 (11.8)	6 (4.7)	
≥40 years (*n*=128)	21 (16.4)	70 (54.7)	17 (13.3)	15 (11.7)	5 (3.9)	
Education attainment						
No formal education (*n*=154)	26 (16.9)	82 (53.2)	22 (14.3)	18 (11.7)	6 (3.9)	
Primary education (*n*=140)	19 (13.6)	71 (50.7)	27 (19.3)	16 (11.4)	7 (5.0)	0.15**
Secondary/Tertiary education (*n*=84)	10 (11.9)	36 (42.9)	14 (16.7)	18 (21.4)	6 (7.1)	
Changes in household income						
Decrease (*n*=185)	22 (11.9)	92 (49.7)	30 (16.2)	32 (17.3)	9 (4.9)	
Constant (*n*=16)	3 (18.8)	6 (37.5)	5 (31.3)	2 (12.5)	-	-0.11*
Increase (*n*=177)	30 (16.9)	91 (51.4)	28 (15.8)	18 (10.2)	10 (5.6)	
Changes in income per capita						
Decrease (*n*=188)	25 (13.3)	97 (51.6)	30 (16.0)	28 (14.9)	8 (4.3)	
Constant (*n*=8)	1 (12.5)	2 (25.0)	2 (25.0)	3 (37.5)	-	-0.03
Increase (*n*=182)	29 (15.9)	90 (49.5)	31 (17.0)	21 (11.5)	11 (6.0)	
Baseline BMI category[§]						
Underweight (*n*=36)	5 (13.9)	21 (58.3)	2 (5.6)	3 (8.3)	5 (13.9)	
Normal (*n*=236)	31 (13.1)	114 (48.3)	44 (18.6)	35 (14.8)	12 (5.1)	$\chi^{2‡}$=4.14
Overweight (*n*=80)	14 (17.5)	41 (51.3)	13 (16.3)	10 (12.5)	2 (2.5)	
Obese (*n*=26)	5 (19.2)	13 (50.0)	4 (15.4)	4 (15.4)	-	

[†]Correlation between changes in % of body weight change and (i) age (years), (ii) years of education, (iii) changes in household income (RM) and (iv) changes in income per capita (RM/person)

[‡]Association between categories of % of body weight change and (i) sex, (ii) BMI categories (underweight/normal vs overweight/obese)

[§]Underweight (BMI <18.50 kg/m^2); Normal (BMI 18.50–24.99 kg/m^2); Overweight (BMI 25.00–29. 99 kg/m^2); Obese (BMI ≥30 kg/m^2)

*p<0.05, **p<0.01

and obesity is growing in the Orang Asli population. It is a health issues that should be addressed.

The higher prevalence of overweight (>30.0%) and obesity (20.0%-45.0%) has been observed in other indigenous groups in developed countries (Ng, Corey & Young, 2011; Hopkins *et al.*, 2015; Thurber *et al.*, 2018) and other developing countries such as Brazil (Oliveira *et al.*, 2015). Indigenous obesity rates vary geographically. The lowest rates of overweight and obesity were found among those living in very remote areas (Australian Institute of Health and Welfare, 2014). This is probably because indigenous peoples living in such areas are more likely to consume traditional foods that are relatively high in nutrients, as reported by previous studies (Ghosh-Jerath *et al.*, 2016; Ferguson *et al.*, 2017). In Peninsular Malaysia, the proportion of Orang Asli living in urban areas has increased. The average percentage of Orang Asli in the urban areas has increased from 1.6% in 1970 to >10.0% since year 2000 (Tuan Pah Rokiah, Devamany & Asan, 2017). It is unclear whether the change in living environment (e.g. lack of access to forest and close proximity to markets) has led to a decrease in traditional food intake. Assessing the dependency on traditional and market foods over time of Orang Asli living in different locations may help to explain the change in overweight and obesity prevalence in this population.

The present study did not examine the impact of environmental factors on weight gain. However, the findings of this study indicated that sociodemographic factors may influence weight gain through their effects on energy intake and energy expenditure. Previous studies have indicated that adults from the lower socioeconomic strata had an increased risk of weight gain (Loman *et al.*, 2013; Bhurosy & Jeewon, 2014; Herzog *et al.*, 2016). Findings of this study showed that income was negatively associated with weight gain among Orang Asli adults living in similar neighbourhoods. A majority of Orang Asli adults in this reserve area were rubber tappers (data not shown). Rather than the reliance on subsistence farming, they were more likely to purchase food from the market. Hence, income could greatly determine the food choices of the adults in the present study. When household income decreases and family budget for food shrinks, food choice tends to shift toward cheaper but more energy-dense foods. It could eventually result in excess calorie intake and weight gain. As shown in this study, Orang Asli adults with lower household income, had a greater percentage of weight gain. An earlier study among Orang Asli women also found that household income was negatively associated with household food security, while household food insecurity was significantly associated with a poorer diet quality and higher BMI (Chong, Geeta & Norhasmah, 2018).

The present study showed that Orang Asli individuals with higher educational attainment had a greater percentage of weight gain. For individuals with lower education, they are more likely to assume highly demanding physical jobs as compared to their counterparts with higher education. Orang Asli adults, who were with higher education, were less likely to perform labour-intensive work and more likely to have less energy expenditure. Hence, they were at greater risk of weight gain. This study showed that younger aged Orang Asli had a greater weight gain, where about one-fourth of adults in the youngest cohort gained >10.0% of weight in a period of four years. It was found that younger aged Orang Asli adults had a lower physical activity as compared to those with older age (data not shown). For example, active transport such as walking was lower among the younger

Orang Asli adults. It has been reported that BMI increases sharply during the transition from adolescence to young adulthood (Lee *et al.*, 2011) and weight gain during early to middle adulthood was significantly associated with an increased risk of chronic diseases (Zheng *et al.*, 2017). To reduce the cost and disease burden associated with obesity, the prevention of overweight obesity in young adults should be prioritized (Dietz, 2017).

This study had some limitations. Some of the Orang Asli adults were highly mobile and efforts were made to address the problem. These included repeated household visits to track the participants as well as working with the community to explore how participants are commonly identified. Another limitation was that there are socioeconomic status indicators identified in the literature such as social class and wealth/assets that were not included in the present study. As with any kind of the self-reported data such as that on income, there is a risk of recall bias.

Despite these limitations, the strength of this study is the reporting of overweight and obesity prevalence at different time points and the changes in weight and BMI among Orang Asli adults over time. It is useful to increase the pool of available health-related information in Orang Asli population and to draw a greater attention to the increased risk of overweight and obesity in this population and its known attendant health risks.

CONCLUSION

This is one of the few studies that has reported the rising prevalence of overweight and obesity as well as the change in body weight in Orang Asli adults. Future studies that investigate the associations between the change in body weight and adverse health effects in Orang Asli population are warranted. Health programmes on the prevention of excess weight gain, particular in younger and normal weight adults who appear to have greater weight gain, are needed. Furthermore, overweight and obese Orang Asli adults should also be monitored closely for the risk factors of chronic diseases and their complications.

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Authors' contributions

CEY, conducted the study, undertook data analysis and interpretation, and prepared the draft of the manuscript; ZMS, participated in the conceptualisation of the study, its design, data interpretation, manuscript preparation and finalisation; NS and GA, reviewed the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Overweight and obesity in patients with cancer: study in Dharmais National Cancer Hospital, Jakarta

Noorwati Sutandyo[1*] & Ririn Hariani[2]

[1]*Department of Hematology and Clinical Oncology, Dharmais National Cancer Centre Hospital Indonesia;* [2]*Department of Clinical Nutrition, Dharmais National Cancer Centre Hospital Indonesia*

Abstract

Introduction: Overweight and obesity are considered risk factors for several solid and blood cancers. However, body mass index (BMI) is rarely assessed in newly diagnosed patients with cancer. This study aimed to evaluate BMI and its associated factors in patients with cancer. **Methods:** This cross-sectional study enrolled newly diagnosed cancer patients over the period January 2015–December 2017 at the Dharmais National Cancer Hospital, Jakarta. Demographic and clinical data were retrieved from the medical records. BMI was calculated for each patient. Comorbidity was evaluated using the Charlson Comorbidity Index. **Results:** In total, 696 newly diagnosed cancer patients were enrolled, with women in predominance (66.2%). The mean patient age was 54.0±12.8 years. Most patients (90.7%) had solid tumours; breast and lung cancers were the most common diagnosis. Among haematological malignancies, lymphoma was the most common (55.4%). Overweight or obesity, noted in 309 (44.4%) patients, was significantly associated with age, sex (women) and haematological malignancies. No association between disease stage and BMI was observed. Among patients with solid tumours, age and sex (women) was associated with overweight and obesity. **Conclusion:** The prevalence of overweight and obesity in newly diagnosed cancer patients was 44.4%. The proportion of this association was more prominent in women and in those with haematological malignancies. Among solid tumours, age and the female sex demonstrated the strongest association with overweight and obesity. Additional studies to assess whether certain dietary patterns and physical activity levels are risk factors for cancer are warranted.

Keywords: Body mass index, cancer, obesity, overweight

INTRODUCTION

Globally, 14.1 million new cases of cancer were reported in GLOBOCAN 2012 (Torre *et al.*, 2012) of which 57% of cases were found in the less developed countries. Data from *Riset Kesehatan Dasar Indonesia* (Kemkes RI, 2013) showed that the national prevalence of cancer was 1.4 per 1000 or approximately 347,792 persons. Cancer ranks third among the non-communicable diseases in Indonesia, after asthma and chronic obstructive pulmonary disease. Wolin, Carson & Colditz (2010) found that

*Corresponding author: Noorwati Sutandyo, MD
Dharmais National Cancer Centre Hospital, Jl. Let. Jend. S. Parman Kav. 84-86,
Slipi, Palmerah, RT.4/RW.9, Kota Bambu Selatan, Palmerah,
Kota Jakarta Barat, Daerah Khusus Ibukota Jakarta, 11420
Tel. No. of Institution: +6221 5681570; Fax No. of Institution: +6221 56969561
Email address: noorwatisoetandyo@gmail.com
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several cancers were associated with obesity, such as endometrial cancer, oesophageal adenocarcinoma, colorectal cancer, breast cancer in postmenopausal women, prostate cancer, and renal cancer. Less common types of cancer that were associated with obesity were melanoma, thyroid cancer, leukaemia, non-Hodgkin lymphoma and multiple myeloma (Lichtman, 2010).

A study by Drake *et al.* (2017) found that high body mass index (BMI) could increase overall cancer incidence, obesity-related cancer incidence, and cancer-related mortality incidence by 3%, 7%, and 5%, respectively. However, another study by Denis & Palmer (2017) showed that people with high BMI but low inflammation and undisturbed metabolism can have a lower risk of obesity-associated cancer compared with normal weight or slightly overweight (per BMI) individuals with inflammation and metabolic abnormalities.

Until recently, data on the nutritional status of patients with cancer in Indonesia and its associated factors were unavailable. Calculating BMI is an inexpensive and concise method that is used to assess nutritional status in patients. This study assessed the BMI status of patients with cancer at the Dharmais National Cancer Hospital, Jakarta, Indonesia, by using BMI and its associated factors.

MATERIALS AND METHODS

This was a cross-sectional study undertaken at the Dharmais National Cancer Hospital. It was reviewed and approved by Ethical Committee of the Hospital and given the approval number Indonesia No. 014/KEPK/I/2018. The patients enrolled in this study were newly diagnosed with cancer between January 2015 and December 2017. The inclusion criteria were age ≥18 years, histologically confirmed malignancy from tissue or bone marrow specimens with available data on initial BMI at the time of diagnosis. Demographic and clinical data were retrieved from the medical records of the patient. There were no exclusion criteria. Clinical data that was extracted consisted of disease stage, histopathology type, comorbidity, and BMI which was calculated by dividing body weight (kg) by the square of body height (m^2). Based on the recommended BMI criteria for the Asian-Pacific region by World Health Organization (WHO), BMI was categorised as underweight (<18.5 kg/m^2), normal (18.5-22.9 kg/m^2), overweight (23.0-24.9 kg/m^2), or obesity (≥25 kg/m^2). The Charlson Comorbidity Index (CCI) was used to assess comorbidity levels.

Descriptive statistics are presented as means and standard deviations for normally distributed variables and as medians and ranges if distribution was skewed. Categorical data are presented as frequency and percentage. Differences in frequencies of variables between groups were analysed using Chi-square or Kolmogorov-Smirnov tests. Differences of means were analysed using the Mann-Whitney U test for skewed data. Associations between variables were considered significant if the *p*-value was <0.05. Data analyses were performed using STATA (version 15.0; STATA Corporation, Texas, USA). Variables with $p<0.25$ in bivariate analysis were included in multivariate analysis.

RESULTS

During the study period, 696 patients were newly diagnosed with cancer, with women showing predominance at 66.2% of the total. The median of age of the patients was 55.0±18.0 years. Most of the cancers (90.7%) were solid tumours, and, breast and lung cancers were the most common diagnoses. Lymphoma

was the most common malignancy (55.4%) among haematological cancers. Among the patients, 71.3% were diagnosed at stage III or IV. Patients with normal BMI were in majority, followed by those classified with obesity (Table 1).

Table 1. Patient characteristics (n = 696)

Variable	*n (%)*
Age group	
<60 years	433 (62.2)
≥60 years	263 (37.8)
Sex	
Women	461 (66.2)
Men	235 (33.8)
Cancer group	
Solid tumors	631 (90.7)
Hematological malignancies	65 (9.3)
Cancer stage	
I	32 (4.6)
II	139 (20.0)
III	251 (36.1)
IV	209 (30.0)
Leukemia (no staging)	27 (3.9)
Unknown	38 (5.5)
BMI	
Underweight (<18.5 kg/m^2)	80 (11.5)
Normal (18.5-22.9 kg/m^2)	307 (44.1)
Overweight (23.0-24.9 kg/m^2)	98 (14.1)
Obese (≥25 kg/m^2)	211 (30.3)
Comorbidity Index (n=540)	
Mild-moderate (CCI <5)	288 (53.3)
Severe (CCI ≥5)	252 (36.2)

Overweight or obesity was found in 309 (44.4%) patients and were significantly associated with the sex and the cancer type. Overweight and obesity were more common in women than in men, haematological malignancies, patients aged >60 years, and those with a CCI of ≥5. No association between disease stage and BMI status was observed (Table 2). Among patients with solid tumours, overweight or obesity was highest among patients with breast cancer (51.4%), gynaecological cancers (44.6%), and lung cancer (41.7%). Head and neck cancer had the fewest patients with overweight or obesity (Table 3).

Multivariate analysis was performed for all variables with p<0.25 in bivariate analysis (Table 2). Age, female sex and hematologic cancer were the variables that were significantly associated with overweight and obesity (Table 4). Sub-analysis was done in solid tumour group. Age and female sex were significant predictive factors to overweight and obesity, while colorectal cancer was a significant protective factor (Table 5).

DISCUSSION

Despite its clinical importance in cancer management, the determination of the BMI status of patients with cancer remains limited in Indonesia. In this study, we attempted to identify patterns of nutritional status among patients with cancer, focusing on those whose BMI calculations indicated overweight and obesity. The calculation of BMI is one of several methods of assessing nutritional status. It is simple and inexpensive and is closely related to body fat level, morbidity, and mortality.

Notably, we found overweight or obesity in >40.0% of our patients with cancer, regardless of disease stage. Furthermore, the proportion tended to be higher in patients above 65 years of age. Epidemiological data by Samper-Ternent & Snih (2012) have shown that the prevalence of obesity among older patients is increasing. However, the 2015 and 2016 National Health and Nutrition Examination Surveys (NHANES) found no significant differences in the prevalence of obesity between younger and older adults (Hales *et al.*, 2017). A study by Kalish (2016) showed that higher total body fat, reduced total

Table 2. Differences in frequencies between BMI status with respect to age, gender, type and stage of cancers and co-morbidity status (*n* = 696)

Variable	*Underweight/Normal n (%)*	*Overweight/Obese n (%)*	*p-value*†
Age (years)			0.049*
<60 years	252 (58.2)	181 (41.8)	
≥60 years	135 (51.3)	128 (48.7)	
Sex			
Male	148 (63.0)	87 (37.0)	0.005*
Female	239 (51.8)	222 (48.2)	
Cancer Group			
Solid tumour	361 (57.2)	270 (42.8)	0.008*
Blood malignancy	26 (40.0)	39 (60.0)	
Stage of disease (n=631)			
Stage I – II	98 (57.6)	72 (42.4)	0.855
Stage III – IV	262 (56.8)	199 (43.2)	
Comorbidity (n=643)			
CCI low-moderate (<5)	177 (61.5)	111 (38.5)	0.079
CCI high (≥5)	136 (54.0)	116 (46.0)	

†Analysis for categorical data using Chi-square or Kolmogorov Smirnov
*p<0.05

Table 3. Distribution of BMI status according to the type of solid tumour *(n* = 531)

Cancer Type	*Underweight/Normal n (%)*	*Overweight/ Obese n (%)*	*Total n (%)*
Breast cancer	123 (48.6)	130 (51.4)	253 (100.0)
Lung cancer	70 (58.3)	50 (41.7)	120 (100.0)
Gynaecological cancer	51 (55.4)	41 (44.6)	92 (100.0)
Colorectal cancer	52 (69.3)	23 (30.7)	75 (100.0)
Head and neck cancer	30 (88.2)	4 (11.8)	34 (100.0)
Others	33 (60.0)	22 (40.0)	55 (100.0)

Table 4. Multivariate analysis of factors associated with overweight and obesity *(n* = 540)

Variables	*OR*	*95% CI*	*p-value*
Age (years)	1.02	1.00-1.04	0.019*
Female	1.82	1.23-2.69	0.003*
Hematologic cancer	2.96	1.61-5.44	<0.001*
CCI score	1.06	0.96-1.16	0.24

Analysis using multiple logistic regression for categorical data and multiple linear regression for numerical data
*p<0.05

Table 5. Factors associated with overweight and obesity among solid tumour patients (*n* = 480)

Variables	*OR*	*95% CI*	*p-value*
Age (years)	1.03	1.01-1.05	0.001*
Female	1.74	1.12-2.71	0.014*
Colorectal cancer	0.31	0.13-0.73	0.008*
Stage III-IV	1.15	0.73-1.81	0.556
CCI score	1.02	0.92-1.13	0.683

Analysis using multiple logistic regression for categorical data and multiple linear regression for numerical data
* $p<0.05$

energy consumption, altered hormonal and basal metabolic rate, and decreased activity associated with aging could explain the higher prevalence of obesity in older compared to younger adults.

We found a significant positive association between the sex of patients and BMI, with women patients being more likely to be overweight or obese. Less physical activity, impulsive diet habits, and psychosocial factors are some explanations for the higher prevalence of obesity in women, as shown by Kanter & Caballero (2012). However, data from the NHANES for 2015 and 2016 showed no significant difference in the prevalence of obesity between men and women (Hales *et al.*, 2017).

Our data showed that overweight and obesity were more common among patients with blood cancers than with solid tumours. Lichtman (2010) found that higher BMIs were associated with greater risks for blood cancers, including lymphoma, leukaemia or multiple myeloma. Another study by Li *et al.* (2017) found that overweight and obesity could increase the incidence of acute myeloid leukaemia and predict poor outcome.

In our study, breast, gynaecological and lung cancers were among the solid cancers that demonstrated the strongest associations with overweight and obesity. In the United States, data by World Cancer Research Fund/American Institute for Cancer Research (2017) showed that the most common solid tumours in patients that are associated with obesity are endometrial cancer; oesophageal adenocarcinoma; and colorectal, breast (in postmenopausal women), prostate, and renal cancers. Another study by Davoodi *et al.* (2013) found that breast, liver, skin, colorectal, ovarian, prostate, renal cell, and endometrial cancer were the solid organ cancers that were strongly associated with obesity.

The association of breast cancer with obesity has been extensively investigated. Chen *et al.* (2016) found that postmenopausal obese women (BMI ≥30 kg/m^2) had an 82% higher risk of triple-negative breast cancer [i.e. negative for estrogen receptors, progesterone receptors, and excess human epidermal growth factor receptor 2 (HER2) protein] compared with women with BMI <25 kg/m^2 (95% CI: 1.32-2.51). This is attributable to hormonal influences, as the conversion of peripheral androgen into estrogen in adipose tissue is the main source of circulating oestrogen in the postmenopausal term. Another study by Khabaz *et al.* (2017) found that increased serum leptin concentration was an independent risk factor for breast cancer. Serum leptin, one of the hormones directly connected to body fat and obesity, was also associated with cell growth, invasion, migration, and

metastasis recurrence in other cancers, such as liver, lung, gastric, thyroid, uterine, and colon cancers.

Tworoger & Huang (2016) reported an elevated incidence of gynaecological cancers, particularly endometrial and ovarian cancers, among patients who were overweight or obese. A study by Feng (2015) showed that overweight is associated with insulin resistance, adiposity and chronic inflammation, all of which increase the risk of gynaecological cancer. However, the association between obesity and cervical cancer remains controversial. In this study, we found that seven of nine (77.8%) patients with endometrial cancer had overweight or obesity. However, only 40.0% of patients with ovarian or cervical cancer were overweight or obese (data not shown). There was no association between BMI and disease stage. Overweight or obesity was found in exactly 50.0% of stage III or IV gynaecological cancer cases.

The association between obesity and risk of lung cancer remains inconclusive. In our series, approximately 40.0% of patients with lung cancer were overweight or obese. A study by Rauscher, Mayne & Janerich (2000) found that increased BMI was significantly associated with an increased risk of lung cancer in both smokers and non-smokers. However, other studies by Duan *et al.* (2015) & Yang *et al.* (2013) have shown different results, such as overweight being a protective factor against lung cancer. This is supported by a study by Patel *et al.* (2017) which found that BMI was not associated with the development of lung cancer.

Our study showed no association between BMI and disease stage in major cancer types (data not shown). Notably, >40.0% of patients in advanced stages, except the patients with colorectal cancer who had mostly normal or low BMIs, were overweight or obese. A study of patients with breast cancer by Cui *et al.* (2002) found that high BMI was significantly associated with the late stage of breast cancer. Women with obesity (BMI >27.3 kg/m^2) had a more advanced stage when first diagnosed compared with women with a BMI of <27.3 kg/m^2 (multivariate-adjusted odds ratio, OR=1.57; 95% CI: 1.15-2.14). Muscaritoli *et al.* (2017) & Wie *et al.* (2010) showed that malnutrition is more commonly found in the advanced stages of cancer. However, study by Prasad *et al.* (2010) showed that malnutrition should be diagnosed not only based on BMI but also by considering changes in body weight and applying laboratory examinations of malnutrition screening tools.

In this study, severe comorbidity (CCI ≥5) was more common in patients with overweight and obesity. To our knowledge, no study has directly assessed the association of comorbidity and BMI in patients with cancer. Pi-Sunyer (2009) found that obesity is a known risk factor for several diseases, including diabetes, stroke, cardiovascular disease, osteoarthritis, liver and renal disorders, sleep apnea, and depression. Other studies by Prasad *et al.* (2010) and Takenaka *et al.* (2014) that have evaluated the nutritional status of patients with and without cancer have found malnutrition to be more common in patients with higher CCI. However, these results remain unexplained.

Several major mechanisms may explain the association between obesity and cancer as stated by Berger (2014) and Pergola & Silvestris (2013), such as increased growth factor levels and bioavailability; increased steroid sex hormones (e.g. oestrogen); altered adipocytokine levels; inflammation and oxidative stress, which influence the cytokines and immune modulation; and changing microbiomes, particularly that of intestinal flora. Excesses of body weight and adipocytes are directly

associated with insulin resistance. Hyperinsulinemia, as compensation for pancreas stimulation, would accelerate growth and increase the aggressiveness of colorectal, pancreatic, liver, (postmenopausal) breast, and endometrial cancers. Insulin possesses an anabolic and anti-apoptotic effect. Most cancer cells are able to respond to the insulin activation effect through the intracellular transduction pathway. Hyperinsulinemia could increase the synthesis of insulin-like growth factor 1 (IGF-1) and decrease the expression of its binding protein. IGF-1 also has a pro-angiogenic effect and induces tumour-related lymphangiogenesis.

This study had some limitations. First, we did not analyse metabolic or nutritional factors related to high BMI because these assessments were not part of routine diagnostic workups in our hospital and thus were not recorded. Second, we could not assess obesity as a risk factor for certain cancers because of the cross-sectional design of our study. However, the results may provide a general picture of BMI status of patients with cancer, which could be affected by eating patterns and physical activity. Future studies to elaborate the associations between energy intake and physical activity and cancer risk are warranted.

CONCLUSION

The prevalence of overweight and obesity among patients with cancer was 44.4%. Overweight and obesity were more prevalent in older age, women and in those with haematological malignancies. They were associated with age and female sex among those with solid tumours and were most common in patients with breast, gynaecological, and lung cancers. Further study is required to assess whether certain dietary patterns and levels of physical activity are risk factors for cancer.

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Authors' contributions

Both NS (principal investigator) and RH conceptualised and designed the study, prepared the draft of manuscript, conducted data collection and reviewed the manuscript.

Conflict of interest

We declare that we have no conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial or not for profit sectors.

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Sensitivity of plasma cholecystokinin and peptide YY in obese and normal weight men

Dian Handayani[1,2*], Inggita Kusumastuty[1], Sri Andarini[3], Nindy Sabrina[4], Achmad Rudijanto[5] & Xu Feng Huang[6]

[1]*Nutrition Department, Faculty of Medicine, East Java Universitas Brawijaya, Malang, East Java , Indonesia;* [2] *Research Center for Smart Molecule and Natural Genetics Resources, Universitas Brawijaya, Malang, East Java, Indonesia.* [3]*Public Health Department, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia;* [4]*Nutrition Program, Faculty of Food Technology and Health, University of Sahid Jakarta, Jakarta, Indonesia;* [5]*Internal Medicine Department, Faculty of Medicine, Universitas Brawijaya, Malang, East Java , Indonesia;* [6]*School of Health Sciences and Illawarra Health and Medical Research Institute, University of Wollongong, Northfield Avenue, Wollongong, NSW 2522, Australia*

ABSTRACT

Introduction: Cholecystokinin (CCK) and peptide YY (PYY) are satiety-stimulating hormones that are released during eating. As such, their levels may be used useful in obesity intervention. The aims of this study were to determine the optimal cut-off values, sensitivity and specificity of plasma CCK and PYY in adult men, in order to determine hormonal dysfunction in obesity. **Methods:** We investigated 16 obese [body mass index (BMI) ≥25.1)] and 16 normal weight (BMI 18.5–22.9) men. They ate isocaloric fast-food for breakfast. Blood for the determination of the hormones was collected at 0 (before), 30, 60, and 120 minutes after consumption. The data that was obtained were analysed using an independent *t*-test or the Mann–Whitney U-test. The receiver operating characteristic (ROC) curve was drawn and the trapezoidal rule analysis was performed to determine the area under the curve, to determine the optimal cut-off values, sensitivity and specificity. **Results:** In obese subjects, CCK was lower compared with normal weight subjects at any time ($p<0.05$). There were no major differences in PYY among subject groups. ROC curve analysis demonstrated that the plasma CCK had an optimal cut-off of 6,310 pg/ml at 120 minutes after eating, with 0.97 area under curve (AUC), sensitivity was 94%, and specificity was 94%. The cut-off for optimal PYY was an average of 294.5 pg/ml at 120 minutes after eating (AUC 0.74; sensitivity 75%; specificity 75%). **Conclusion:** Our findings suggest that the plasma CCK level is a better potential predictor of obesity and constantly decreased over time compared to PYY.

Keywords: Cholecystokinin, peptide yy, obese, receiver operating characteristic

INTRODUCTION

The high prevalence of obesity has emerged as a health concern since it is a risk factor of chronic disease (Heymsfield & Wadden, 2017). In Indonesia, 19.7% of the men and 32.9% of the women were obese in 2013 (NIHRD, 2013). The combined prevalence of men and women increased to 21.8% in 2018 (NIHRD, 2018). Between 2013 and 2018, overall central obesity increased by about 5.0%

*Corresponding author: Dian Handayani
Nutrition Department, Faculty of Medicine, Universitas Brawijaya, Malang-Indonesia
Email: handayani_dian@ub.ac.id
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(26.6% to 31.0%). Excessive energy intake, as the cause of obesity, involves the brain-gut axis in the central nervous system. Hormones such as peptide YY (PYY) and cholecystokinin (CCK) play a role in decreasing appetite (Posovszky & Wabitsch, 2015).

PYY and CCK have been known as satiety hormones contributing to the delay and inhibition of gastric emptying (Adamska *et al.*, 2014). In humans, low levels of PYY have been associated with obese patients (Batterham *et al.*, 2003). An animal study found low PYY levels in obese rats and high PYY in lean rats, suggesting that PYY deficiency plays a role in the pathogenesis of obesity (Moghadam, Moran & Dailey, 2017). A human study also showed that CCK concentrations in metabolic syndrome that followed morbid obesity were significantly lower than those in lean control subjects (Zwirska-Korczala *et al.*, 2007). It can thus be inferred that the alteration in PYY and CCK hormones are intimately associated with obesity.

The gut hormones have been investigated as a potential therapeutic target for obesity. Recently, acylated ghrelin (AG), an energy homeostasis regulator hormone, has been one of the targets for obesity therapy using ghrelin O-acyltransferase (GOAT) inhibitor (Khatib *et al.*, 2015). Andarini, Kangsaputra & Handayani (2017) investigated the AG levels and optimal cut-off values in normal and obese patients using receiver-operating characteristic (ROC) curve analysis. The investigation revealed that there was a significant difference in AG levels, and the optimal cut-offs were 2,332 pg/ml before eating (sensitivity 88%; specificity 100%) and 2,710 pg/ml at 30 minutes after eating (sensitivity 88%; specificity 100%) (Andarini *et al.*, 2017). Determination of gut hormone cut-offs would assist in establishing rational therapeutic principles in obesity therapy management. Some researchers have found that appetite control hormones, CCK and PYY, can also be potential therapeutic targets in the management of obesity (Perry & Wang, 2012; Olszanecka-Glinianowicz *et al.*, 2013; Prinz & Stengel, 2017). CCK and PYY deficiency in obese subjects, treatment with CCK and PYY has been attempted. However, an animal study demonstrated that chronic CCK administration induced pancreatitis in rats, and high dose PYY infusion produces vomiting and nausea (Degen *et al.*, 2005; Jia, Yamamoto & Otsuki, 2015).

The current literature provides limited information on CCK and PPY cut-off levels. This aim of this study was to investigate CCK and PYY levels between obese men and control, and to assess the optimal cut-off, sensitivity and specificity of CCK and PYY by using ROC analysis.

MATERIALS AND METHODS

Study design

This study was conducted in Malang, Indonesia. It aimed to obtain and compare the optimal cut-off values, sensitivity, and specificity of CCK and PYY plasma levels using ROC analysis to evaluate pre- and post-prandial plasma CCK and PYY. Participants were given isocaloric food (51% carbohydrate, 33% fat, and 13% protein) and water for breakfast after overnight fasting. Fasting blood samples were collected before breakfast (0 minutes), and 30, 60, and 120 minutes after breakfast. The study was approved by the Ethical Committee of Medical Faculty of Universitas Brawijaya (No. 389/EC/KEPK/2015).

Test meals

One serving of commercial fast-food containing similar amounts of energy (546–593 kcal) was provided to the 32 participants at breakfast time. The

meals were obtained from a popular fast-food franchise restaurant in Malang, Indonesia. This study used commercial fast-food since the community expressed a preference for it.

Previous studies have reported that the consumption of fast-foods correlated with obesity (Janet *et al.*, 2010; Andreyeva, Kelly & Harris, 2011). The total energy and macronutrient content are presented in our previous study (Handayani *et al.*, 2017). The amount of energy was analysed using a bomb calorimetric, protein was assayed by the Kjeldahl method and fibre by enzymatic analysis.

Participants

Sixteen men with normal weight and 16 obese men were recruited. Males were chosen as the participants due to their stable energy intake, since females have a fluctuating energy intake within their menstrual cycle. The physiological responses to hunger and satiety also differ between male and female (Bédard *et al.*, 2015). Pre-screening was performed to determine healthy participants without a previous history of diabetes mellitus, hypercholesterolemia, or hypertension. Blood pressure, blood glucose and plasma cholesterol levels were measured using sphygmomanometer, blood glucose test kit and the multi-monitoring system Autocheck FDA-CE197, respectively. Plasma leptin was measured using competitive enzyme-linked immunosorbent assay (ELISA) (KIT: CAN-L-4260, DB Canada). The percentage (%) body weight and % waist fat were measured using bioelectrical impedance analysis (BIA)-Omron type HBF 375. All participants provided informed consent for the study.

Blood biochemistry examination

Fasting blood samples were collected for CCK and PYY from participants before eating, and at 30, 60 and 120 minutes after. Plasma was separated from blood samples by centrifugation at 3,000 revolutions per minute (rpm) for 10 minutes at 25°C using PLC-05 tabletop centrifuge (Gammy Industrial Corporation, Taiwan, in association with Cannic, Inc., USA) and then was stored at -80˚C until analysis.

Plasma CCK (pg/ml) was measured using competitive ELISA (E-EL-H0723; Elabscience, Biotechnology, Beijing). Plasma PYY (pg/ml) was measured using sandwich ELISA (E-EL-H1237; Elabscience, Biotechnology, Beijing). The plasma samples were added onto a pre-coated microtiter plate and combined with biotinylated detection antibody. After incubation at 37°C, horseradish peroxidase (HRP) conjugate was added and incubated. The substrate reagent was added for the colour reaction. The optical density (OD) was measured at a wavelength of 450.0±2.0nm. CCK and PYY hormone levels were obtained from the OD of the samples and the standard curve.

Statistical analysis

The data are presented as a mean±standard deviation (SD). The baseline comparison between obese and normal weight men was examined using the independent *t*-test. For CCK and PYY levels, differences between obese and normal weight were examined by using the independent *t*-test for normally distributed data and Mann-Whitney U test for abnormally distributed data. The area under the curve (AUC) of CCK and PYY was performed by calculating the graphic trapezoid. The AUC scores were interpreted as excellent if ≥0.90, good if 0.80–0.89, fair if 0.70–0.79, and poor if <0.70 (Kendzor, Caughy & Owen, 2012). The optimal cut-off values of CCK and PYY were determined using the ROC curve. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version

Table 1. Characteristics of research participants

Characteristics	*Obese (n=16) (Mean±SD)*	*Normal weight (n=16) (Mean±SD)*	*p-value*
Age (years)	21.4±1.9	20.6±1.1	0.18
Weight (kg)	97.0±1.9	60.9±5.7	<0.01
Height (cm)	170.0±5.5	169.0±6.3	0.89
BMI (kg/m^2)	33.6±4.8	21.2±1.1	<0.01
Body fat (%)	30.2±3.8	17.0±5.1	<0.01
Waist fat (%)	16.7±4.1	4.7±1.0	<0.01
Waist circumference (cm)	105.0±12.0	75.4±4.2	<0.01
Systolic blood pressure (mm/Hg)	124.0±9.6	117.0±6.8	0.04
Diastolic blood pressure (mm/Hg)	80.6±4.4	75.0±5.2	<0.01
Random blood sugar (g/dL)	122.0±21.1	103.0±12.2	0.16
Total cholesterol (g/dL)	135.0±27.7	94.8±49.8	<0.01
Leptin (pg/mL)	61.9±21.8	21.6±9.1	<0.01

16 (IBM, Chicago, IL, USA). Statistical analyses were considered as significant at $p<0.05$.

RESULTS

Characteristics of the research participants

In total, 32 adult men (16 normal weight and 16 obese) were recruited for the study. The mean age was 21.4±1.9 years for the obese men and 20.6±1.1 years for the normal weight men. Obese men were heavier and had significantly higher ($p<0.01$) BMI (≥25.1), body fat percentage, and waist fat percentage than did normal weight men whose BMI was 18.5–22.9 (Table 1). The obese men had higher levels of systolic blood pressure, diastolic blood pressure (DBP), total cholesterol, and leptin (Table 1) (Andarini *et al.*, 2017).

CCK and PYY

The mean±SD of CCK and PYY levels in time points are shown in Table 2. In CCK, there was significant difference in the mean values of the obese and normal weight groups ($p<0.002$). Moreover, there was a decrease in CCK concentration in the obese group at the different time points (before eating, 30 minutes after eating, 60 minutes after eating, and 120 minutes after eating) but this did not show in the normal weight group. There

Table 2. CCK and PYY concentrations before eating and 30, 60, and 120 minutes after eating

Variables	*Obese (Mean±SD)*	*Normal weight (Mean±SD)*	*p-value*
CCK 0 min (pg/ml)	7045.6±738.1	8234.4±950.8	<0.002
CCK 30 min (pg/ml)	6371.8±738.1	8033.1±1334.3	<0.001
CCK 60 min (pg/ml)	6195.0±746.7	8395.6±1134.1	<0.001
CCK 120 (pg/ml)	5275.0±789.6	8799.4±1595.9	<0.001
PYY 0 min (pg/ml)	251.9±190.8	155.9±92.3	0.036
PYY 30 min (pg/ml)	279.0±190.8	346.9±113.9	0.121
PYY 60 min (pg/ml)	478.8±263.0	363.1±137.2	0.129
PYY 120 min (pg/ml)	248.3±187.6	353.1±119.1	0.069

Table 3. Cut-off, sensitivity, and specificity of CCK and PYY content before and after eating at each time point

Time (min)	*CCK*				*PYY*			
	Cut off (pg/ml)	*AUC*	*Sensitivity*	*Specificity*	*Cut off (pg/ml)*	*AUC*	*Sensitivity*	*Specificity*
0	7665	0.82	75%	75%	191.5	0.27	38%	38%
30	7040	0.82	75%	75%	266.5	0.31	69%	69%
60	7015	0.96	88%	88%	369.0	0.37	44%	44%
120	6310	0.97	94%	94%	294.5	0.74	75%	75%

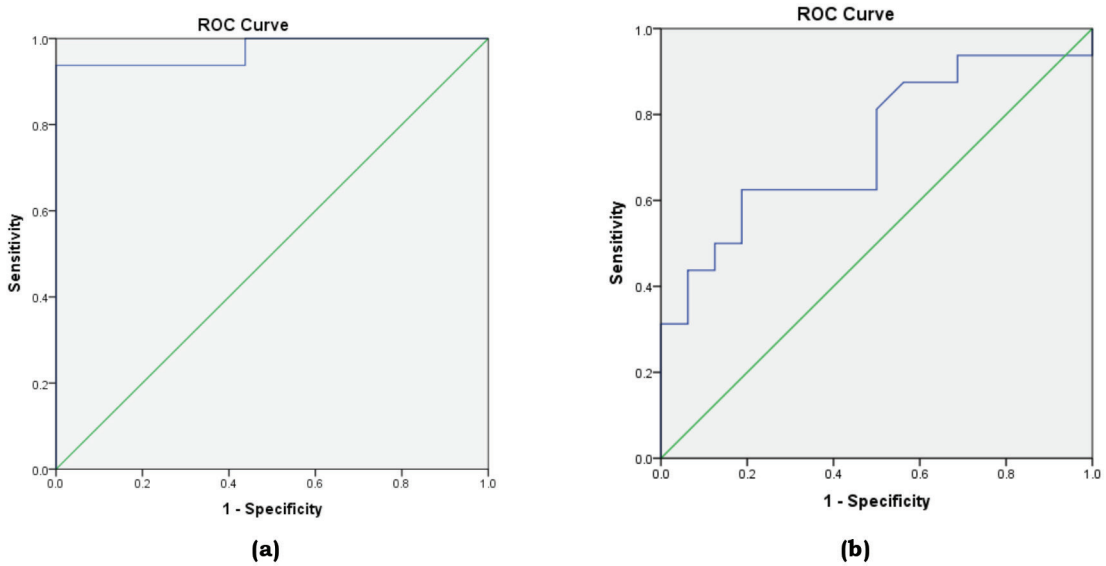

Figure 1. Receiver characteristics curve (ROC) of CCK (a) and PYY (b) at 120 minutes after eating

were no significant differences in PYY levels between the obese and normal weight groups.

CCK and PYY as an alternative biomarker test for obesity

Table 3, Figure 1(a), and Figure 1(b) shows optimal cut-off points and area under ROC curve for CCK and PYY on each time point. The ROC curve of CCK and PYY showed the highest sensitivity and specificity at 120 minutes after eating. ROC analysis of CCK showed a cut-off value of 6,310 pg/ml, with area under curve (AUC) of 0.97, sensitivity was 94% and specificity was 94%. The highest PYY levels showed highest sensitivity and specificity with a cut-off value of 294.5 pg/ml (AUC, 0.74; sensitivity, 75%; specificity, 75%).

DISCUSSION

In recent years, the alteration of gut hormones that are related to obesity such as CCK and PYY has been documented. Those hormones play an important role in mediating satiation. The present study revealed that when sensitivity and specificity were 94% each, the optimal cut-off value of CCK was 6,310 pg/ml at 120 minutes after eating. At the same time, the optimal cut-off of PYY was at a lower sensitivity and specificity value

(75% and 75%, respectively) than CCK. There was a statistically significant difference between the CCK levels in obese and normal weight men at each of the time points of 0, 30, 60, and 120 minutes, suggesting that obese people have lower CCK levels. However, there were no significant differences in PYY. The increasing CCK concentration indicates a lower gastric emptying rate through an appetite suppression mechanism and food intake reduction. Gastric motility will be suppressed by CCK release, resulting in slower gastric emptying which leads to prolonged gastric distension and satiety (Ma *et al.*, 2009; Li, Ma & Wang, 2011; Robert *et al.*, 2015). Plasma CCK increased within 15 minutes after meal initiation, went higher in 30 minutes and remained elevated over the 120 minutes initiation, and then decreased gradually. Our study confirmed that the decreased CCK concentration at time points contributes to a decrease of satiety and an increase of hunger in obese subjects (Ma *et al.*, 2009; Adamska *et al.*, 2014; Robert *et al.*, 2015).

Obesity has been significantly associated with rapid gastric emptying rates (Acosta *et al.*, 2015). In this study, CCK levels were reported to be lower in obese than in normal-weight men. Our observations were consistent with those of Lean & Malkova (2016) who reported decreased CCK in obese and overweight compared to lean subjects after dietary intervention. Even though Brennan *et al.* (2012) demonstrated no major differences in CCK responses to macronutrients between lean and obese subjects, high protein content stimulated CCK production in their obese respondents compared to high fat. Another investigation found a delayed CCK response to oleic acid infusion in obese subjects compared to control groups (Stewart *et al.*, 2011). These observations suggest that CCK in obese subjects is sensitive to protein and fat intake. Our finding confirms that the high-fat content in fast foods contributed to lower CCK in obese subjects compared to lean subjects. Also, some investigations found a synergistic interaction between CCK and leptin that resulted in short-term satiety. Heldsinger *et al.* (2011) established that the Phosphoinositide 3-kinase (PI3K) and Signal transducer and activator of transcription 3 (STAT3) signaling pathway can be mediated for CCK and leptin to activate vagal neurons which may contribute to food intake. The significant difference in leptin between obese and lean subjects may affect CCK in controlling satiation. Plasma leptin level in this study as reported in Table 1 is higher in the obesity group compared to the normal weight group.

The amount of CCK gradually decreased over time but increased in sensitivity and specificity with a peak at 120 min following the consumption of food. The mechanism underlying the increases of sensitivity and specificity over time remains unclear. However, the previous finding which revealed the inverse relationship between hunger and plasma CCK but not PYY explains that CCK is more sensitive in controlling satiety (Brennan *et al.*, 2012). The result was consistent with the present study that PYY does not have good specificity and sensitivity as CCK, and the ROC curve appears to fluctuate. The better specificity and specificity over time in CCK also showed that time could determine the amount CCK released into circulation.

PYY acts in the reduction of gastric emptying and the delay in intestinal transit, which is called 'ileal brake'. PYY also has biological functions including reducing food intake, decreasing gastric emptying, and slowing gastrointestinal motility. The lack of endogenous PYY

secretion may lead to obesity development (Li *et al.*, 2011; Adamska *et al.*, 2014; Troke, Tan & Bloom, 2014). Some studies reported that obese subjects have lower fasting and postprandial plasma PYY than normal weight subjects. It has been reported that protein or fat has the strongest stimulant of PYY secretion and thus contribute to its increase in blood concentrations (Mittelman *et al.*, 2010; Li *et al.*, 2011; Lean & Malkova, 2016). The obese subjects had significantly higher hunger and lower satiety than the normal weight subjects. However, the postprandial PYY profiles were not translated into the feeling of less hungry or more fullness that led to a reduction in food intake (Lomenick, Clasey & Anderson, 2008; Lomenick *et al.*, 2009; van der Klaauw *et al.*, 2013). The PYY response was reported slightly higher immediately after exercising both in obese men and women. Lifestyle and environment factors do not have a significant impact on plasma PYY in men, but some factors such as smoking, medication use, and a post-menopause condition in women lead to an increase of circulating PYY concentration (Cahill *et al.*, 2014; Lean & Malkova, 2016). Our data were also consistent with the latest study in older and middle-aged men by Madsen *et al.* (2019) who found that there was no difference in plasma concentration of PYY in response to lipid load. The 30-minute interval of test meal had been chosen based on previous studies which showed a significant difference in gut hormones (le Roux *et al.*, 2006).

This study had several limitations. The first limitation was the small number of participants in each group. Secondly, even though the study used fast food meals with similar energy density, the serving size among the foods was different. A previous study revealed that food form and portion size affect postprandial hormonal response in normal weight and obese subjects (Leidy *et al.*, 2010). It is suggested that in future studies, the confounding factor of the test meal could be minimised by choosing the same food with similar serving size and macronutrient content. In this way, the focus would be more on analysing the sensitivity and specificity of the hormonal response.

This study showed that CCK is more sensitive and specific than PPY in the study of gut hormones in obese subjects. This finding may enhance the development of appropriate gut hormonal testing and intervention to help to combat obesity.

CONCLUSION

At present, CCK is rarely considered as a gut hormone target in obesity management. However, we have established that CCK is better than PYY in predicting obesity from the significantly different amounts of CCK in obese and lean subjects at all time points. Furthermore, we have demonstrated that CCK has better sensitivity and specificity over time, unlike PYY, which had a fluctuating ROC curve. Our findings suggest that CCK has potential in obesity treatment. Future studies are required to clarify the nutrients and hormones contributing to CKK release and the side effect(s) of CCK administration.

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Authors' contributions

DH, the principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; IK, led the data collection and statistical analysis; SA, led

the data collection and statistical analysis; AR and NS, assisted in drafting the manuscript, advised on the data analysis and interpretation and reviewed the manuscript; XFH, conducted data analysis and interpretation, assisted in drafting the manuscript and reviewed the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Plain water and beverage consumption patterns among university students in Puncak Alam, Malaysia

Nur Islami Mohd Fahmi Teng[1*], Norsham Juliana Nordin[2] & Aisyah Suraya Muhammad Shah[1]

[1]*Faculty of Health Sciences, Universiti Teknologi MARA, Cawangan Selangor, Kampus Puncak Alam, Selangor;* [2]*Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia, Kuala Lumpur*

ABSTRACT

Introduction: Data on water and sugar sweetened beverages (SSB) intake among young adults in Malaysia is sparse. This study aimed at measuring the intake of plain water and SSB among undergraduate students in a Malaysian university and examine its association with body mass index (BMI). **Methods:** A total of 376 undergraduate students aged 18-30 years were recruited. A self-administered questionnaire was used to determine the SSB consumption pattern. The questionnaire consisted of five sections that included the background of the participants, knowledge about SSB, SSB preferences, frequency and portion size. **Results:** 23.9% of subjects in this study were overweight. Almost all of the subjects took outside food (93.1%) and drink (74.2%). The highest daily consumption was plain water (92.3%), with a majority drinking more than two cups at each intake. Caffeinated drinks (coffee or tea) were the most popular SSB among the students (18.4%). Most students (79.7%) did not consume SSB on a daily basis. A significant association was found between the proportion of plain water consumption and BMI ($p<0.05$). Those who were overweight consumed a greater amount of plain water as compared to those underweight. **Conclusion:** Our findings of low plain water intake among the underweight may be used to tailor intervention efforts to increase its intake and reduce that of SSB, especially among underweight young adults.

Keywords: Sugar sweetened beverages, SSB, plain water, obesity, undergraduate students

INTRODUCTION

The prevalence of obesity has increased drastically to epidemic proportions in Malaysia. Based on a systematic analysis for the Global Burden of Disease Study (2013), 44.2% of Malaysian adults have been found to be overweight or obese (Ng *et al.*, 2014). Similar trends have been seen among Malaysian children and adolescents. These trends could lead to serious health complications in adulthood. Various public health co-morbidities are associated with overweight and obesity, including hypertension, cardiovascular disease, diabetes, depression and multiple type of cancers. Generally, obesity arises from an imbalance of energy homeostasis with the complex interaction between genetic, metabolic,

*Corresponding author: Dr Nur Islami Mohd Fahmi Teng
Faculty of Health Sciences, Universiti Teknologi MARA, Cawangan Selangor, Kampus Puncak Alam, 42300 Puncak Alam, Malaysia
Tel: (6)03-32584540; Fax: (6)(03)32584599; E-mail: nurislami@uitm.edu.my
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cultural, environmental, socioeconomic, and behavioural factors (Heitmann *et al.*, 2012). Recent highlights from the Prospective Urban Rural Epidemiology (PURE) study showed that the intake of carbohydrates is high among Malaysians (Dehghan *et al.*, 2017). National survey data has indicated that the consumption of carbohydrates in the form of added sugars has also increased (Amarra, Khor & Chan, 2016; Dehghan *et al.*, 2017).

Multiple studies have reported that current estimates of added sugar intake among Malaysian children and adults range from 9.0% to 28.4%, with the highest intake found among adolescents (Amarra *et al.*, 2016). The reported intake is worrying, as it exceeds World Health Organization (WHO) (2015) recommendations which state that added sugar must not exceed 10% of the total intake of energy. This WHO guideline also recommended to further reduce the intake of free sugars to below 5% of total energy, if conditions warrant this. This recommendation translates into 25 grams of added sugar or approximately six teaspoons of sugar per day for a 2000 kcal diet. Fructose and non-fructose-rich corn syrups, cane and sucrose, honey and other edible sugar are common types of sugar added to food or beverages.

In the United States, soft drinks are the leading source of added sugar, each serving of which is a high glycaemic load that increases the risk of type 2 diabetes mellitus (T2DM) (Hu & Malik 2010). However, in Malaysia, the Malaysian Adult Nutrition Survey (MANS) 2003 has pointed out that intake of sweetened beverages such as coffee, *teh tarik*, and chocolate beverages are the highest contributors to the consumption of added sugar in the daily dietary intake of Malaysian adults' (Norimah *et al.*, 2008).

Water consumption is important for adequate hydration, body function and health. Increased water consumption, specifically plain water, is used as a key message in many weight reduction programmes (Muckelbauer *et al.*, 2013). The Malaysian Dietary Guidelines has recommended drinking 6-8 glasses of plain water daily (NCCFN, 2010). Studies have reported that substituting SSB with plain water plays a significant role in reducing energy intake, and thus can aid in weight management and diabetes prevention (Muckelbauer *et al.*, 2013). As Malaysian's prevalence of T2DM in 2015 (17.5%) has doubled since 1996, in parallel with obesity, it has become important to identify the extent of SSB intake in the population (Cheah *et al.*, 2018).

This study specifically aimed to determine plain water and SSB intake, and their association with body mass index (BMI), among university undergraduate students in an urban area in Malaysia.

MATERIALS AND METHODS

Participants

Undergraduate students at the Puncak Alam campus of Universiti Teknologi MARA, were invited to participate in this cross-sectional study. We advertised the study by using flyers and also approached students at the common areas of the university such as library, student lounge and cafeteria. As there were approximately 16000 registered students, we estimated that a sample size of 376 was needed by using the Raosoft Calculator Software (95% of confidence level with a 5% margin error). The participants were required to be free from diet-related disease, mental disorder and physical disabilities. Those who met the study criteria were recruited as subjects and asked to complete a self-administered questionnaire and consent form. The study was approved by the Ethics Committee of Universiti Teknologi MARA.

Instruments

SSB was defined as any beverage with added sugar or calories. This definition covered fruit drinks, milk, carbonated/ soft drinks, coffee/tea and sports and energy drinks.

A self-administered questionnaire that was adapted from a study by Hedrick *et al.* (2010) was used to obtain data about plain water and SSB consumption pattern. We modified the questionnaire to better reflect local drinking habits in Malaysia, and ran a pilot study to assess the reliability of the modified questionnaire. A Cronbach's alpha of >0.7 was obtained for all sections in the questionnaire, which indicated a good reliability. The questionnaire was entirely in English, and definitions or explanations about the food items were also given. The questionnaire consisted of four sections, which included the background participants' (including self-reported weight and height), SSB preferences, frequency of SSB consumption and portion size.

Statistical analysis

All analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 22. We reported the data descriptively and used the

Table 1. Characteristics and background of the students (N=376)

Characteristic (mean±SD)	*Frequency (n)*	*Percentage (%)*
Age (22.23±1.17)		
≤ 22	260	69.1
> 22	116	30.9
Gender		
Male	44	11.7
Female	332	88.3
Faculty		
Health science	188	50.0
Non-health science	188	50.0
Marital status		
Single	374	99.5
Married	2	0.5
Year of Study		
1st-year student	41	10.9
2nd-year student	125	33.2
3rd-year student	187	49.7
Final year student	23	6.1
Place of living		
With family	23	6.1
Hostel resident	324	86.2
Non-resident/rental house	29	7.7
Body mass index (BMI)		
Underweight	68	18.1
Normal	218	58.0
Overweight	90	23.9
Physical activity		
Not exercise	203	54.0
Irregular exercise	157	41.8
Regular exercise	16	4.3

chi-square test to compare between categorical variables. The significance level was defined as $p<0.05$.

RESULTS

A total of 376 subjects aged 18-30 years participated in this study. There was an equal number of subjects (n=188) from both the health science and non-health sciences faculties. More than half of them had normal BMI (58.0%) and 54% of the total did not exercise. The prevalence of overweight among the subjects was high at 23.9%. Table 1 summarises the characteristics and background of our subjects.

Most of the subjects eat outside food (93.1%) and did not prepare their food (92.2%). They showed a similar preference between buying outside drinks (74.2%) and preparing their own drinks (52.4%) (Table 2).

Table 3 reveals the beverage consumption patterns of the subjects. Plain water (92.3%) was the most commonly consumed beverage. A majority of the subjects drank >2 cups each time. This was followed by sweetened coffee or tea (18.4%) and full cream milk (9.6%). Besides plain water, most of the subjects consumed only ≤1 cup of the beverage each time. The lowest frequency of intake was alcoholic drinks, with 97.1% of subjects consuming such beverages either once or not at all in a week. The low intake may have been because of religious reasons since most of the subjects were Muslims who are forbidden from consuming alcohol. Based on Table 3, soft drinks and milk were least favourable among our subjects.

Table 4 summarises the factors related to the consumption of SSB's among the subjects. Approximately two-thirds of the subjects (69.7%) did not consume SSB on a daily basis. They preferred cold SSB (66.5%) purchased from

Table 2. Sources of food and drinks (N=376)

Characteristic	*Frequency (n)*	*Percentage (%)*
Source of food		
Prepared by family members		
Yes	38	10.1
No	338	89.9
Purchased from the cafeteria/ restaurant		
Yes	350	93.1
No	26	6.9
Self-prepared		
Yes	67	17.8
No	309	82.2
Source of drinks		
Prepared by family members		
Yes	27	7.2
No	349	92.8
Purchased from cafeteria/restaurant		
Yes	279	74.2
No	97	25.8
Self-prepared		
Yes	197	52.4
No	176	47.6

Table 3. Pattern of consumption of beverages (N=376)

Types of beverages	*≤ 1 time per week*		*≤ 6 times per week*		*Daily*	
	n	*%*	*n*	*%*	*n*	*%*
Plain water	5	1.3	24	6.4	347	92.3
100% fruit juice	259	68.9	87	23.1	30	8.0
Sweetened fruit beverages	247	65.7	106	28.2	23	6.1
Full cream milk	252	67.0	88	23.4	36	9.6
Low fat milk	276	73.4	71	18.9	29	7.7
Skimmed milk	307	81.6	48	12.8	21	5.6
Regular soft drink	303	80.6	56	14.9	17	4.5
Diet soft drink	335	89.1	25	6.6	16	4.3
Sweetened coffee or tea	187	49.7	120	31.9	69	18.4
Energy or sports drinks	283	75.3	74	19.7	19	5.1
Alcoholic drinks	365	97.1	7	1.9	4	1.1
Other beverages	362	96.3	7	1.9	7	1.9

Table 4. Consumption pattern of sugar-sweetened beverages (N=376)

Parameters	*Frequency (n)*	*Percentage (%)*
Frequency of consumption		
Daily	114	30.3
Not daily	262	69.7
Types of beverages		
Hot	27	7.2
Cold	262	69.7
Both	87	23.1
Location of purchase		
Supermarket	250	66.5
Cafeteria	198	52.7
Vending machine	133	35.4
Others	2	0.5
Location of consumption		
Home	31	8.2
Outside	262	69.7
Both	83	22.1
Time of consume		
Breakfast	74	19.7
Lunch	192	51.1
Evening tea	119	31.6
Dinner	123	32.7
Reason for consume		
To reduce thirst	164	43.6
To complement a meal	123	32.7
As a refreshment	140	37.2
To stay up	117	31.1
Others	14	3.7
Factors influencing to choice of SSB		
Taste	320	85.1
Price	81	21.5
Brand	69	18.4
Health concerns	55	14.6
Others	3	0.8

Table 5. Portion size of beverage consumption according to BMI status

	Total (N=376)		BMI Underweight (n=68)		Normal (n=218)		Overweight (n=90)		p-value†
	n	%	n	%	n	%	n	%	
Plain Water									
≤1 cup each time	49	13.0	19	27.9	26	11.9	4	4.4	
≤2 cups each time	70	18.6	10	14.7	45	20.6	15	16.7	0.000*
>2 cups each time	257	68.4	39	57.4	147	67.4	71	78.9	
100% fruit juice									
≤1 cup each time	291	77.4	55	80.9	172	78.9	64	71.1	
≤2 cups each time	75	19.9	10	14.7	41	18.8	24	26.7	0.319
>2 cups each time	10	2.7	3	4.4	5	2.3	2	2.2	
Sweetened fruit beverages									
≤1 cup each time	280	74.5	50	73.5	164	75.2	66	73.3	
≤2 cups each time	87	23.1	15	22.1	50	22.9	22	24.4	0.809
>2 cups each time	9	2.4	3	4.4	4	1.8	2	2.2	
Full cream milk									
≤1 cup each time	300	79.8	54	79.4	178	81.7	68	75.6	
≤2 cups each time	72	19.1	13	19.1	39	17.9	20	22.2	0.572
>2 cups each time	4	1.1	1	1.5	1	0.5	2	2.2	
Low fat milk									
≤1 cup each time	312	83.0	57	83.8	182	83.5	73	81.1	
≤2 cups each time	58	15.4	9	13.2	33	15.1	16	17.8	0.815
>2 cups each time	6	1.6	2	2.9	3	1.4	1	1.1	
Skimmed milk									
≤1 cup each time	336	89.4	63	92.6	194	89.0	79	87.8	
≤2 cups each time	38	10.1	5	7.4	22	10.1	11	12.2	0.650
>2 cups each time	2	0.5	0	0.0	2	0.9	0	0.0	
Regular soft drink									
≤1 cup each time	304	80.9	55	80.9	183	83.9	66	73.3	
≤2 cups each time	62	16.5	13	19.1	28	12.8	21	23.3	0.103
>2 cups each time	10	2.7	0	0.0	7	3.2	3	3.3	
Diet soft drink									
≤1 cup each time	337	89.6	62	91.2	198	90.8	77	85.6	
≤2 cups each time	36	9.6	6	8.8	18	8.3	12	13.3	0.614
>2 cups each time	3	0.8	0	0.0	2	0.9	1	1.1	
Sweetened coffee or tea									
≤1 cup each time	252	67.0	51	75.0	140	64.2	61	67.8	
≤2 cups each time	103	27.4	15	22.1	65	29.8	23	25.6	0.511
>2 cups each time	21	5.6	2	2.9	13	6.0	6	6.7	
Energy or sports drink									
≤1 cup each time	278	73.9	51	75.0	164	75.2	63	70.0	
≤2 cups each time	87	23.1	17	25.0	48	22.0	22	24.4	0.322
>2 cups each time	11	2.9	0	0.0	6	2.8	5	5.6	
Alcoholic beverages									
≤1 cup each time	367	97.6	67	98.5	213	97.7	87	96.7	
≤2 cups each time	9	2.4	1	1.5	5	2.3	3	3.3	0.742
>2 cups each time	0	0.0	0	0.0	0	0.0	0	0.0	
Others									
≤1 cup each time	373	99.2	67	98.5	217	99.5	89	98.9	
≤2 cups each time	3	0.8	1	1.5	1	0.5	1	1.1	0.665
>2 cups each time	0	0.0	0	0.0	0	0.0	0	0.0	

†Analysis using x^2 test
*p<0.05

supermarkets (66.5%), and they typically consumed these when away from home or in their rooms (69.7%). The preferred time for SSB consumption was during lunch (51.1%), in order to reduce thirst (43.6%), as that was the prime time for hot weather. SSBs were predominantly chosen based on taste (85.1%).

Further analyses on the association between beverage consumption and BMI showed significant differences between plain water consumption portion size and BMI ($p<0.05$) (Table 5). Those who were overweight consumed a significantly higher amount (>2 cups) of plain water each time (78.9%) compared to those who were underweight (27.9%), who typically drank <1 cup of water each time.

DISCUSSION

This study evaluated plain water and SSB consumption patterns among undergraduates from a Malaysian university. Vella-Zarb & Elgar (2009) have reported that college and university students were susceptible to unhealthy weight-related behaviours including consumption of SSB. Various studies in multiple countries have shown that the trend of increasing SSB intake among university students is worrying (Vilaro *et al.*, 2018; Joh, Lim & Cho, 2015). Highly urbanised countries such as the United States and Australia have reported high intakes of sweetened beverages, at 40-50% of university students who were studied (O'Leary *et al.*, 2012; Block *et al.*, 2013). Similar trends have also been reported among Turkish university students (Deliens *et al.*, 2015). Thus, the trend affects the students' intake on plain water. Presently, studies on sugary beverages and plain water consumption within Malaysia remain scarce.

Most of the students consumed foods and drinks from outside their homes. The result is to be expected, as most of the subjects lived on campus, with limited access to food preparation facilities. As discussed by in a previous study (Greaney *et al.,* 2009), food choices of students were influenced by the availability and accessibility of foods and cooking facilities. It has also been shown that such living arrangements, particularly when living outside of their family home, are associated with unhealthy dietary habits, including increased SSB consumption (An, 2016). In line with one study (Vilaro *et al.,* 2018), our subjects also reported taste as one of the determinants in choosing type of SSB. Taste is an important reason for high SSB consumption and unhealthy diets.

Caffeinated drinks and sweetened fruit beverages (including cordial drink) were the most consumed SSBs among our subjects. This is in line with a study by Norimah *et al.* (2008) which reported a high consumption of coffee and tea among Malaysians. In Malaysia, such beverages are often prepared with added sugar and condensed milk, which eventually contributes to high fat and calorie. Our study found that although most of the subjects did not consume SSB on a daily basis, almost 70% consumed SSBs multiple times in a week. Again, this may be related to the high frequency (74.2%) of students who bought their drinks from outside at cafeteria/restaurants. As reported by previous study, eating/drinking out was the largest contributor to SSBs intake (An, 2016). Frequent SSB consumption is associated with an increased risk of obesity and related non-communicable diseases (NCDs). There have been many reports over this decade of the early onset of NCDs among Asians (Dans *et al.*, 2011; Misra & Khurana, 2011). Thus, strategies to prevent the onset of NCDs must start earlier as a healthy lifestyle during childhood and youth can prevent the onset of NCDs later in adulthood.

This study revealed that overweight students were highly cautious of their dietary intake, particularly of SSBs. They drank significantly more plain water compared to the underweight subjects. This is consistent with previous study, which reported that overweight and obese young adults were found to be high water consumers (Lee, Park & Kim, 2014). Obese persons attempt to reduce their energy intake by substituting plain water for SSBs (Park *et al.* 2012). In contrast, there were fewer underweight subjects who did not take plain water at all on a daily basis. This is unfortunate, as having normal or underweight BMI should not be taken to mean that healthy eating is to be ignored. A randomised controlled trial among 646 children reported that the reduction of sweets and sweetened beverages observed in the intervention group significantly reduced their BMI and blood pressure (Chan & Woo, 2010). Another recent study conducted in Japan on water intake revealed that there was an inverse correlation between high water intake and risk of cardiovascular diseases (Cui *et al.*, 2018). Therefore, a high intake of water particularly plain water must be promoted seriously in our community lifestyle. In Malaysia, the consumption of 1ml of water for each calorie eaten is recommended (NCCFN, 2010). This amount is equivalent to 7 to 11 glasses of water or fluid per day.

Another result that is of concern in this study is the very low intake of milk. Fewer than 10% of our subjects drank milk on a daily basis. The revelation is in agreement with studies by Norimah *et al.* (2008) & Talaei *et al.* (2018), who also reported a low level of milk intake among the Malaysian and Asian populations. As milk is the primary source of calcium, it is critical to ensure that the recommended level of daily calcium intake is fulfilled. However, previous study have shown that calcium intake among Malaysians is low and does not meet recommended nutrient intake (RNI) (Alam, 2012). Low calcium and vitamin intake, together with a sedentary lifestyle, are risk factors for osteoporosis. Therefore, an innovative approach should be made to promote proper nutrition and physical activity for healthy bones among young adults.

Many studies have compared dietary intake of SSBs with gender (Grimes *et al.*, 2013; Ha *et al.*, 2016; Ranjit *et al.*, 2010). However, our study did not find any gender differences in SSB consumption.

An important limitation of this study was the preponderance of females over males. More than 80% of the subjects were female. In general, females are more concerned about their calorie intake. This may explain the low intake of daily SSBs among them. Another limitation was that we did not take into account the food intake habits by way of daily total calorie intake of the subjects.

CONCLUSION

Understanding trends in plain water and SSB consumption, together with healthy and balanced dietary intake among young adults is imperative to formulate effective nutritional intervention strategies to achieve a healthy lifestyle. Ultimately, this may help to prevent the risk of obesity and related diseases later in life. The present study highlights that lowering SSB intake and increasing plain water intake, especially among the underweight, is crucial. The results from this study may help by providing information for future intervention studies. In addition to SSB, future interventions should also focus on a strategy to increase calcium intake among young adults.

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Authors' contributions

TNIMF, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; NJN, assisted in design the study, assisted in drafting the manuscript, reviewed the manuscript; ASM conducted the study, data analysis and interpretation, and prepared the draft of the manuscript.

Conflict of interest

There is no conflict of interest to declare.

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Bioaccumulation of heavy metals in different tissues of Nile tilapia (*Oreochromis niloticus*) in Bangladesh

Afroza Parvin[1], Md Kamal Hossain[1]*, Shabiha Islam[2], Saborni Swarna Das[2], John Liton Munshi[1], Priyanka Dey Suchi[1], Mohammad Moniruzzaman[1], Badhan Saha[1] & M Golam Mustafa[3]

[1]*Soil and Environment Research Section, Biological Research Division, BCSIR Laboratories Dhaka, Bangladesh Council of Scientific and Industrial Research, Dr. Qudrat-i-Khuda Road, Dhanmondi, Dhaka-1205;* [2]*Department of Fisheries & Marine Science, Noakhali Science and Technology University, Sonapur, Noakhali-3814, Bangladesh;* [3]*Department of Oceanography, Noakhali Science and Technology University, Sonapur, Noakhali-3814*

ABSTRACT

Introduction: The culture of Nile tilapia *(Oreochromis niloticus*) has become wide spread because of its high productivity over a short period of time. Its production partially fulfills the demand for food in rural people in Bangladesh. However, the accumulation of toxic heavy metals in the human body through consumption of fish contaminated by it causes various diseases. The aim of this study was to evaluate the bioaccumulation of five heavy metals, namely, cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni) and copper (Cu) in cultured Nile tilapia in the Noakhali region of Bangladesh. **Methods:** Fish were collected from three different fish farms in the Noakhali region and samples of gill, muscles and liver of tilapia were assayed for Cd, Cr, Pb, Ni and Cu using atomic absorption spectroscopy. Proximate composition of the tilapia was also determined. **Results**: Metal accumulation in different tissues was as follows: liver > gill > muscle. The accumulation of metals in the muscle, gill and liver was Ni > Pb > Cr > Cu > Cd, Pb > Ni > Cu > Cr > Cd and Pb > Cu > Ni > Cr > Cd, respectively. The bioaccumulation of lead was significantly increased in liver and gill while muscle showed the lowest value. **Conclusion:** It can be concluded that bioaccumulation of Pb, Cr and Ni in Nile tilapia in this study exceeds the permissible limits set for heavy metals by Food and Agriculture Organization (FAO) and International Atomic Energy Agency (IAEA)-407. This is potentially risky for consumers.

Keywords: Bioaccumulation, heavy metal, gill, muscles, liver

INTRODUCTION

Malnutrition in Bangladesh remains a challenge. Fish is the largest source of animal food accounting for approximately 60% of animal protein intake, at 18.1kg consumed per person per year (Belton *et al.*, 2014). It contributes to a healthy diet that provides high value amino acids, nutrients and essential omega-3 fatty acids. The American Heart Association has recommended that fish should be eaten at least twice per week in order to reach the required daily intake of omega-3 fatty acids (Kris-Etherton,

*Corresponding author: Dr. Md. Kamal Hossain
Biological Research Division, BCSIR Laboratories Dhaka, Bangladesh Council of Scientific and Industrial Research, Dr. Qudrat-i- Khuda Road, Dhanmondi, Dhaka-1205, Bangladesh
Tel: +8802-8625038, Ext-295; Email:kamalbcsir@gmail.com
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Harris & Appel, 2002). Fish meat is an important source of such nutrients as protein, unsaturated fatty acid and minerals (Zhao *et al.,* 2010).

Fishery is an important industry in Bangladesh. Fish is important from the aspect of national food security since it is estimated that 60% of animal protein is afforded through fish. Furthermore 11% of total population of the country obtained their livelihood through fisheries or related activities. Nearly 2% of national foreign currency earning is contributed by fisheries sector. Over the last five years, fish production has grown by 5.9% (DoF Bangladesh, 2014).

Nile tilapia (*Oreochromis niloticus*), is one of the most important fish species that is consumed and is very important in world fisheries (FAO, 2011). It is extensively cultured in Bangladesh to meet the protein demand from all walks of life because of its cost-effectiveness. Fish muscle contains 19.5g/100g protein, 2g/100g fat, 0.70g/100g vitamin B_{12}, 6.3g/100g vitamin D_3 and 0.40g/100g vitamin E, in the raw edible parts. Apart from proximate composition, Nile tilapia contains iron, zinc, calcium, iodine, selenium, phosphorus, magnesium, sodium, potassium, manganese, sulphur, copper (Cu) and at the levels of 1.1mg, 1.2mg, 95mg, 11g, 26g, 190mg, 26mg, 81mg, 280mg, 0.052mg, 240mg and 0.031mg per 100g raw edible part, respectively (Bogard *et al.*, 2015). Bangladesh is currently generating about 0.5 million metric tons (MT) of Nile tilapia and Pangus annually together from its fresh water and brackish water aquaculture system. According to the Yearbook of Fisheries Statistics of Bangladesh, there was a rapid development in Nile tilapia farming which showed an increase in annual production in this country from 2,140 MT to 370,017 MT during the period 1999 to 2016-2017 (FRSS, 2017). The production of Nile tilapia was 16.7% of total pond fish production in the year of 2016-2017.

Heavy metals are high-density non-biodegradable metals and metalloids with prolonged toxic effects, which, upon accumulation in the aquatic environment, are transferred to the aquatic biota through various pathways (Khalifa *et al.*, 2010). This ecotoxicity can impact changes on species diversity and the ecosystem (Türkmen *et al.*, 2009; Storelli *et al.*, 2005). Heavy metals eventually enter the food chain and their bioaccumulation and magnification can cause physiological and morphological alterations not only in aquatic animals but in human beings as well (Vinodhini & Narayanan, 2008). Bioaccumulation results in an increase in the concentration of a toxic xenobiotic substance in an organism with time that parallels the xenobiotic concentration in the environment. These substances can, in turn, have carcinogenic, cytotoxic and mutagenic effects on humans who consume these organisms (Rauf, Javed & Ubaidullah, 2009). Although fish is highly nutritious, its high consumption rate can have significant deleterious effects on human health because of accumulated toxic metals beyond permissible safety limits.

The production of this fish was 93,132 MT in the Noakhali region of Bangladesh, during the period 2016-17. The aim of the present study was to evaluate the level of contaminant heavy metals such as cadmium (Cd), chromium (Cr), lead (Pb), nickel (Ni) and Cu of Nile tilapia from three different fish farms of Noakhali region, at the three organ sites namely muscles, gill and liver, and, in addition, in the feed and sediments.

MATERIALS AND METHODS

Sample collection

Nile tilapia fingerlings were cultured in three different commercial farms in the

Noakhali region for one year from August 2015 to August 2016. The average pH, temperature, turbidity and dissolved oxygen in different ponds were 7.1, 30^0C, 117 Nephelometric Turbidity Units (NTU) and 4.7mg/L respectively. The fingerlings in each farm were fed once a day at a fixed feeding rate. In the first 20 days of the experiment, feeds were given at 10% of body weight, 5% in next 20 days and 1% for the remaining experimental days. The fish were sampled by following an appropriate sampling procedure. Eighteen samples of fish were randomly collected from August 23, 2015 to August 28, 2016 through cast-net and stored at -20^0C to prevent deterioration. After recording the wet body weight (150-200g) and total body length (22-25 cm), the samples were washed, preserved in ice boxes, and transported to the Bangladesh Council of Scientific and Industrial Research (BCSIR) for heavy metal analysis where they were stored in a freezer. Feed, sediment and water samples were also simultaneously collected in plastic containers from each farm. The physicochemical parameters of the farm water, the heavy metal content of the feed, sediment and water samples have been discussed in our previous paper (Das *et al.*, 2017).

Sample preparation

The frozen fish samples were thawed at room temperature and dissected using stainless steel scalpels. Muscle, the entire liver and gill from each sample were dissected for analysis. For the proximate components (protein, fat, moisture, ash), standard analytical methods as per the Association of Official Analytical Chemists (AOAC) were used. The determination of protein (block digestion - AOAC 981.10), fat (acid hydrolysis - AOAC 948.15), moisture (air drying – AOAC 950.46), ash (direct method – AOAC 920.153) were performed in triplicate.

The dissected samples were taken separately and kept in an oven at 70^0C for about 24 hours. The samples were then ground into a fine powder.

Digestion processes

Fish samples were digested according to the AOAC (1995) technique. A 0.5g dried powder of muscle, liver and gill from each sample were separately weighed in different glass beakers, to which was added 10ml of concentrated nitric acid and then covered with a watch glass. The samples were digested on a hot plate at 80-90°C. After 2 hours when solutions became almost transparent, the watch glasses were removed from beaker. The beakers were then removed from hot plate. When solution became cooler, 5ml perchloric acid was added after 5-15 minutes. All beakers were then placed on the hot plate under fume hood at 100-150°C temperature till the solution achieved a jelly-type steadiness. After complete digestion, the samples were cooled to room temperature and diluted to 25ml with distilled water. A blank solution was prepared in the similar way. Solutions were then filtered and collected into sterilized plastic bottles, before the analysis for heavy metals.

Heavy metal analysis

The concentrations of Cd, Cr, Cu, Pb and Ni were determined using an atomic absorption spectrophotometer (Simadzu: AA-7000). Standard solutions for calibration were prepared from commercially available chemicals. The heavy metal concentration in the unknown was determined by using following formula:

Actual concentration of heavy metal (mg/kg) in sample = Reading X Dilution Factor

where,

Reading = AAS reading of digest

Dilution Factor = volume of digest used / weight of digested sample

Bioaccumulation factor

The bioaccumulation factor (BAF) is an important parameter of environmental contamination. It is the extent to which from water pollutants get deposited into aquatic organisms such as fish. The BAF is the ratio of the pollutant concentration in the fish to that in water (Chiou, 2002). It was calculated as follows:

$$BAF = \frac{\text{Concentration of heavy metals in dry \% of fish muscle (mg kg}^{-1}\text{)}}{\text{Concentration of heavy metals in water (mg kg}^{-1}\text{)}}$$

Statistical analyses

All samples were collected and analysed in duplicate. Data were analyzed using Statistical Package for the Social Sciences (SPSS) software (version 15) (IBM Corp., Armonk, NY). The t-test for duplicate paired samples (at 95% significance) was performed to determine if the individual results in each pair were statistically similar. Differences in metal concentrations in the samples of fishes studied were determined using analysis of variance (ANOVA). Significant level was determined at $p<0.05$. Other calculations and graphs were performed by Microsoft Excel 2010 and origin 8.5.

RESULTS

The proximate composition of Nile tilapia fish was as follows: protein 19.46g, fat 2.10g, moisture 77.02g and ash 1.20g per 100 g raw edible parts. The results of our present study were consistent with those of Bogard *et al.* (2015).

Metal contamination in Nile tilapia fish is an important issue as this fish is an important source of nutrients for poor as well as rich people. A previous paper (Das *et al.*, 2017) had identified heavy metal contamination in this fish. Before remedial measures could be taken, the identification of the route by which heavy metals enter into fish tissues needed to be done. Since the level of heavy metals was below detection limit in three different farmed waters, they had to come from either feed or sediment. The content of heavy metals (Cd, Cr, Pb, Ni and Cu) in feed, sediment and Nile tilapia that were collected from three different fish farms and the permissible limits set by the International Atomic Energy Agency (IAEA)-407 (Wyse, Azemard & Mora, 2003) are presented in Table 1.

Table 1 shows the concentrations of heavy metals in feed, sediment and fish. The highest concentration of metal in feed sample was Cu which was present at a concentration of 12.14±0.60mg/kg and the lowest was Cd. In sediment sample, order of metal concentrations was Ni (25.00±1.10 mg/kg) was present in the highest concentration while Cd was the lowest. The heavy metal present in the highest concentration in the fish was Pb (3.56±0.71 mg/kg) followed by Ni, Cu, Cr and Cd. By comparing concentration of heavy metals in the Nile tilapia samples with the tolerable

Table 1. Concentration (mg/kg) of heavy metal in various tissues of farmed tilapia, feed and sediment, with IAEA-407 values for comparison

Heavy metals	*Feed* †	*Sediment* †	*Fish* †	*IAEA-407*
Cd	0.25±0.02	0.28±0.05	0.34±0.04	0.18
Cr	5.99±1.06	8.09±0.77	1.67±0.19	0.73
Pb	1.29±0.22	7.07±0.46	3.56±0.71	0.12
Ni	2.00±0.48	25.43±1.10	2.59±0.62	0.60
Cu	12.14±0.60	23.67±1.90	2.13±0.28	3.28

†Values are shown as mean±standard errors, n=3

Table 2. Comparison of heavy metal content in various tissues of fish sample (mg/kg) from our study and from other studies reported in the literature (Rajeshkumar & Li, 2018).

Sample	*Description*	*Heavy metals*					*Reference*
			Pb	*Cr*	*Cd*	*Cu*	*Ni*	
1	Noakhali fish farm, Bangladesh	Muscle	2.26	1.99	0.25	1.58	2.39	Our study
		Gill	2.59	1.26	0.10	1.50	2.32	
		Liver	4.63	0.77	0.72	2.96	2.68	
2	Dhanmondi Lake, Bangladesh	Muscle	2.08	-	-	-	-	Begum *et al.*, 2005
3	Taihu lake fish sample, China	Muscle	0.61	0.34	0.12	0.21	-	Rajeshkumar & Li, 2018
		Gill	0.49	0.16	0.12	0.24	-	
		Liver	0.60	0.35	0.12	1.45	-	
4	Kolleru lake, Kerala, India	Muscle	1.84	11.00	0.11	-	-	Sekhar *et al.*, 2004
		Gill	2.98	19.00	0.22	-	-	
		Liver	3.77	30.00	0.37	-	-	

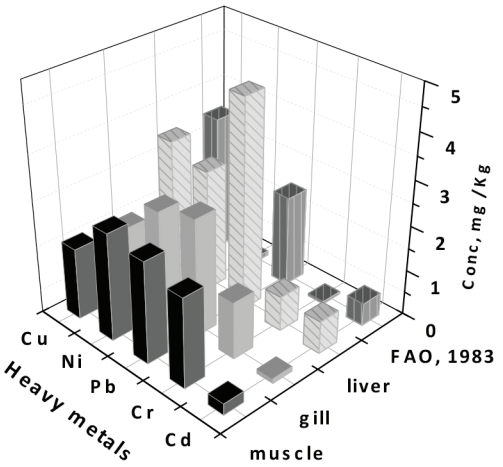

Figure 1. Concentrations of Cd, Cr, Pb, Ni and Cu in the different tissues of Nile tilapia compared with permissible values by FAO

values stated by IAEA-407, it was found that accumulated metals had reached beyond permissible levels for human consumption. The concentration of Pb showed a value that was very harmful to human health as well as other aquatic organisms also and thus is a matter of concern.

Table 2 depicts the comparison of Cd, Cu, Cr, Ni and Pb content in mg/kg in the muscle, gill and liver tissue of fish that were observed in this and other published studies by different authors. The concentrations of Cd, Cr, Pb, Ni and Cu with acceptable values by Food and Agriculture Organization (FAO) are presented in Figure 1. Lead is a toxic metal and its adverse health effects are well known. Of the different parts of Nile tilapia, the liver accumulated the highest Pb concentration at 4.63±0.54mg/kg, while muscle accumulated the lowest at 2.26±0.89mg/kg Pb. According to FAO (1983), the permissible level of Pb was 2mg/kg. The present study showed that the Pb levels in all the analyzed fish parts were well above the acceptable limit for human consumption. Concentrations of Cd in this study were low with the highest recorded value being 0.72±0.18mg/kg in liver followed by 0.25±0.03mg/kg in muscle and 0.10±0.04mg/kg in the gills. The Cd concentration in liver exceeded the permissible values by FAO. The presence of Cr in the diet is

essential, due to its active involvement in metabolism. According to FAO, the recommended maximum permitted value of Cr is 0.05mg/kg. In this study, the muscle accumulated the highest amount (1.99±0.09mg/kg) of Cr and liver accumulated the lowest amount (0.77±0.17mg/kg), which are several fold higher than the acceptable value. Copper is an essential trace element. However, very high levels of Cu can cause acute toxicity. Concentration of Cu in muscle, gill and livers were 1.58±0.24mg/kg, 1.50±0.49mg/kg and 2.96±0.15mg/kg, respectively. The safe level for Cu that is recommended by the FAO is 3mg/kg, which is slightly higher than our determined values. The concentration of Ni in the environment is normally very low, but can cause a variety of adverse health effects. The FAO prescribed the permissible limit of Ni to be 0.1mg/kg. The maximum (2.68±0.52mg/kg) concentration of Ni was measured in the liver and the minimum concentration (2.32±1.53mg/kg) was found in the gill. Our values for Ni were higher than the safety levels established by the FAO. Overall, the heavy metal accumulation in the muscle, gill and liver was Ni > Pb > Cr > Cu > Cd, Pb > Ni > Cu > Cr > Cd and Pb > Cu > Ni > Cr > Cd, respectively.

The bioaccumulation factor, which is an indicator of aquatic pollution, was not from water to fish as heavy metal content in water was below detection limit.

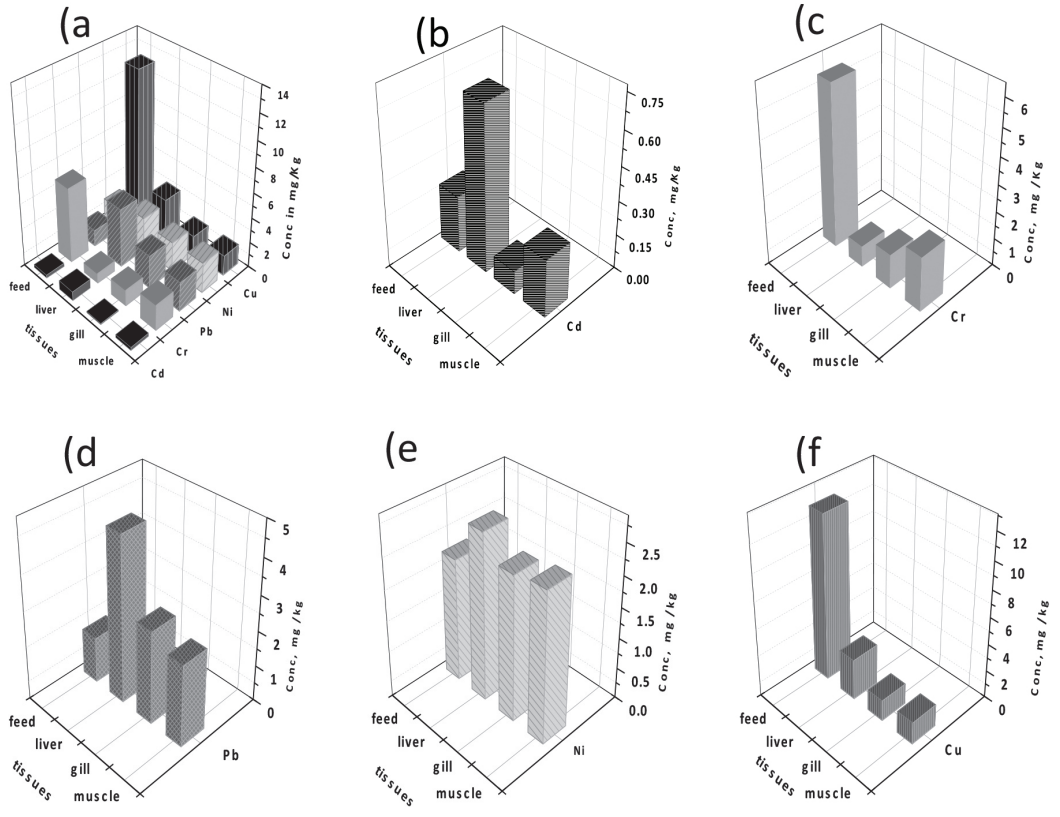

Figure 2. Bioaccumulation from feed to different tissues in Nile tilapia fish of (a) five selected heavy metals; (b) Cd; (c) Cr; (d) Pb; (e) Ni; and (f) Cu

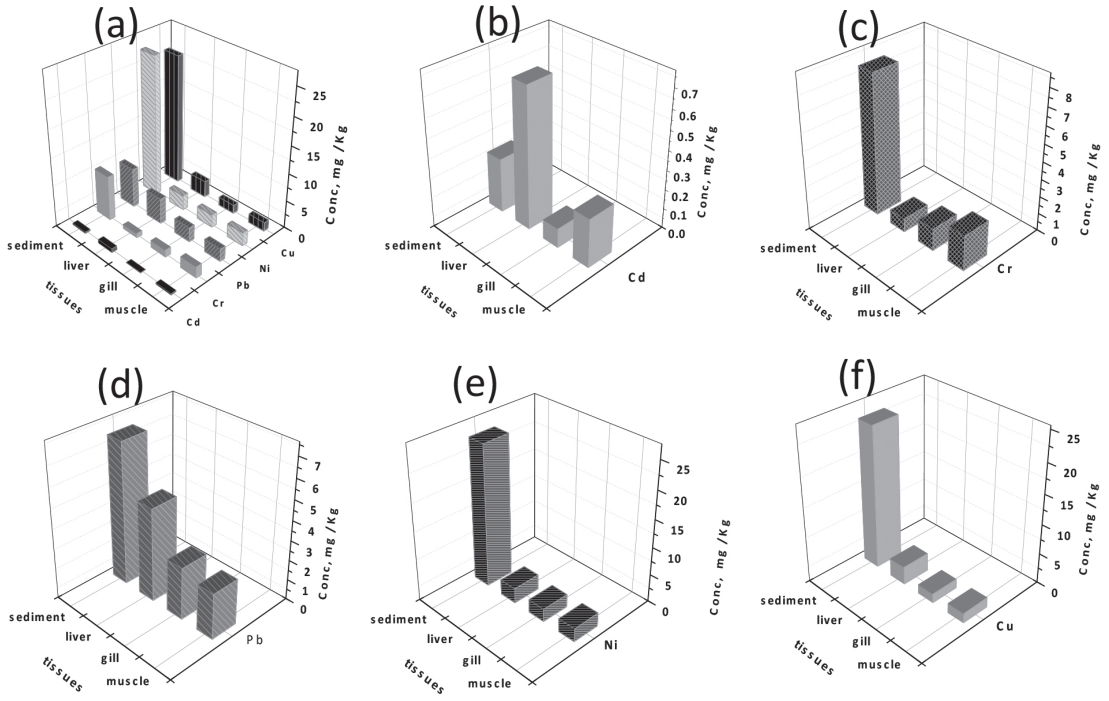

Figure 3. Bioaccumulation from sediment to different tissues in Nile tilapia fish of (a) five selected heavy metals, (b) Cd, (c) Cr, (d) Pb, (e) Ni and (f) Cu

There were significant differences in the muscle, gill and liver of the Nile tilapia as a result of the selective bioaccumulation by the tissues. Figure 2a shows the bioaccumulation of five selected trace metal from feed to different tissues of fish. As shown in Figure 2b, the liver accumulated the highest concentration of Cd followed by muscle and gill. Based on the distribution of heavy metals in various tissues of Nile tilapia, the sequence seems to be as follows: muscle > gill > liver (Figure 2c). Figure 2d shows the distribution of Pb in various tissues and it is in the following order liver > gill > muscle. Figure 2e shows the order of metal concentrations in the various tissues, which is as follows: liver > muscle > gill. In case of Cu distribution in different tissues, the sequence is shown in Figure 2f, which is liver > muscle > gill. In general, concentration of heavy metals is higher in feed than various tissues of fish. However, the scenario is reversed in case of Cd (Figure 2c), Pb (Figure 2d) and Ni (Figure 2e) where the concentration of the metal is higher in tissues than in feed. Thus, feed is not only source of heavy metals accumulation in fish. Another source of metal distribution in fish is sediment. Figure 3a shows the bioaccumulation of five selected trace metals from sediment to different tissues of fish. With the exception of Cd, the distribution of remaining four metals is several fold higher in sediment than in fish tissues. As shown in Figure 3b, the order of Cd distribution in various tissues is liver > muscle > gill. The concentration Cr in different tissues is liver > gill > muscle (Figure 3c). Based on the distribution of Pb in various tissues of fish, the sequence is shown in Figure

3d and the concentrations are liver > gill > muscle. Ni accumulation in fish tissue from sediment is shown in Figure 3e and it is liver > muscle > gill. As shown in figure 3f, liver accumulated the highest concentration of Cu followed by muscle and gill accumulated the lowest.

In general, the concentration of heavy metals was lower in the muscle tissues compared to other organs (liver, gill) of Nile tilapia.

DISCUSSION

The accumulation of heavy metals in fish occurs in many of its organs. Among the different organs, the liver accumulates higher concentrations of metals and has been used widely to investigate the process of bioaccumulation. Liver, kidney and gills were found to have the highest concentrations of heavy metals (Golovanova, 2008). The different organs in the body accumulate a specific metal to a high level though others do not accumulate the metal though present in the medium (Al-Kahtani, 2009). In present study, Pb accumulated in the order gill > liver > muscle. The gill is the one of sites which creates a path for the heavy metal to enter into the fish body (Bols *et al.,* 2001). Our results show that the liver accumulates highest level of metals followed by the gill and muscle. Bioaccumulation of heavy metal in muscles is lower. Fish liver and gills, both of which are metabolic active tissues, showed significantly higher abilities for the accumulation of all metals than the muscle. Similar results were also described by Jabeen, Javed & Azmat (2012). Yacoub (2007) reported this may be as the result of elevated metal–binding protein synthesized in gills and liver.

There are two reasons for lower metals accumulation in muscle: 1) The presence of a mucous layer coating the fish skin surface creates barrier that protects fish muscle tissue by forming complexes with the heavy metals that are present in surrounding environment (Uysal, 2008); 2) Jagakumar & Paul (2006) have stated that detoxification does not directly occur in the muscle. Hence, other tissues do not transport heavy metals to muscle.

Our results showed that the highest concentration of heavy metals are accumulated in the liver. This may be due to interaction of metals with the constituents of the target organ (Sorensen, 1991). As fish liver was seldom consumed, it was thus less of a threat to human health.

The presence of heavy metals in sediment and feed was found to be higher than in the different part of fish body. The Cu and Ni were in higher concentrations in both compared to the other three heavy metals. Besides their presence in fish feed, chemicals that are used near the fish culture pond for agricultural crops and vegetables also contain heavy metals that leach and contaminate them. The consequent accumulation in fish in turn then harms humans who consume them. In this study, Cd was abundant in fish feed and high levels of Cd were also detected in fish farm sediments. This finding is in agreement with that of a previous study by Kalantzi *et al.* (2013). The concentration of Cd in liver is higher than in feed and sediment. When fishes survive in high level of metal ions in aquatic environment, their tissues tend to take up these metal ions through various routes from their surroundings. In general, metals occur in very low concentrations (in nanogram to microgram per liter concentrations) amount in the natural aquatic ecosystems. Diet and water are two main pathways of metal accumulation in fish (Bury, Walker & Glover, 2003). The present study has shown that Nile tilapia accumulates metals to concentrations

many times higher than that which is present in their surrounding water.

CONCLUSION

Fish is widely consumed as a main source of nutrition in many coastal communities like Noakhali, in Bangladesh. The unconscious consumption of metal contaminated fish may result in severe chronic and acute diseases. This consumption is the main route for human exposure to heavy metals compared to other routes such as inhalation and dermal contact, if the fish contain excessive amount of toxic metals. The pollution of the aquatic environment by heavy metals has become a worldwide problem in recent times. In this study undertaken in the Noakhali region, the order of heavy metal accumulation in the muscle, gill and liver was Ni > Pb > Cr > Cu > Cd, Pb > Ni > Cu > Cr > Cd and Pb > Cu > Ni > Cr > Cd, respectively. In case of feed and sediment it was Cu > Cr > Ni > Pb > Cd and Ni > Cu > Cr > Pb > Cd, respectively. Of all the heavy metals, the bioaccumulation of lead was significantly increased in gill and liver of Nile tilapia. This study determined the concentration of heavy metals (Cd, Cr, Pb, Ni and Cu) of Nile tilapia sediment and fish feed. The highest levels of all the metals in the present study were detected in sediment and followed by feed. As such, there was a high possibility of bioaccumulation through commercial fish feed and sediment. Among the fish tissues, higher levels were found in liver and gill while muscle showed the lowest value of metal accumulation.

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Authors' contributions

HMK, one of the principal investigator, conceptual and designed the study, prepared the draft of the manuscript and reviewed the manuscript; conducted the study, data analysis and interpretation, assisted in drafting of the manuscript and reviewed the manuscript; MMG, another principal investigator, conceptual and designed the study, prepared the draft of the manuscript and reviewed the manuscript; IS, led the data collection in the Noakhali region, and sample digestions and analysis, conducted the study, data analysis and interpretation, assisted in drafting of the manuscript and reviewed the manuscript; PA, led the data collection in the Noakhali region and sample digestions and analysis; DSS, led the data collection in the Noakhali region and sample digestions and analysis and reviewed the manuscript; MJL, advised on the data analysis and interpretation and reviewed the manuscript; SPD, MM, and SB performed the heavy metals analyses using the atomic absorption spectrometer.

Conflict of interest

There is no conflict of interest.

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Orange-fleshed sweet potato (*Ipomoea batatas*) extract attenuates lipopolysaccharide-induced inflammation in RAW264.7 cells via inactivation of MAPKs and IκB signalling

Yada Saokosol[1], Kemika Praengam[2], Monruedee Sukprasansap[2] & Siriporn Tuntipopipat[2*]

[1]*Graduate student in Master of Science Program in Nutrition, Faculty of Medicine Ramathibodi Hospital and Institute of Nutrition, Mahidol University, Thailand*;
[2]*Institute of Nutrition, Mahidol University, Salaya, Nakhonpathom 73170, Thailand*

ABSTRACT

Introduction: Orange-fleshed sweet potato (OFSP) is an excellent source of β-carotene. Due to its health benefits, β-carotene-rich plants are receiving attention. This study aimed to assess the inhibitory effect of the ethanol extract of steamed OFSP on lipopolysaccharide (LPS)-induced inflammation in murine macrophage cell line (RAW 264.7 cells). **Methods:** β-carotene, total phenolics and total flavonoids of OFSP were measured by high performance liquid chromatography (HPLC), the Folin-Ciocalteu assay and the aluminum chloride colorimetry, respectively. RAW264.7 cell monolayers were pre-treated with 0.5-2.0 mg/mL ethanol extract from steamed OFSP prior to co-incubation with or without LPS for 24 h. Culture media and cell lysate were collected to measure nitric oxide, interleukin-6 (IL-6), IL-1β, tumour necrosis factor-α, inducible nitric oxide synthase, cyclooxygenase-2, mitogen-activated protein kinases (MAPKs) and inhibitory kappa B (IκB), respectively. **Results:** The ethanol extract from steamed OFSP significantly suppressed LPS-induced production of such pro-inflammatory mediators by the inactivation of MAPKs and IκB signalling pathway. The ethanol extract from steamed OFSP contained 226 μg/g DW (dry weight) of β-carotene, 2.13 mg gallic acid equivalent/g DW of total polyphenolics and 0.24 mg quercetin equivalents/g DW of total flavonoids. **Conclusion:** These results indicated that bioactive compounds in steamed OFSP have anti-inflammatory potential.

Keywords: Orange-fleshed sweet potato, β-carotene, anti-inflammation, RAW264.7 cells, lipopolysaccharide

INTRODUCTION

Chronic inflammation has an association with the pathogenesis of obesity, metabolic syndrome and type 2 diabetes mellitus (Esser *et al.,* 2014). During an inflammatory response, various transcription factors including nuclear factor-kappa B (NF-κB) are activated resulting in up-regulated expression of pro-inflammatory mediators (Liu *et al.,* 2017). The over-expression of pro-inflammatory mediators during chronic

*Corresponding author: Siriporn Tuntipopipat, Ph.D.
Institute of Nutrition, Mahidol University, Salaya, Nakhonpathom, Thailand 73170
Tel: +66(0)-2800-2380; Fax: +66(0)-2441-9344; E-mail: siriporn.tun@mahidol.ac.th
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inflammation has been hypothesised to be an initiating or aggravating factor for development of some chronic diseases (Aggarwal & Shishodia, 2004). The macrophage is an innate immune cell that plays an important role during inflammatory responses against noxious stimuli, including lipopolysaccharide (LPS). When macrophages are exposed to LPS, NF-κB and mitogen-activated protein kinases (MAPKs), including p-38, extracellular signal-regulated kinases (ERK1/2) and c-Jun N-terminal kinases (JNK) are activated, resulting in up-regulation of various pro-inflammatory mediators including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (Guha & Mackman, 2001).

Various dietary phytochemicals including carotenoids, phenolic acids and flavonoids in colourful dietary plants have demonstrated multiple benefits including antioxidant, anti-cancer and anti-inflammatory activities (Aggarwal *et al.,* 2009; Pan, Lai & Ho, 2010). The sweet potato (*Ipomoea batatas*) with various coloured tuberous roots is an important staple food in Sub-Saharan Africa, Asia and the Pacific Islands. In particular, the orange-fleshed sweet potato (OFSP) is a good source of several nutrients, β-carotene, phenolic acids and flavonoids (Park *et al.,* 2016). Being a rich source of β-carotene, previous studies have investigated its potential to improve vitamin A status in poor communities (Gurmu, Hussein & Laing, 2014; Jamil *et al.*, 2012). Recently, the impact of pasteurisation and sterilisation on its β-carotene content and bioaccessibility were evaluated in an OFSP-based baby puree (Dhuique-Mayer *et al.*, 2018). Currently, the health benefits of β-carotene, such as its antioxidant and anti-inflammatory activities are gaining interest. In addition, the effect of thermal processing on carotenoid stability, total phenolics and antioxidant capacity of OFSP cultivars have been evaluated (Donado-Pestana *et al.,* 2012). However, the anti-inflammatory activity of OFSP has never been investigated. This present study aims to assess anti-inflammatory activity of extract from OFSP in LPS-stimulated murine macrophage cell line (RAW264.7 cells).

MATERIALS AND METHODS

Chemicals and reagents

The chemicals that were used were analytical and high performance liquid chromatography (HPLC) grade. Enzyme-linked immunosorbent assay (ELISA) kits were purchased from BioLegend (San Diego, CA, USA). Antibodies against iNOS, COX-2, inhibitory kappa B (IκB) and MAPKs were purchased from Cell Signalling Technology (Danvers, MA, USA). Anti-IL-1β was purchased from Peprotech (USA). Carotenoid standards, anti–β-actin–horseradish peroxidase (HRP), secondary antibody, Dulbecco's Modified Eagle's Medium (DMEM), LPS (E. coli O11:B4), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Sigma. Fetal bovine serum (FBS) was obtained from Merck (Darmstadt, Germany). Penicillin-streptomycin solution was purchased from Caisson labs (Smithfield, UT, USA). Trypsin-Ethylenediaminetetraacetic acid (EDTA) was purchased from Gibco (Grand Island, NY, USA).

Preparation of steamed OFSP

OFSP tubers were bought from three major distributors (10 kg from each distributor) at a wholesale market at Pathum Thani province, Thailand. Each distributor, in turn, had bought the OFSP from three different growing areas (Nakhon Ratchasima, Lopburi and Suphanburi province). The tubers were washed with tap water and peeled

prior to washing again with tap water. Approximately 1 kg of peeled OFSP from each distributor was steamed in a steaming basket over boiling water for 45 min, cooled down at 25°C and blended with an electric kitchen blender, prior to lyophilization by freeze dryer (GEA Lyophil GmbH, Germany) to preserve stability of phytochemicals. The dried samples were homogenised by a kitchen electronic blender (Philips). An equal amount of dry sample from each distributor was pooled together and thoroughly mixed prior to being packed in aluminum foil under vacuum and stored at -20°C until usage.

Carotenoid analysis

To 1 ml deionised (DI) water, 0.02±0.001g of dried sample was added. The resulting suspension was homogenised on ice by an ultrasonic processor (130 Watt, 20 kHz; Sonics & Materials, Inc., Newton, USA) for three cycles of 30 sec on/off pulsing (personal communication with Failla *et al.,* 2018 at the Ohio State University). Nine mL of absolute ethanol (resulting in a 90% final concentration) was added to the suspended sample. Separately, to another suspension, 10 mL of mixed solvent [hexane:acetone:ethanol (2:1:1)] (Kubola & Siriamornpun, 2011) was added. Both were thoroughly mixed for 2 min followed by sonication in an ultrasonic bath for 10 min (Daihan Scientific Co., Ltd., WUC-A02H, Korea) and centrifugation (Hettich, Rotina 38R, Tuttlingen, Germany) at 4,140 g at 25°C for another 10 min. The extraction procedures were repeated two times. The combined supernatants were evaporated until dry by using rotary evaporator (Buchi Rotavapor-Re-124, Switzerland) under vacuum at 38-40°C. The dry film was reconstituted with 1.5 mL methyl-tert-butyl-ether (MtBE) and 500 µL methanol before it was vigorously mixed and sonicated. The sample was then passed through 0.22 µm polytetrafluoroethylene (PTFE) membrane filter and diluted to an appropriate concentration with MtBE:methanol (3:1, v/v) solution prior to analysis by HPLC. The carotenoid content was determined by the previously described method of Failla, Thakkar and Kim (2009) by using an HPLC system (Agilent 1100 series, Santa Clara, CA, USA) with photodiode array detector and separated in C30 reverse-phase column (YMC 150 mm x 4.6 mm ID, 5 µm, Japan) with a C18 cartridge guard column (4 mm x 3 mm ID, Phenomenex, USA) at 25 °C. Carotenoids were eluted at a flow rate of 0.6 mL/min with 20 µL injection volume. The mobile phases consisted of 98% methanol in ammonium acetate buffer (Solvent A) and MtBE (Solvent B) with the following solvent gradient profile: 80% A for 0-1 min, 60% A for 1-10 min, 40% A for 10-20 min, 25% A for 20-30 min and 80% A for 30-37 min. The eluted carotenoids were identified by comparison of their retention time and absorption spectra at 450 nm with carotenoid standards and quantified by comparison of the peak area of sample with those of standard curves of lutein, zeaxanthin, β-cryptoxanthin, lycopene, α-carotene and β-carotene.

Determination of total polyphenol and flavonoid content

To 2 mL deionised water was added 0.5±0.01g of dried sample and the suspension homogenized on ice by an ultrasonic processor for three cycles of 30 sec on/off pulsing. This was followed by the addition of 18 mL absolute ethanol and the mixture shaken at 25°C for 2 h before centrifugation at 1,400 *g* for 20 min. The supernatant was collected to measure total polyphenols and flavonoids.

The total polyphenol content was determined by the Folin-Ciocalteu assay (Alhakmani, Kumar & Khan, 2013). Twenty-five µL of extract or gallic acid (standards) or DI water (blank) were

each transferred into 96-well microplate and mixed with 50 µL of diluted Folin-Ciocalteu reagent (1:10) at 25°C for 5 min followed by addition of 200 µL of 7.5% sodium carbonate (Na_2CO_3) and incubated at 25°C for 2 h in the dark. The absorbance of the reaction mixture was measured at the wavelength of 760 nm. The total phenol content was calculated by reading off the absorbance of sample from the gallic acid standard curve and expressed as mg gallic acid equivalent per gram of dry weight (mg GAE/g DW).

Total flavonoid content was measured by the colorimetric method of Prommuak, De-Eknamkul & Shotipruk (2008). The 25 µL of extract was mixed with 75 µL of 90% ethanol, 5 µL of 10% aluminum chloride, 5 µL of 1 M potassium acetate and 140 µL of DI water. The mixture was incubated at 25°C for 30 min in the dark. Sample blank of extract and standard quercetin solutions were prepared in the same procedure by replacing aluminium chloride solution with DI water. The absorbance of the reaction mixture was measured at the wavelength of 415 nm and total flavonoid content was determined by comparing the absorbance of sample with those of quercetin standards and expressed as mg quercetin equivalents per gram dry weight (mg QE/g DW).

Sample extraction for cell treatment

Two sets of 0.50±0.02 g dried samples were suspended in 2 mL DI water and homogenised on ice by an ultrasonic processor for three cycles of 30 sec on/off pulsing. Eighteen mL absolute ethanol was added in a suspended sample (90% ethanol) and 20 mL of mixed solvent was added to another suspended sample (less polar extract) and mixed for 2 min before sonication in an ultrasonic bath for 10 min and centrifugation at 4,140 *g* for 10 min at 25°C. The extraction procedures were repeated three times. The supernatants were evaporated until dry by using a rotary evaporator at 38-40°C and kept at -20°C until use. Yields of 90% ethanol and mixed solvent extracts were 0.11±0.002 g and 0.05±0.002 g, respectively. The dried extract was reconstituted with 0.2% dimethyl sulfoxide (DMSO) and further diluted to designated concentrations with serum/phenol free medium before passing through a sterile 0.2 µm membrane filter for treatment with cell monolayers.

Cell growth and activation

Murine macrophage RAW264.7 cells were purchased from ATCC (Rockville, MD, USA). This cell line was established from a tumour induced by the Abelson murine leukemia virus derived from BALB/c mice. Stock RAW264.7 cells at passage number 6 were stored in liquid nitrogen. Cells with passage numbers of 10-20 were used in the experiments. The cells were grown in complete medium (DMEM supplemented with 10% FBS, 15 mM HEPES, 100 U/mL of penicillin and 100 µg/mL of streptomycin) at 37°C in humidified atmosphere of 5% CO_2/95% air. Cell monolayers were seeded for 24 h prior to incubation with or without non-toxic doses of extract or ferulic acid (FA), a well-known anti-inflammatory phenolic acid, in serum/phenol red free media for 1 h followed by co-culturing with or without 2 ng/mL LPS for another 24 h.

Cytotoxicity test

The cytotoxicity of the OFSP extracts were assessed by sulforhodamine B (SRB) assay (Vichai & Kirtikara, 2006), to select the non-toxic doses of the extract prior to conducting other experiments. Briefly, RAW264.7 cells were pre-treated with 0.5-2.0 mg/mL OFSP extracts from 90% ethanol or mixed solvent or 0.2% DMSO (vehicle control) or FA in serum/phenol red free media for 1 h prior to addition with or without 2 ng/mL LPS for another

24 h. Cell monolayers were washed with cold phosphate-buffered saline (PBS) prior to fixing with 50% trichloroacetic acid and then incubated at 4°C for 2 h. After excessive washing with DI water and air-drying, the cellular protein was stained with 7 mM SRB in 1% acetic acid for 20 min before extensively rinsing with 1% acetic acid to remove excess SRB and air-drying. The protein stained with SRB was solubilized with 10 mM Tris-hydro-methyl-aminomethane for 5 min on gyratory shaker. The absorbance was read at the wavelength of 500 nm and reference wavelength of 690 nm by a microplate reader. The absorbance was observed to be proportionate to the cell number. The absorbance of cells treated with LPS in the control vehicle was defined as 100% viability. The non-toxic doses of the extract were selected when the cells treated with extract and LPS had more than 90% cell viability relative to cells treated with LPS in the control vehicle.

Measurement of nitric oxide (NO), TNF-α and IL-6

After LPS stimulation, the culture media were collected to measure the nitrite concentration (a stable product of NO) by the Griess reagent, and, TNF-α and IL-6 by ELISA kits (BioLegend, San Diego, CA, USA).

Briefly, 100 µL of culture medium, standard sodium nitrite ($NaNO_2$) and DI water were each mixed with an equal volume of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% *N*-(1-Naphthyl) ethylenediamine dihydrochloride in DI water) and incubated for 10 min at 25°C. The absorbance was measured at 520 nm. NO level was estimated from a $NaNO_2$ standard curve ($y = 0.0226X + 0.0023$, $R^2 = 1$).

Briefly, high-binding 96-well plates (NUNC, Roskilde, Denmark) were incubated overnight with capture antibody for mouse TNF-α and IL-6 at 25°C. After washing with 0.05% Tween-20 in PBS (PBST), the unbound sites were blocked with 1% bovine serum albumin (BSA) in PBS for 1 h at 25°C. After washing with PBST, culture medium or recombinant mouse TNF-α or IL-6 standards or DI water were added to each well and incubated for 2 h at 25°C prior to addition of biotinylated TNF-α or IL-6 antibodies to each well. After 1 h incubation and washing with PBST, the immune complex was detected with streptavidin HRP–tetramethylbenzidine detection system by incubating at 25°C for 30 min. Reactions were terminated with sulfuric acid (H_2SO_4) and the absorbance at 450 nm was measured by microtiter plate reader. Concentrations of TNF-α and IL-6 in samples were calculated from their standard curves. The equations of TNF-α and IL-6 standard curves were $y = 0.0015X + 0.0481$ ($R^2 = 0.9978$) and $y = 0.0016X + 0.0092$ ($R^2 = 0.9981$), respectively.

Western blot analysis

After LPS activation, the treated cell monolayers were washed with cold PBS and treated with ice-cold lysis buffer [50 mM Tris-hydrochloride pH 7.4, 150 mM sodium chloride (NaCl), 1 mM EDTA, 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 1% phosphatase inhibitor cocktail (Bio Basic Inc., Ontario, Canada) and 0.5% protease inhibitor cocktail (Sigma)] at 4°C for 30 min, on an orbital shaker. The supernatants of cell lysate were collected after centrifugation at 12,000 g at 4°C for 5 min. The protein concentration was determined by bicinchoninic acid assay. Samples [20 µg, 40 µg or 80 µg protein/well for IL-1β, iNOS, COX-2, IκB and MAPKs in loading buffer, respectively] were separated by 8% (for iNOS and COX-2), 10% (for IκB and MAPKs protein) or 12% (for IL-1β) of SDS-PAGE and transferred onto 0.45 µm nitrocellulose membranes (Whatman

GmbH, Dassel, Germany). Other procedures were followed as previously described (Tuntipopipat *et al.,* 2011).

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 19.0 (SPSS Inc., Chicago, IL) was used to analyze data. All data were presented as mean±standard deviation (SD) from at least two separate experiments conducted on separate days. The statistical significance was determined by one-way analysis of variance (ANOVA) with Tukey's HSD (honestly significant difference) test for multiple comparisons to identify mean differences among treatment groups. Statistical significance was set at $p<0.05$.

RESULTS

Bioactive compounds in steamed OFSP extract

The HPLC chromatograms identified that β-carotene was the predominant carotenoid in OFSP extracted with 90% ethanol and solvent mixture of hexane, acetone and ethanol (in supplementary material). The β-carotene content of ethanol extract from steamed OFSP was 226±11 μg/g DW, while that of mixed solvent extract was 283.8±8.6 μg/g DW. Total polyphenol and flavonoid content in the ethanol extract of OFSP were 2.13±0.05 mg GAE/g DW and 0.24±0.01 mg QE/g DW, respectively.

Effect of 90% ethanol or mixed solvent extract from OFSP on cell viability

The cells treated with 0.5-2.0 mg/mL of 90% ethanol or mixed solvent extracts or ferulic acid followed by LPS treatment had a cell viability of more than 90% as compared with those treated with LPS alone. These results indicated that 0.5-2.0 mg/mL of 90% ethanol or mixed solvent extracts or ferulic acid did not show a significant effect on cell viability.

Comparison of 90% ethanol and mixed solvent extracts from steamed OFSP on LPS-induced NO production

RAW264.7 cell monolayers treated with ethanol extract from steamed OFSP at 0.5-2.0 mg/mL significantly inhibited LPS-induced NO production in a dose-dependent manner (Figure 1a) as compared to those treated with LPS alone ($p<0.05$), whereas cells treated with the extract from mixed solvent did not show any suppressive effect (Figure 1b). Thus, only the ethanol extract was used to assess other anti-inflammatory activities in the present study.

OFSP ethanol extract decreased NO production and iNOS expression

RAW264.7 cells exposed to LPS significantly produced NO while vehicle treated cells or the cells treated with ethanol extract alone had no significant effect (Figure 2a). Compared to the LPS treatment group, cell monolayers treated with OFSP ethanol extracts or ferulic acid significantly decreased LPS-induced NO production ($p<0.05$). NO is the product of enzyme iNOS. As expected, cells exposed to LPS up-regulated iNOS protein expression (Figure 2c) and the extracts significantly suppressed LPS-induced iNOS protein expression in a dose dependent manner when compared to LPS-only treatment group ($p<0.05$). Thus, the OFSP ethanol extract decreased NO secretion by inhibiting iNOS protein expression.

OFSP ethanol extract inhibited TNF-α, IL-6, IL-1β and COX-2 production

Exposure of RAW264.7 cells to LPS significantly produced TNF-α, IL-6 and IL-1β whereas the control vehicle or cells treated with extract alone had no significant effect (Figure 2b, d and f). Cell monolayers treated with OFSP ethanol extracts or ferulic acid before co-incubation with LPS significantly

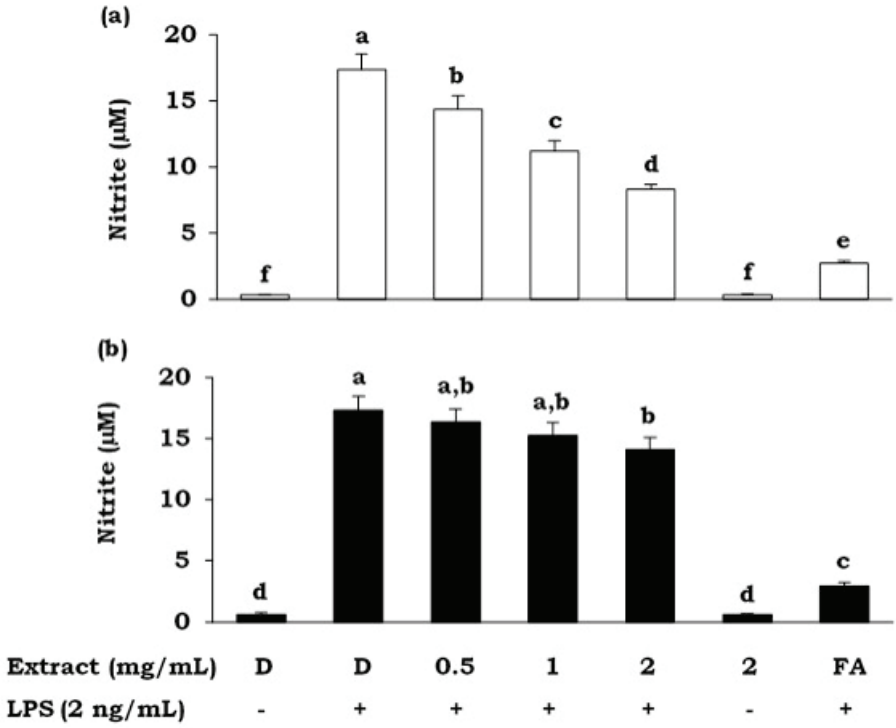

Figure 1. Effect of ethanol and mixed solvent extracts on LPS-induced NO production. Cells were pre-treated with 0.5-2.0 mg/mL of **(a)** ethanol or **(b)** mixed solvent extract from steamed OFSP or 0.2% DMSO (D) or 25 μM ferulic acid (FA) in serum/phenol red free media for 1 h, followed by 2 ng/mL LPS for 24 h. Ferulic acid was used as a control system. Nitrite in culture media was measured by Griess reagent. Data were expressed as mean±SD (n=6). Different letters above the error bars indicated significant differences among treatment groups ($p<0.05$)

decreased TNF-α, and IL-6 production and IL-1β expression when compared to LPS-only treatment group ($p<0.05$).

COX-2 is an inducible pro-inflammatory enzyme produced by macrophages during the inflammatory process. As expected, RAW264.7 cells exposed to LPS significantly enhanced COX-2 expression, whereas cells pre-treated with OFSP ethanol extracts or ferulic acid significantly inhibited LPS-induced COX-2 protein expression (Figure 2e) as compared to LPS-only treatment group ($p<0.05$). These results indicated that bioactive compounds in the ethanol extract of OFSP exerted anti-inflammatory activity by the suppression of LPS-induced pro-inflammatory mediator production.

OFSP ethanol extract suppressed MAPKs phosphorylation

Cell monolayers treated with LPS for 24 h markedly activated phosphorylation of ERK1/2, JNK and p38 (Figure 3a-c) without effecting their total ERK1/2, JNK and p38. Cell monolayers treated with the OFSP extract significantly inhibited phosphorylation of ERK1/2 and JNK in a dose-dependent manner (Figure 3a, b) whereas phosphorylation of p38 was significantly inhibited only at 1-2 mg/mL of the OFSP extract (Figure 3c) as compared to LPS-only treatment

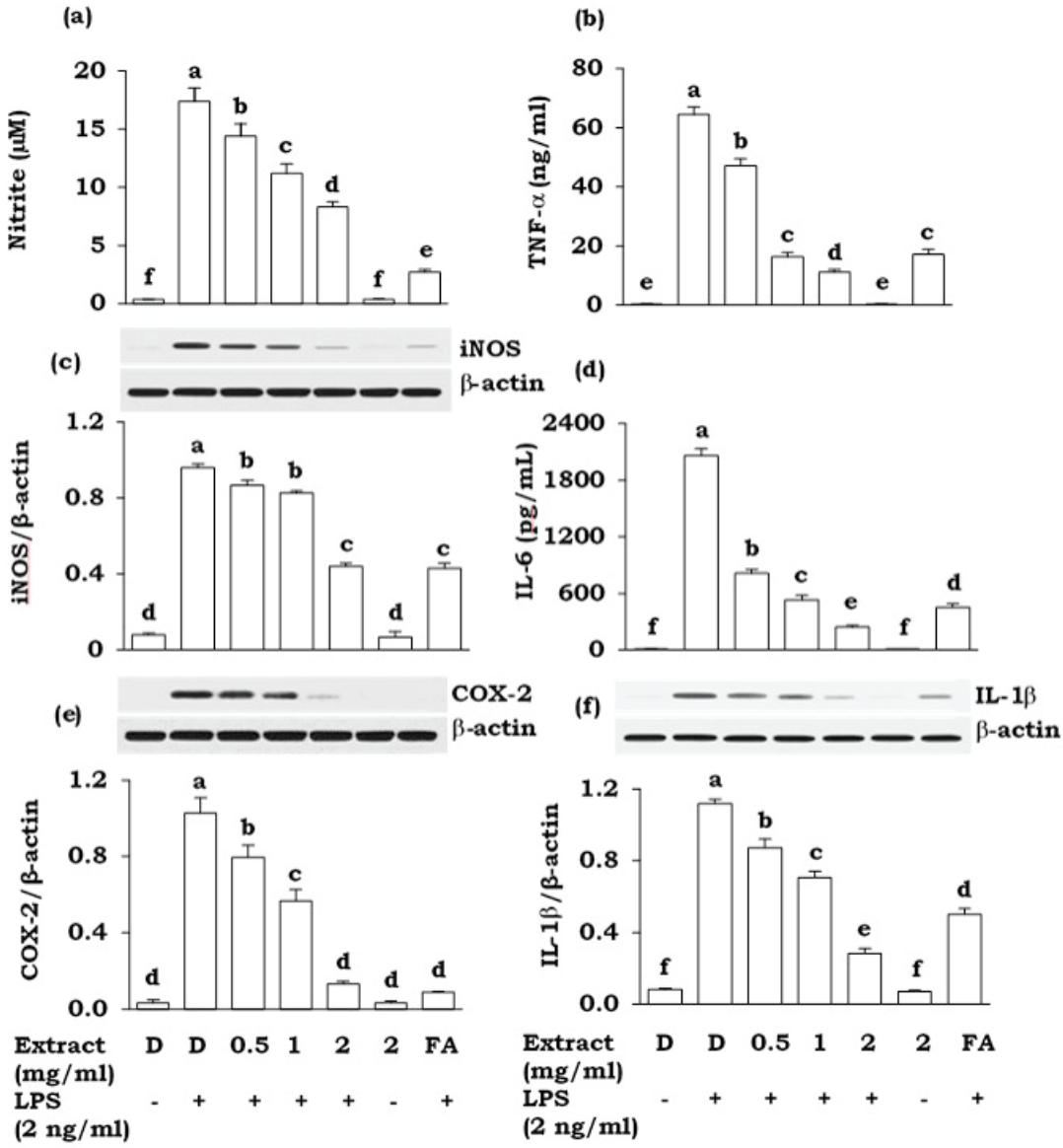

Figure 2. Ethanol extract from steamed OFSP inhibited LPS-induced NO, TNF-α, IL-6 production, iNOS, COX-2 and IL-β expression. Cells were treated with 0.5-2.0 mg/mL ethanol extract or 0.2% DMSO (D) or 25 μM ferulic acid (FA) in serum/phenol red free media for 1 h, followed by 2 ng/mL LPS for 24 h. Culture media were collected to measure **(a)** nitrite, **(b)** TNF-α and **(d)** IL-6 and cell lysates were collected to measure **(c)** iNOS/β-actin, **(e)** COX-2/β-actin and **(f)** IL-1β/β-actin. Data were expressed as mean±SD (n=6). Different letters above the error bars indicated significant differences among treatment groups ($p<0.05$)

group (p<0.05). Ferulic acid treated cells also significantly suppressed phosphorylation of ERK1/2, JNK and p38 (Figure 3a-c). Thus, the steamed OFSP ethanol extract inhibited pro-inflammatory mediator expression by blocking MAPKs phosphorylation.

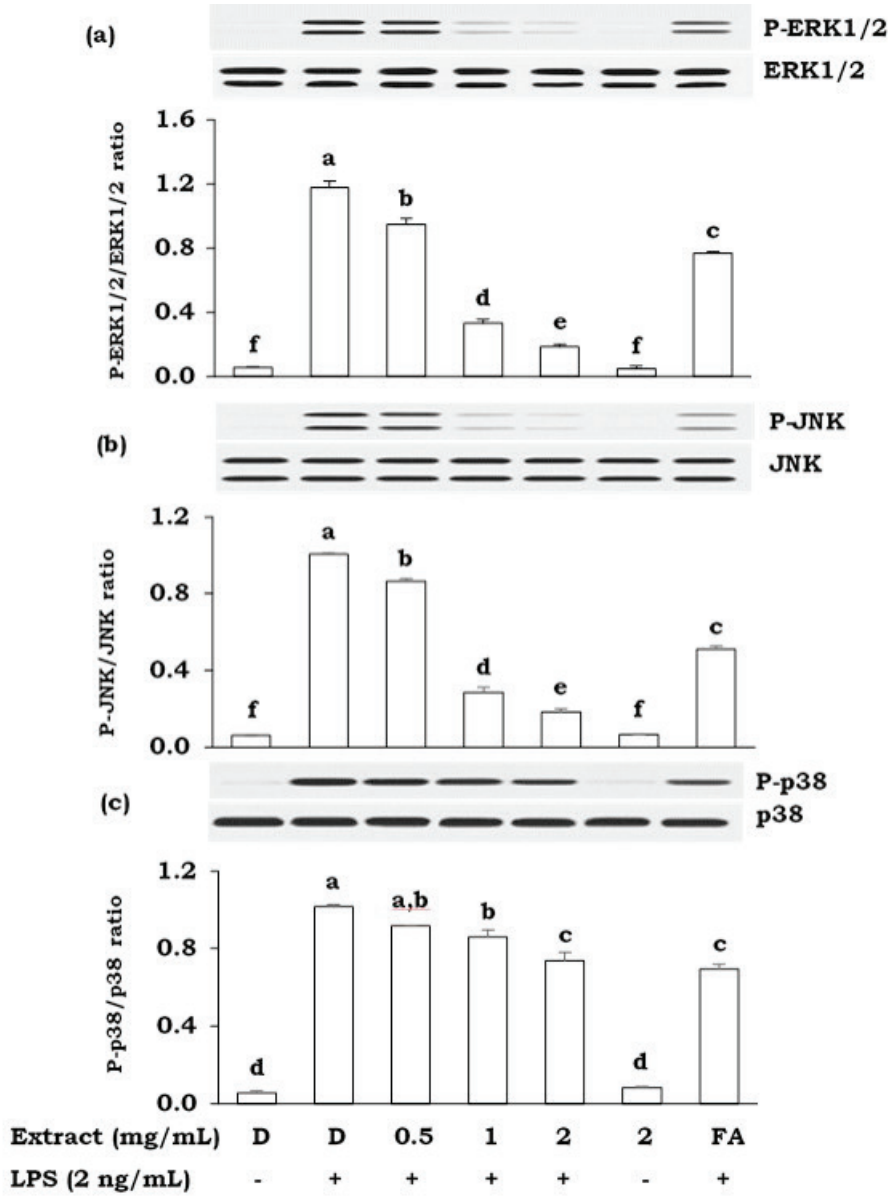

Figure 3. Ethanol extract from steamed OFSP inhibited LPS-activated MAPKs signalling. Cells were treated with 0.5-2.0 mg/mL ethanol extract or 0.2% DMSO (D) or 10 μM ferulic acid (FA) in serum free media for 1 h, followed by 2 ng/mL LPS for 24 h before collecting cell lysate to measure **(a)** phospho-ERK1/2 and total ERK1/2, **(b)** phospho-JNK and total JNK and **(c)** phospho-p38 and total p38. Data were expressed as mean±SD (n=4). Different letters above the error bars indicated significant differences among treatment groups ($p<0.05$)

OFSP ethanol extract inhibited IκB phosphorylation and degradation

LPS activated RAW264.7 cells induced IκB phosphorylation and degradation (Figure 4a, b). Pretreatment of cell monolayers with OFSP extract significantly inhibited LPS-activated IκB phosphorylation and degradation at 1-2

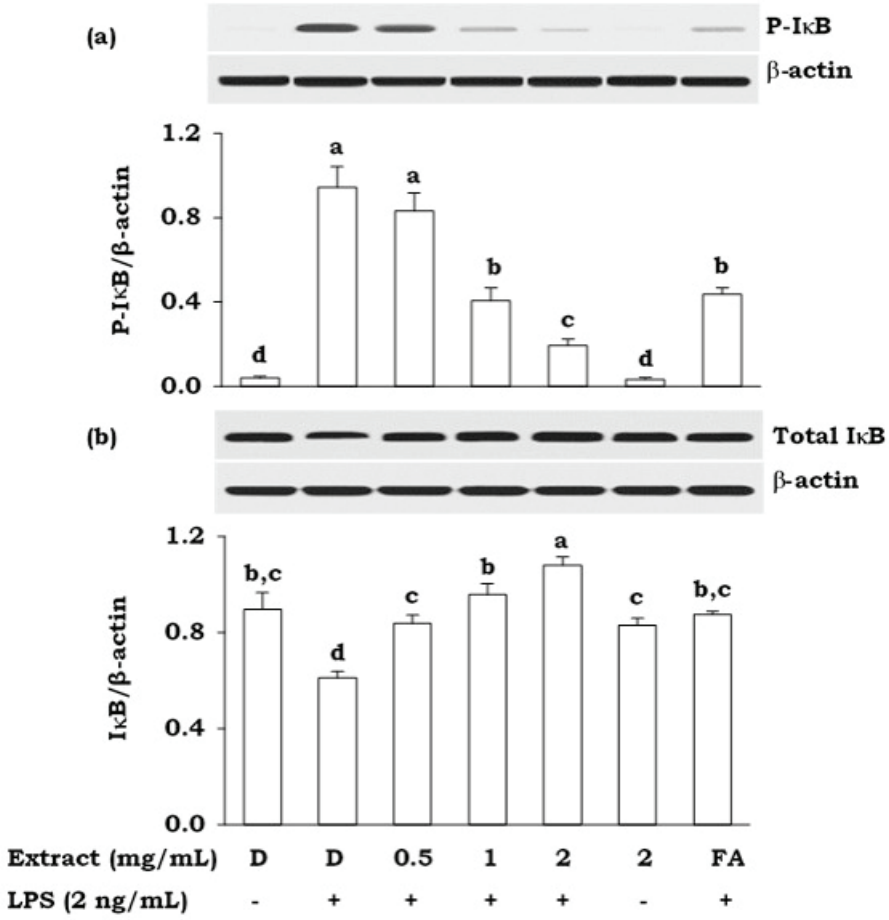

Figure 4. Ethanol extract from steamed OFSP inhibits LPS-activated IκB phosphorylation and degradation. Cells were pre-treated with 0.5-2.0 mg/mL ethanol extract or 0.2% DMSO (D) or 25 μM ferulic acid (FA) in serum free media for 1 h, followed by 2 ng/mL LPS for 24 h before collecting the cell lysates to measure **(a)** phospho-IκB/β-actin and **(b)** total IκB/β-actin. Data were expressed as mean±SD (n=6). Different letters above the error bars indicated significant differences among treatment groups (p<0.05)

mg/mL (Figure 4a, b) when compared with LPS-only treatment group (p<0.05). These results indicated that steamed OFSP ethanol extract inhibited pro-inflammatory mediator production via suppression of IκB phosphorylation and degradation.

DISCUSSION

OFSP is a good source of macro- and micro- nutrients and various phytochemicals (Wang, Nie & Zhu, 2016). The present study found that OFSP extract contained β-carotene, polyphenols and flavonoids, which were consistent with data from previous studies (Failla *et al.,* 2009; Tang, Cai & Xu, 2015). β-carotene is the predominant carotenoid, which is consistent with a previous study (Failla *et al.,* 2009). The ethanol extract contained 226 μg/g DW of β-carotene while the mixed solvent extract contained 284 μg/g DW of β-carotene. A previous study in

Taiwan (Liu, Lin & Yang, 2009) reported the presence of 127-258 µg/g DW of β-carotene in mixed solvent extract of OFSP (Tainung 66 variety). However, the amount of mixed solvent extract in this study was slightly higher than that of the Taiwanese variety.

The ethanol extract from steamed OFSP contained 2.13 mg GAE/g DW of total polyphenolic compounds. This figure was lower than the Chinese variety (5.19 GAE/g DW) (Tang *et al.,* 2015) but was significantly higher than the Brazilian variety (1.05-1.56 mg GAE/g DW) (Donado-Pestana *et al.,* 2012). Our study demonstrated that the total flavonoid content of steamed OFSP extract was 0.24 mg QE/g DW which was slightly lower than the Taiwanese variety which had 0.33 mg QE/g DW of total flavonoids (Huang, Chang & Shao, 2006). The amount of β-carotene, polyphenol and flavonoid compounds in OFSP is dependent on many factors such as the variety, environmental conditions and agricultural management. In addition, the solvent used for extraction and the method used for the measurement of carotenoids by HPLC also influenced the measurable β-carotene content as reported in previous studies (Rautenbach *et al.,* 2010). Therefore, it is difficult to compare our results for the amounts of bioactive compounds present in OFSP with those reported in other studies.

Besides β-carotene, other compounds found in OFSP which have demonstrated anti-inflammatory activity include phenolic acids such as caffeic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid, FA, sinapic acid and flavonoids including quercetin, myricetin, kaempferol and luteolin (Ambriz-Pérez *et al.,* 2016; Pan *et al.,* 2010; Li, Hong & Zheng, 2018). As OFSP contains various bioactive compounds such as phenolic acids, flavonoids (polar organic compounds) and carotenoids (non-polar organic compounds), the comparative effect of its extracts from ethanol (polar) and hexane:acetone:ethanol mixture (non-polar) on LPS-induced NO production in RAW264.7 cells were investigated to select the potent solvent. The results revealed that the ethanol extract significantly inhibited NO production, whereas the mixed solvent extract did not show any suppressive effect (Figure 1a, b). Although mixed solvent extract contained a higher amount of β-carotene than the ethanol extract, its suppressive effect on LPS-induced NO production did not correlate with the β-carotene content. It implied that organic compounds in mixed solvent extract did not play major role in suppressive effect or mixed solvent extract may contain interfering compounds that neutralize such an inhibitory effect. Conversely, the polar organic compounds in the ethanol extract may play an important role in this inhibitory effect. Therefore, the ethanol extract was selected to further assess anti-inflammatory activity in the present study.

The ethanol extract also decreased NO secretion by inhibiting iNOS protein expression (Figure 2a, c). NO is a vital free radical that plays an important role in the progression of inflammation. It is synthesized by the oxidation of L-arginine to L-citrulline through the activity of NOS (Phaniendra, Jestadi & Periyasamy, 2015). iNOS is up-regulated during inflammation leading to NO generation. LPS can stimulate macrophages to produce NO, which is mediated by activation of transcription factor "NF-κB". Our results also demonstrated that OFSP ethanol extract inhibited the production of TNF-α and IL-6 (Figure 2b, d) and suppressed IL-1β and COX-2 expressions (Figure 2f, e). These pro-inflammatory cytokines and enzymes play crucial roles in activating the acute phase of immune response. They promote tissue damage in the pathogenesis of

chronic inflammatory diseases and facilitate tumour progression and invasiveness. Additionally, prolonged overexpression of such pro-inflammatory mediators is mainly associated with the loss of apoptosis, uncontrolled cell proliferation, growth, metastasis, neovascularization and angiogenesis, leading to development of pathogenesis of various inflammatory diseases.

In addition, pre-treatment of RAW264.7 cells with OFSP extract inhibited LPS-induced pro-inflammatory protein expression by suppressing the phosphorylation of MAPKs including ERK, JNK and p38 (Figure 3a-c) and IκB activation (Figure 4a, b). These inflammatory effects of LPS are widely known as an important inducer for triggering the phosphorylation of MAPKs, resulting in NF-κB activation. MAPKs are serine/threonine protein kinases that mediate intracellular signalling related with the regulation of biological processes and cellular activities such as cellular stress, inflammatory responses, gene induction, cell proliferation, differentiation, survival, apoptosis and transformation (Kim & Choi, 2010). Additionally, JNK activation has been associated with the regulation of cellular functions such as cell proliferation, survival and differentiation. JNK is activated by LPS, environmental stress, growth factors and inflammatory cytokines such as IL-1β and TNF-α. JNK plays an important role in the transcriptional regulation of many inflammatory mediators such as IL-2, iNOS and COX-2. Short-term JNK activation can promote cell survival, but prolonged JNK activation induces cellular apoptosis (Arndt *et al.,* 2004). Furthermore, p38 activation has been implicated in the production and activation of inflammatory mediators for initiating leukocyte recruitment and activation. Also, p38 plays a key role in regulating the expression of various genes related to inflammation such as those encoding TNF-α, IL-1β, IL-6, IL-8, iNOS and COX-2 (Guha & Mackman, 2001; Neuder *et al.,* 2009).

The master transcription factor "NF-κB" is a dimer protein that is bound to IκB protein in cytoplasm during quiescent state. When macrophages are exposed to LPS, NF-κB is activated and IκB undergoes phosphorylation and degradation by the action of the IκB kinase (IKK) complex. NF-κB becomes free and is translocated into the nucleus, which then binds to responsive elements and induces many genes encoding inflammatory mediators including TNF-α, IL-1β, IL-6, COX-2 and iNOS (Liu *et al.,* 2017). The present results indicated that the suppressive effects of OFSP extract on pro-inflammatory mediator expression were mediated partly by inactivation of MAPKs (Figure 3a-c) and IκB (Figure 4a, b) signalling pathway. Therefore, the suppression of these signalling pathways may reveal the potent activity of our extract as an inhibitor of inflammatory mediators and cytokines.

The bioactive compounds present in the steamed OFSP extract may act synergistically to suppress inflammatory mediator production observed in the present study. According to a previous report, mouse peritoneal macrophages treated with phytonutrient mixture (lycopene or Lyc-O-Mato and carnosic acid, lutein, and/or β-carotene) revealed a synergistic inhibition of LPS-induced TNF-α, NO, prostaglandin E_2 (PGE_2) and superoxide production derived from down-regulation of iNOS, COX-2 and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in both messenger RNA (mRNA) and protein expression. A combination of phytonutrients is known to exhibit an anti-inflammatory effect by the synergistic inhibition of LPS-induced internal superoxide production leading

to a marked reduction in ERK and NF-κB activation, probably due to their antioxidant activities (Hadad & Levy, 2012). This study identified and quantified the predominant carotenoid in the extract bioactive compound in OFSP, namely β-carotene. Supporting evidence indicated that synthetic β-carotene inhibited LPS-stimulated COX-2, iNOS and TNF-α gene expression in RAW264.7 cells (Kawata *et al.,* 2018). β-carotene treatment inhibited the production and expression of various pro-inflammatory mediators in LPS stimulated RAW 264.7 cells by suppressing the phosphorylation and degradation of IκB/NF-κB pathway (Li *et al.,* 2018). However, phenolic acids including caffeic acid, *p*-hydroxybenzoic acid, vanillic acid, syringic acid, *p*-coumaric acid, FA and sinapic acid and flavonoids namely, quercetin, myricetin, kaempferol and luteolin were also found in OFSP (Park *et al.,* 2016) which might exert anti-inflammatory activity along with β-carotene. Due to the potent anti-inflammatory effect that were observed in the present study, OFSP may be an alternative promising functional food for preventing or reducing the risk of inflammatory diseases.

CONCLUSION

The present study indicated that the ethanol extract of steamed OFSP inhibited the production and expression of several pro-inflammatory mediators by suppressing the MAPKs and IκB activation on LPS-induced murine macrophage cell line. These findings clearly demonstrated that OFSP had anti-inflammatory potential, and that regular consumption of OFSP may reduce risk of inflammatory diseases. However, this study is an *in vitro* experimental model. Further studies in animal models and humans are needed to confirm whether OFSP can be used as an alternative food supplement to prevent or alleviate severity of inflammatory diseases.

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Authors' contributions

YS, conducted the study, performed data analysis and interpretation, and prepared draft of the manuscript; KP, gave advice on the analytical methods and data analysis; MS, reviewed and edited the manuscript; ST, designed the study, gave advice on the analytical methods and data analysis, edited and overviewed the manuscript.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Under-reporting of energy and nutrient intake is a persistent issue in the Malaysian Adult Nutrition Surveys

Ahmad Ali Zainuddin[1,2], Norazmir Md. Nor[1*], Safiah Md Yusof[3], Adriana Irawati Nur Ibrahim[4], Tahir Aris[2] & Foo Leng Huat[5]

[1]*Universiti Teknologi MARA, Puncak Alam;* [2]*Institute for Public Health, Ministry of Health Malaysia, Kuala Lumpur;* [3]*International Medical University, Kuala Lumpur;* [4]*Universiti of Malaya, Kuala Lumpur;* [5]*Universiti Sains Malaysia, Kubang Kerian*

ABSTRACT

Introduction: Under-reporting of energy intake is a common cause of bias in nutritional studies. This study was aimed at examining the extent of under-reporting of energy intake and its related characteristics among respondents in the Malaysian Adult Nutrition Survey (MANS) 2003 and MANS 2014. **Methods:** The present study analysed energy intakes of 9,624 adults aged 18-59 years from the MANS in year 2014 (2,890 respondents) and 2003 (6,734 respondents) using a single 24-hour diet recall. Basal metabolic rates (BMR) were calculated from the age- and gender-specific equations of Schofield. Under-reporting was defined as an energy intake:BMR ratio of <1.2 as proposed by Goldberg. **Results:** Under-reporting was found to have increased significantly from 53% in 2003 to 61% in 2014. In both surveys, under-reporting increased with higher body mass index (BMI) and older age groups. It was higher among women than men, lowest among those with primary schooling or below, and those living in Peninsular Malaysia. It was higher among rural respondents in 2014 but higher among urban respondents in 2003. The intake of energy and micronutrients increased when under-reporters were excluded. **Conclusion:** Under-reporting was prevalent in both the nationwide MANS, and is associated with BMI, age, gender, education level, location strata, zone. It is important to take this factor into account when assessing dietary intake in population-based studies.

Keywords: Energy intake, 24-hours diet recall, under-reporting, nutrition survey, adults

INTRODUCTION

The under-reporting of energy intake (EI) is a major concern in dietary assessment. Based on the analysis of numerous dietary intake surveys that were conducted among respondents aged 15-84 years old, Black *et al.* (1991) concluded that self-reported EI tends to be under-reported. In addition, more recent studies have pointed out that the under-reporting of EIs resulting from the use of the self-reported method from population nutrition surveys is a considerable problem that has distorted the findings of several surveys. Among these were the United States National

*Corresponding author: Norazmir Md Nor
Associate Professor, Centre of Nutrition and Dietetics, Faculty of Health Sciences
Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia.
Tel: +6-03-3258 4510, +6019-666 4534; Fax: +6-03 3258 4599; Email: azmir2790@uitm.edu.my
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Health and Nutrition Examination Survey (Briefel *et al.*, 1997), the Canadian Community Health Survey (Garriguet, 2008), the New Zealand Adult Nutrition Survey (Gemming *et al.*, 2014), the Finnish Adults Dietary Survey (Hirvonen *et al.*, 1997), the Korean National Health and Nutrition Examination Survey (Kye *et al.*, 2014), the Australian Children's Survey (Rangan *et al.*, 2011) and the Brazil Nutrition, Physical Activity and Health Survey (Souza *et al.*, 2015). In a French dietary survey using a 7-day food record, 22.5% adults were under-reported (Berta Vanrullen *et al.*, 2014). The extent of under-reporting has ranged from 10-50%.

The validity of the self-reporting of diet histories, food records, 24-hour dietary recalls and food frequency questionnaires (FFQ), are obviously dependent on the degree of accuracy with which the respondents report or recall their food consumption. Several factors appear to be associated with the under-reporting of EIs, including obesity, age, gender, social status, controlled eating habits and the consumption of certain food groups (Azizi, Esmaillzadeh & Mirmiran, 2005; Briefel *et al.*, 1997; Garriguet, 2008; Hirvonen *et al.*, 1997; Johansson *et al.*, 1998; Kye *et al.*, 2014). Specifically, relative to the comparison group, overweight and obese respondents, women and older people were found to under report EI (Briefel *et al.*, 1997; Garriguet, 2008; Hirvonen *et al.*, 1997; Kye *et al.*, 2014). This reporting bias may lead to a misinterpretation of the individual's nutritional state and may also result in misleading associations between diet and disease.

Thus, it is important to assess the extent of under- or over-reporting of EI in nutritional surveys. Most studies, including those cited above, have applied the Goldberg equation (Black, 2000) to distinguish between under-reporting and acceptable reporting. This equation calculates the ratio between EI and basal metabolic rate (BMR). A ratio below 1.2 is considered inadequate for the maintenance of body weight and, thus, identifies the low energy reporters. A further distinction between under-reporting and over-reporting can be made using alternative cut-off points. These cut-off values were obtained by calculating the EI:BMR ratio for each respondent. The cut-off values were then used to identify three ranges: EI:BMR of <1.34 (under-reporting), 1.35–2.39 (normal range) and >2.40 (over-reporting) (Black, 2000).

BMR can be measured based on different equations, e.g. Schofield's equation (Ramirez-Zea, 2005) and Henry's equation (Henry & Rees, 1991; Ramirez-Zea, 2005). In 1985, the Food and Agriculture Organization / World Health Organization/United Nations University (FAO/WHO/UNU) Committee introduced BMR as the basis for calculating the energy requirement for populations aged over ten years old. The FAO/WHO/UNU applied Schofield's equation in computing the BMR of individuals according to age (0-3 years, 3-10 years, 10-18 years, 18-30 years, 30-60 years and >60 years), gender and body weight (WHO, 2001). However, Schofield's predictive equation corroborated mainly with people from Europe and North America and with only 5.2% of those from other parts of the world (Ismail *et al.*, 1998). Another limitation was that the Italian population, who had higher BMR values compared to the other populations, was over-represented in the data (47%) (Ramirez-Zea, 2005).

Henry and Rees (1991) developed a new set of predictive equations to calculate the BMR of people living in tropical countries all over the world. They found that the FAO/WHO/UNU predictive equations had overestimated the BMR of tropical peoples by 8%. Their equation was also tested among the Malays and Chinese populations and pointed to a lower BMR value than that predicted by the FAO/WHO/UNU

equation. In other words, it would be more favourable to use the equation developed using the data obtained from our own population rather than the aforementioned predictive equations. Thus, the predictive BMR equation developed by Ismail *et al.* (1998) using data obtained from 656 Malaysian adults (men = 307, women = 349), aged 18-60 years old was used for this study.

The Malaysian Adult Nutrition Survey (MANS) was a series of nationwide surveys to monitor the nutritional status of the Malaysian population. It was carried out first in 2003 and subsequently in 2014. The objectives of MANS were to determine the socio-demography of meal pattern, habitual food intake, dietary intake, vitamin, mineral and food supplement intakes food security, nutritional status and physical activity pattern among Malaysian adults aged 18-59 years old (IPH, 2014c). Mirnalini *et al.* (2008) found that 54.8% of the respondents in MANS 2003 had under-reported their EIs. Despite this being the case, the previous MANS reports (IPH, 2014b; Mirnalini *et al.*, 2008) only published EIs based on total respondents without excluding under- and/ over-reporting.

The present study aimed to examine the level of under-reporting in EIs among Malaysian adults in both MANS 2003 and 2014. It compared the nutrient intakes and other relevant characteristics of those who had under-reported.

MATERIALS AND METHODS

This research was approved by the Ethics Committee of the Ministry of Health Malaysia [NMRR-17-888-34549(IIR)].

Study populations

This study was based on data from two nationwide MANS that were carried out in 2003 and 2014 on a representative sample of Malaysian adults. Briefly, for each survey, a stratified random sample of men and women aged 18-59 years was drawn from the population sampling frame. The sample sizes were 6,887 respondents in 2003 and 2,973 in 2014. Data collection was undertaken covering both weekdays and weekends. All surveys were paper-and-pencil, interviewer-administered and anonymous. Details of the survey methodology for MANS 2003 and MANS 2014 are described elsewhere (IPH, 2014c; Mirnalini *et al.*, 2008). For the present study, respondents with missing data on body weight, 153 in 2003 and 83 respondents in 2014 were excluded. The total number of respondents was, thus, 9,624 (2,890 in MANS 2014 and 6,734 in MANS 2003).

Subject characteristics

The variables analysed in this study were as follows: age classified into four groups (18-29, 30-39, 40-49 or 50-59 years); gender (men or women); education level (primary school, secondary school, and tertiary level being college or university); strata (urban or rural, based on classification of the Department of Statistics Malaysia); zone (resident in Peninsular or East Malaysia); day of dietary recall interview (weekday or weekend), and physical activity (active or not active, based on International Physical Activity Questionnaire). Anthropometric measurements were height in cm measured by a SECA Bodymeter 208 and weight in kg measured by a TANITA 319 weighing scale. Body Mass Index (BMI) was calculated by dividing weight by height in meters squared and classified as normal and underweight (<25.0 kg/m^2), overweight (≥25.0 to <30.0 kg/m^2) and obese (≥30.0 kg/m^2). Energy and nutrient intake data were obtained from a single 24-hour dietary recall interview. The dietary recall questionnaire was adapted from Gibson and Ferguson (2008). Conversion to nutrients was done using Nutritionist Pro™ Diet Analysis Software (Axxya Systems, 2014).

Cut-off for under-reporting

BMR was calculated based on age- and gender-specific equations proposed by Ismail *et al.* (1998). By definition, The EI was considered under-reported when the EI:BMR ratio was below 1.2. The cut-off that was considered as inadequate for the maintenance of body weight (Goldberg *et al.*, 1991). As the MANS 24-hour dietary recall was designed to estimate short-term habitual intake, the value of 1.2 was selected because it was proposed as the minimum value of habitual EI to fulfil a normal, not bedridden, lifestyle, that can be representative of short-term habitual intake. For the present analysis, respondents were categorised as under-reporters (URs) (<1.2) and non-under-reporters (non-URs) (≥1.2).

Statistical analysis

All statistical analyses were undertaken using the Statistical Package for the Social Sciences (SPSS) version 19.0 (IBM SPSS Statistics, Armonk, NY). The distribution (%) of socio-demographic and health-related variables for URs and non-URs, and summary measures for nutrients were calculated. The statistical significance of differences at $p<0.05$ between the UR and non-UR groups were tested by the chi-square test for categorical variables, and the *t*-test for continuous variables.

RESULTS

The socio-demographic and health characteristics of respondents (URs vs non-URs) for both Surveys are presented in Table 1. Overall, there was a 7.4% increase in the proportion of URs from 2003 compared to 2014, from 53.6% to 61.0%. In both surveys, there were significant differences in the percentages of URs compared to non-URs when the respondents were categorised according to age, gender, education level, strata, zone and BMI. In both MANS 2003 and MANS 2014, the following observations regarding URs were made:

- there was an increasing trend from the youngest age groups (18-29 years old) to the oldest age groups (50-59 years old);
- more among women than men;
- an increasing trend from those with the highest education level (tertiary education) to lowest education level (primary school or less);
- more in urban than rural respondents;
- more in Peninsular Malaysia than East Malaysia; and,
- more among respondents with higher BMI than those respondents with lower BMI.

There were no significant differences in the proportion of URs and non-URs by day of recall, or between active and non-active respondents.

The largest increase in the prevalence of URs from 2003 to 2014 was in the respondents from East Malaysia (13.6%), followed by subjects living in rural areas (12.0%), the age group 30-39 years old, women, respondents with secondary education, those living in Peninsular Malaysia, who offered weekday recall, were physically active and non-active subjects showed higher than the average increase in the proportion of URs. The smallest increase was seen urban respondents (1.3%).

Table 2 shows the results of logistic regression analysis on each socio-demographic characteristic for both surveys. The highest odds ratio was seen among subjects with BMI >30.0 kg/m² who were 4.55 times more likely to under report. Meanwhile, overweight respondents were twice as likely to under-report compared to those with BMI <25.0 kg/m² (the reference group). Similar to the patterns shown in Table 1, the likelihood of under-reporting increased significantly from younger to older age group, in women compared to men. The likelihood of under reporting decreased significantly among those

Table 1. Percentage distribution of URs (<1.2 EI:BMR) and non-URs (≥1.2 EI:BMR) in the Malaysian Adult Nutrition Surveys by sociodemographic and health characteristics

Characteristics	*2003 (n=6734)*			*2014 (n=2890)*		
	URs (n=3609)	*Non URs (n=3125)*	*p*†	*URs (n=1762)*	*Non URs (n=1128)*	*p*†
Overall	53.6	46.4		61.0	39.0	<0.001
Age group						
18-29	47.2	52.8	<0.001	54.3	45.7	<0.001
30-39	52.2	47.8		60.6	39.4	
40-49	60.2	39.8		63.4	36.6	
50-59	62.2	37.8		67.5	32.5	
Gender						
Men	50.1	49.9	<0.001	56.0	44.0	<0.001
Women	57.0	43.0		65.4	34.6	
Education						
Primary or less	60.5	39.5	<0.001	66.0	34.0	0.002
Secondary	51.5	48.5		60.8	39.2	
Tertiary	50.0	50.0		56.9	43.1	
Strata						
Urban	55.7	44.3	<0.001	59.0	41.0	0.019
Rural	51.2	48.8		63.2	36.8	
Zone						
Peninsular Malaysia	56.3	43.7	<0.001	63.6	36.4	<0.001
East Malaysia	43.2	56.8		56.8	43.2	
Days of recall						
Weekdays	52.3	47.7	0.224	62.4	37.6	0.350
Weekends	54.0	46.0		60.5	39.5	
Physical Activity						
Active	53.4	46.6	0.765	60.8	39.2	0.734
Not active	53.8	46.2		61.4	38.6	
BMI						
< 25.0 kg/m^2	44.0	56.0	<0.001	49.1	50.9	<0.001
≥ 25.0 to <30.0 kg/m^2	62.4	37.6		67.0	33.0	
≥ 30.0 kg/m^2	78.1	21.9		81.3	18.7	

† Obtained from chi-square test; Data given as %; UR = under-reporter; Non UR = non-under-reporter

Table 2. Logistic regression analysis of factors predicting under-reporting (<1.2 EI:BMR) status by year in the Malaysian Adult Nutrition Survey

Variable	*2003*		*2014*	
	Odds ratio	*95% CI*[†]	*Odds ratio*	*95% CI*
Age group				
18-29	1.00		1.00	
30-39	1.22	1.08-1.38	1.29	1.06-1.58
40-49	1.70	1.49-1.93	1.46	1.19-1.79
50-59	1.84	1.57-2.15	1.75	1.40-2.19
Gender				
Men	1.00		1.00	
Women	1.32	1.20-1.45	1.48	1.28-1.72
Education				
Primary	1.00		1.00	
Secondary	0.69	0.62-0.78	0.79	0.66-0.96
Tertiary	0.65	0.56-0.75	0.68	0.55-0.84
Strata				
Urban	1.00		1.00	
Rural	0.83	0.76-0.92	1.19	1.03-1.39
Zone				
Peninsular Malaysia	1.00		1.00	
East Malaysia	0.59	0.52-0.66	0.75	0.64–0.87
Days of recall				
Weekdays	1.00		1.00	
Weekends	1.07	0.96-1.19	0.92	0.77-1.09
Physical Activity				
Active	1.00		1.00	
Not active	1.02	0.92-1.12	1.03	0.88-1.20
BMI				
< 25.0 kg/m^2	1.00		1.00	
≥ 25.0 to <30.0 kg/m^2	2.12	1.89-2.37	2.10	1.77-2.50
≥ 30.0 kg/m^2	4.55	3.83-5.41	4.50	3.54-5.72

[†] Obtained from logistic regression analysis; data given as CI = confidence interval

with higher education levels compared to those with lower education, and Peninsular Malaysia compared to East Malaysia. However, the likelihood of under-reporting among urban respondents was significantly higher in 2003 but significantly lower in 2014.

The mean energy and micronutrient intakes for 2003 and 2014 are presented in Table 3. The under-reporting of EIs represents the under-reporting of all nutrients that were estimated in MANS. There were significant differences in EIs between URs and non-URs in both 2003 and 2014. In terms of nutrient intake, only the protein intake in 2014 showed no significant difference between URs and non-URs; all other nutrients showed significant differences between UR and non-URs in both 2003 and 2014. If the URs were excluded from the analysis, the mean EIs (for non-URs) in 2003 and 2014 were 2097 (SE±9.3) kcal and 2123 (SE±16.1), respectively.

The data comparing the mean EIs for the total study subjects between 2003 and 2014 is not shown on Table 3. Briefly, the values were 1617 (SE±7.5) kcal in 2003 and 2123 (SE±16.1) kcal in 2014, and they were significantly different (*p*-value <0.0001).

DISCUSSION

The prevalence of under-reporting in large nutritional surveys ranges from 18-

Table 3. Mean nutrient intake (kcal) for under-reporters (<1.2 EI:BMR) and non-under-reporters (≥1.2 EI:BMR) of energy intake in the Malaysian Adult Nutrition Survey in 2003 and 2014.

MANS (year)	*Variable*	*URs (n=1762) (Mean±SE)*	*Non URs (n=1128) (Mean±SE)*	*p-value*
2003	Energy (kcal)	1203±5	2097±9	<0.001
	Carbohydrate (% energy)	59.6±9.8	56.7±8.9	<0.001
	Protein (% energy)	14.9±4.3	14.7±3.8	0.01
	Fat (% energy)	25.5±8.4	28.6±7.4	<0.001
	Sodium (mg)	1949.0±19.0	3341.0±29.0	<0.001
	Calcium (mg)	305.0±2.9	501.0±4.4	<0.001
	Iron (mg)	7.8±0.1	14.1±0.2	<0.001
	Vitamin C (mg)	51.8±1.1	73.6±1.4	<0.001
	Vitamin A (µg)	395.0±8.7	659.0±12.5	<0.001
	Thiamine (mg)	0.6±0.0	0.9±0.0	<0.001
2014	Energy (kcal)	1198±8	2123±16	<0.001
	Carbohydrate (% energy)	55.7±0.2	53.4±0.3	<0.001
	Protein (% energy)	16.3±0.1	15.9±0.1	0.07
	Fat (% energy)	28.0±0.2	30.7±0.2	<0.001
	Sodium (mg)	1756.0±29.0	3022.0±52.0	<0.001
	Calcium (mg)	339.0±5.3	540.0±9.0	<0.001
	Iron (mg)	12.1±1.3	16.4±0.3	0.008
	Vitamin C (mg)	60.1±2.3	76.9±3.0	<0.001
	Vitamin A (µg)	554.0±20.0	899.0±26.0	<0.001
	Thiamine (mg)	0.6±0.0	0.9±0.1	<0.001

54 percent of the overall sample but can be as high as 70 percent in particular subgroups. This wide variation between studies is partly due to different criteria that were used to identify URs and also because of non-uniformity of under-reporting across populations (Macdiarmid & Blundell, 1998).

The proportion of URs in our study was one of the largest (53.6% in 2003 and 61% in 2014), compared to national surveys elsewhere (Briefel *et al.*, 1997; Garriguet, 2008; Kye *et al.*, 2014; Souza *et al.*, 2015), where the proportion of URs ranged from 9.6% in Canada (Garriguet, 2008) to 50% in Brazil (Souza *et al.*, 2015). All these studies used single 24-hour diet recalls similar to that used in our study. However, most of them used computer-based, interviewer-assisted and/or the multiple pass technique to improve the accuracy of dietary recall (Briefel *et al.*, 1997; Garriguet, 2008; Gemming *et al.*, 2014).

Among the socio-economic and anthropometric variables included in the study, the most notable risk factor was the higher BMI. This study has revealed that overweight and obese respondents were two and four times more likely to under-report, respectively, compared to respondents with normal BMI. This finding is similar to that of other studies where there was an inverse association between BMI and self-reporting of EI (Kye *et al.*, 2014; Macdiarmid & Blundell, 1998; Orcholski *et al.*, 2015; Souza *et al.*, 2015).

In both MANS surveys, more women were found to under-report EI than

men. Macdiarmid and Blundell (1998) revealed that in 11 of the 12 studies that they reviewed, women were significantly more likely to under-report their dietary intake than men. In the United States, 28% women were URs compared to 18% in men (Briefel *et al.*, 1997), while in New Zealand 25% of the women were URs compared to 21% in men (Gemming *et al.*, 2014). The same trend was also be seen in South Korea, where 23.0% women were URs compared to just 14.4% in men (Kye *et al.*, 2014). It is believed that women tend to be more concerned about their body weight, food, and eating than men (Macdiarmid & Blundell, 1998) and they perceive under-reporting to be a socially acceptable behaviour (Schoeller, 1990) in order to conform to a healthy diet.

Our study has identified age as a strong independent predictor of under-reporting. Older respondents group (50-59 years old) under-reported EI more than younger respondents group (18-29 years old). The true impact of this relationship is unknown as age tended to be associated with other characteristics such as BMI (Macdiarmid & Blundell, 1998).

Our results have also identified educational level as a strong independent predictor of under-reporting. Under-reporting is more common among respondents with lower educational background (primary school or less). Generally, other studies have also found under-reporting to be associated with lower educational levels (Briefel *et al.*, 1997; Klesges, *et al.*, 1995; Kye *et al.*, 2014). This finding is not surprising as most methods for recording food intake depend heavily on literacy. In contrast to this study, Hirvonen *et al.* (1997) found that under-reporting was associated with high level of education among Finnish adults. While Azizi *et al.* (2005) found educational level of under-reporters did not differ significantly among Iranians adults.

No relationship was found between self-reported physical activity and under-reporting of EI. Self-reporting of physical activity is probably subject to similar error to those in reporting food intake, but BMR is unlikely to explain the lower EIs reported by under-reporters (Bedard, Shatenstein & Nadon, 2004).

The inclusion of weekends is expected to raise EIs since it has been shown that food intakes were higher during the weekends compared to weekdays (Macdiarmid & Blundell, 1997). However, our study could find no significant relationships between food intakes during weekends and weekdays.

Both surveys showed higher under-reporting in Peninsular Malaysia than in East Malaysia. This was due to higher prevalence of obesity in the former (Azmi *et al.,* 2009; IPH, 2014b).

The only discrepancy between MANS 2003 and MANS 2014 involved the proportion of under-reporting among urban respondents. MANS 2003 showed a higher proportion of under-reporting among urban respondents in 2003 compared to 2014, whereas MANS 2014 shows the opposite. These differences cannot be explained by BMI of the urban and rural respondents as there were no significant differences between them in both surveys (Azmi *et al.*, 2009; Institute for Public Health, 2014b).

Finally, our study showed a significant increase in energy and micronutrient intakes when URs were excluded. The previous findings before removing the mis-reporting showed a median EI of 1,540 kcal in MANS 2003 (Mirnalini *et al.*, 2008) decreasing to 1,466 kcal in MANS 2014 (IPH, 2014a) which contradicted the increasing obesity in the Malaysian population over that period. In fact, the intake of all micronutrients was reported to have decreased from 2003 to 2014 to below the Malaysian Recommended Nutrient Intake (IPH, 2014a; Mirnalini *et al.*, 2008). Based on our findings, it is clear that the effects of the under-reporting EI will carry over to almost all other nutrients, that is, the under-reporting of EI will indirectly affect the

under-reporting of other nutrients. This important conclusion opens the door to better assessments and interpretations of self-reported dietary intake. It also provides the reason for future researchers to develop strategies to minimize inaccuracies.

The EI:BMR ratios used as cut-off points also vary between studies. This study defined a ratio of 1.2 as the minimum ratio for the maintenance of body weight, which is in accordance with previous studies conducted in Malaysia (Lee, Norimah & Ismail, 2010; Mirnalini *et al.*, 2008; Sahathevan *et al.*, 2015; Sharif, Wen & Rajikan, 2016). For instance, the second National Health and Nutrition Examination Survey (NHANES II), a national survey that used 24-hour diet recall, had also utilised the same cut-off point of 1.2 (Klesges, Eck & Ray, 1995). There are other studies that have used the same cut-off points of <1.2 to identify under-reporting among Asian populations, such as Southern Indian (Sudha *et al.*, 2006) and Malaysian populations (Lee *et al.*, 2010; Mirnalini *et al.*, 2008; Sahathevan *et al.*, 2015; Sharif *et al.*, 2016).

The magnitude of misreporting in EI varies according to the choice of cut-off points and the methodologies used in the collection of dietary data (Goldberg *et al.*, 1991; Johansson *et al.*, 1998). The cut-off point that was used in this study (<1.2) would have resulted in a higher percentage of under-reporting compared to other cut-off point choices. In addition, the use of a 24-hour diet recall could result in a lower EI:BMR ratio compared to other methods (Goldberg *et al.*, 1991). This is a possible explanation for this study's findings, in which there is a high prevalence of under-reporting of EIs. Without technological innovation, the under-reporting of EIs will remain a major limitation of the 24-hour dietary recall method used in large-scale nutrition surveys (Gemming *et al.*, 2014).

Gender differences have been shown to be important in under-reporting. In the Canadian study, 54% men compared with 35% women were URs; the disparity may be due to the fact that EI was estimated using food frequency questionnaires (Bedard *et al.*, 2004). In a survey in Finland, 46% of women and 42% of men were URs based on three-day food record method (Hirvonen *et al.*, 1997).

Inaccuracies and unreliability in self-reporting are features in which human beings monitor various aspects of their own behaviour or the impact of their behaviour on themselves or the environment. Often, there is no way of checking the validity of self-reporting. However, in nutrition, the use of formulations based on biological processes such as EI:BMR for example, provide a guide to the reliability of the self-reporting (Macdiarmid & Blundell, 1997). The strength of this study was the use of EI:BMR ratio as it provided a reasonable guide to check the validity of self-reported dietary intake. The limitation of this study, however, was the use of a single 24-hour diet recall that was not able to capture the usual day to day variability in food intake. It is suggested that future studies should use a computer-based interviewer-assisted method of 24-hour dietary recall using multiple pass technique.

This study will should provide the basis in the analysis of nutrient intake for any future MANS that may be undertaken. It would do so by taking under-reporting into account in order to derive more accurate data on nutritional intake.

CONCLUSION

This study has shown that under-reporting of EI increased from the MANS 2003 to the MANS 2014 in Malaysian adults. There was under-reporting in almost all major nutrients. The magnitude of under-reporting tends to distort the relationship between EI and obesity. Under-reporting was more

evident in higher BMI, women, older adults, those who had a lower education level, and living in Peninsular Malaysia. Therefore, URs must be taken into account when assessing dietary intake in population-based studies, and efforts made to reduce its occurrence in the sub-groups identified at higher risk, especially those with high BMI. The accuracy of dietary intake assessment can be improved with better techniques.

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Author's contributions

AAZ, wrote the manuscript with support and supervised from all authors. AINI and SMY, verified the analytical methods. All authors provided critical feedback, discussed the results and contributed to the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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Changes in energy and nutrient intakes among Malaysian adults: findings from the Malaysian Adult Nutrition Survey (MANS) 2003 and 2014

Ahmad Ali Zainuddin[1,2], Norazmir Md. Nor[1*], Safiah Md Yusof[3], Adriana Irawati Nur Ibrahim[4], Tahir Aris[1] & Foo Leng Huat[5]

[1]*Universiti Teknologi MARA, Puncak Alam;* [2]*Institute for Public Health, Ministry of Health Malaysia, Kuala Lumpur;* [3]*International Medical University, Kuala Lumpur;* [4]*Universiti of Malaya, Kuala Lumpur;* [5]*Universiti Sains Malaysia, Kubang Kerian*

ABSTRACT

Introduction: Monitoring changes in energy and nutrient intakes of the population over the course of time is essential to help healthcare providers develop effective dietary policies. The aim of this study was to assess the changes in the nutrient intake and Recommended Nutrient Intake (RNI) achievements by using the data obtained from the Malaysian Adult Nutrition Surveys (MANS) that were carried out in 2003 and 2014. Mis-reporting of energy intake was taken into account. **Methods:** Dietary data were obtained from MANS 2003 and MANS 2014, which involved a combined total of 4,044 randomly selected respondents, aged 18-59 years, using a single 24-hour diet recall. Energy and nutrients calculations were based on the Malaysian Food Composition database using the Nutritionist Pro software. The results were compared against the RNI for Malaysia to assess dietary adequacy. **Results:** The proportions of calories derived from macronutrients were within the recommendations for a healthy diet. The consumption of protein, fat, calcium, iron and vitamin A was significantly higher in 2014 than in 2003. The consumption of protein, iron, vitamin C, and vitamin A was found to exceed the RNIs in 2014. However, carbohydrate and sodium intakes had significantly decreased. Despite the decrease, sodium intake still exceeded RNI recommendations. **Conclusion:** Signs of changing energy and nutrient intakes were found, including increases in protein and fat intakes since 2003, and decreased carbohydrates. This could be an alarming indicator of the tendency to eat energy dense food among the population.

Keywords: Nutrient intake, Malaysian population, 24-hours diet recall

INTRODUCTION

The estimation of nutrient intake is an essential component of monitoring nutritional status. It identifies groups which are nutritionally at risk due to insufficient or excessive intake of specific nutrients. In addition, it helps planners to target, plan and evaluate nutrition intervention programmes, and, to establish dietary recommendations, food regulations and nutrition policies (Sandström, 2001).

In Malaysia, the Ministry of Health carried out the Malaysian Adult Nutrition Survey (MANS), a cross-sectional survey that was conducted for the first time in 2003 on a representative sample of the Malaysian adult population. This survey

*Corresponding author: Norazmir Md Nor
Associate Professor, Centre of Nutrition and Dietetics, Faculty of Health Sciences
Universiti Teknologi MARA, 42300 Puncak Alam, Selangor, Malaysia
Tel: +6-03-3258 4510, +6019-666 4534; Fax: +6-03 3258 4599; Email: azmir2790@uitm.edu.my
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allowed researchers to estimate the nutrient intake (Mirnalini *et al.*, 2008), meal patterns (Manan *et al.*, 2012), nutritional status (Azmi *et al.*, 2009), physical activity (Poh *et al.*, 2010), and use of dietary supplements (MOH Malaysia, 2008a). MANS also provided data for the food consumption database (MOH Malaysia, 2006).

The second MANS was carried out in 2014 and its results were published in three reports (IPH, 2014c; 2014b; 2014a). This second survey was undertaken to evaluate changes in dietary patterns and monitor the nutritional status of the Malaysian population, particularly with respect to the increasing prevalence of non-communicable diseases. The dietary patterns of the population may deviate from those indicated in MANS 2003 due to urbanisation and the recent increase in diet-related chronic diseases, both of which have resulted in rapid changes in dietary intakes among the Malaysian population (IPH, 2015). A comprehensive study on the energy and nutrient intakes of the Malaysian population would provide the additional information that could then be used to develop more effective food and nutrition policies.

While surveys on nutritional intakes are carried out regularly in various countries, several methodological problems are well known. These include the use of 24-hour dietary recalls and food frequencies in large-scale surveys. The mis-reporting of energy intakes (EIs) (both under and over-reporting) is a common problem encountered in a number of national surveys conducted among adults (Garriguet, 2008; Klesges, Eck & Ray, 1995; Mackerras & Rutishauser, 2005).

This study was conducted in order to assess changes in nutrient intake by comparing the survey data of 2014 to that of 2003. It also evaluated the current nutrient intake among the adult population in Malaysia using only reliable data on EI. The findings of this study should serve as a foundation for the formulation of dietary intervention programmes, educational projects, and nutritional guidelines for both healthcare providers and the general public.

MATERIALS AND METHODS

This study was approved by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia [NMRR-17-888-34549(IIR)].

Sampling design and study population

MANS was conducted by the Ministry of Health Malaysia. It consisted of a series of cross-sectional nutrition surveys representing non-institutionalised Malaysian adults aged 18-59 years old. The MANS data collected in 2003 involved 6,928 respondents (3,523 male and 3,405 female), and that in 2014 involved 2,973 respondents (1,553 male and 1,420 female). Each survey followed a stratified multistage sampling design. The surveys combined face-to-face interviews with anthropometry measurements. The large majority of respondents were interviewed and physical measurements were taken at their home. In a small number of cases, data were collected at their offices. The overall response rates for the survey were 94% in 2003 (MOH Malaysia, 2008b) and 80% in 2014 (IPH, 2014c). Informed consent was obtained from all participants. For the present study, analyses were restricted to adults who had a reliable, self-reported 24-hour diet recall (after excluding misreporting of EI) and had completed anthropometric measurements. This paper included a reanalysis of the data from the two studies mentioned.

Socio-demographic and anthropometric characteristics

The socio-demographic information of the respondents in this study referred to their gender (male-female) and strata (urban-rural) only. Anthropometric

measurements included body weight and height. The reliability and validity of all these measurements are well-established (Baharudin *et al.*, 2017; Geeta *et al.*, 2009). Body mass index (BMI) was calculated as weight (in kilogram) divided by height (in meter squared). The BMI was used to estimate the basal metabolic rate (BMR) of the respondent. Mis-reporting of EI was determined based on the ratio of reported EI to estimated BMR, or EI:BMR.

Dietary assessment

Energy and nutrient intake were measured from a single 24-hour dietary recall. Trained nutritionists interviewed the subjects and collected detailed information on food items and the quantities consumed during the previous day. Where possible, food recipes were recorded. The interactive 24-hour dietary recall was conducted to assess all foods and drinks consumed by the respondent during the preceding 24-hour period, included cooking methods, brand names and portion sizes.

Dietary assessment aids, such as an album of food pictures (IPH, 2011; MOH Malaysia, 2002) and household measures, were used to facilitate the identification of foods and quantification of portion sizes consumed. The album consisted of actual sized photographs of individual foods, which were useful in helping subjects estimate amounts eaten as fractions or multiples of the illustrated reference portions.

The dietary analysis software, Nutritionist Pro™ Nutrition Analysis Software version 5.3 (Axxya Systems, 2014), was used for this study. For local complex mixed cooked dishes that were not available in any of the food databases, local recipe books were used to identify at least two recipes for each dish. For each recipe, it was ensured that the quantitative information on oils, fats and salt were available. The energy and nutrient content of these recipes were analysed using the Malaysian Food Composition Tables (Tee *et al.*, 1997) and the average of these values was entered into the Nutritionist Pro software. For example, two recipes for fish curry (gravy) were obtained and the ingredients were analysed for energy and nutrient values (per 100 gram). The average values of the two recipes were then used as the standard for nutrient content of fish curry. For processed and packaged foods, information on energy and nutrient content on their labels was entered into the software for analysis. For all foods consumed by the subjects, steps were taken to ensure that oils, fats and salt were accounted for. The macro- and micro-nutrient intakes that are reported in this paper are based exclusively on the consumption of food and fluids and do not include contributions from vitamin and mineral supplements.

Mis-reporting of EI

To estimate the mis-reporting of EI from the 24-hour diet recall, the ratio of reported total daily EI to BMR, or EI:BMR was calculated. The calculation for BMR for the Malaysian population was done using the predictive equation by Ismail *et al.* (1998). Respondents were classified as follows according to their EI:BMR ratio: under-reporters (EI:BMR< 1.2); plausible (EI:BMR 1.2–2.4); and over-reporters (EI:BMR >2.4) of EI, as suggested by Black (2000) and Goldberg *et al.* (1991). Other studies on the Malaysian population have also applied the cut-off points <1.2 and >2.4, to classify the under- and over-reporting of individuals (Sahathevan *et al.*, 2015; Sharif, Wen & Rajikan, 2016).

Dietary adequacy

The most recent version of Recommended Nutrient Intakes (RNI) for Malaysia (NCCFN, 2017) was used to assess dietary adequacy among Malaysian adults according to age, gender and physical activity level (PAL). For the general population group, a PAL score of

1.6 (i.e. moderately active) was used for this study, as recommended.

The percent contribution of macronutrients towards total daily EI was considered achieved if the respondent's mean intake was within the following recommendations of the 2017 Malaysian RNI: 50-65% of energy from carbohydrate, 10-20% from protein and 25% to 30% from fat. For comparison, the previous 2005 Malaysian RNI guidelines had the following cut-offs: 55-70% of energy from carbohydrate, 10% to 15% from protein and 20-30% from fat. The adequacies of macronutrients (carbohydrate, protein, and fat) intake were compared between RNI 2005 and RNI 2017 by using the respective cut-off points for both years, in order to ascertain and analyse their differences.

For the adequacy of sodium intake, the 2017 Malaysian RNI for sodium of 1500 mg per day for adults was compared to the World Health Organization (WHO) guidelines of 2012, which recommends a sodium intake of <2000 mg per day for adults.

Statistical analysis

Data on energy and nutrient intakes were transferred from the Nutritionist Pro to the Statistical Package for Social Sciences (SPSS) version 21.0 for statistical analysis. The total EI data of the respondents were converted to *z*-score to identify outliers. After identifying the outliers, all cases within normal range were selected and the rest discarded; this was to ensure the normality of the data.

The means and standard errors (SE) of the nutrient intake, as well as percentages meeting the RNI for selected nutrients were calculated. Student's *t*-test was performed to assess the significance of differences in mean intakes between the two study periods. For each nutrient, the findings were compared with the Malaysian RNIs for the respective groups. Subsequently, the average intake for both groups combined, gender (male-female) and strata (urban-rural) were determined. Statistical significance was accepted at $p < 0.05$.

RESULTS

In this study, we found that under-reporting of EI were 53.6% and 61% from MANS 2003 and MANS 2014, and 2.4% and 1.7% of over-reporting, respectively.

Changes in energy and macronutrient intakes

Table 1 shows the comparison of energy and nutrients intake of MANS 2003 and MANS 2014. Based on the table, there are no significant changes in EI between the MANS in terms of gender and respondents from both groups. The same is also true for respondents from both urban and rural areas.

Table 2 shows the percentages of RNI achievement for subjects for both years. The mean percentage of RNI achievement for energy was significantly higher ($p = 0.04$) in the year 2014 (100% RNI) than in 2003 (99% RNI). There were no significant differences in the percentages of RNI achievement for EI in both gender groups. A significant increase ($p = 0.03$) in the percentage of RNI achievement for EI was noted among respondents in urban areas. In 2003, both strata showed similar trends in terms of EI. However, in 2014, there was a larger increase in the mean EI among urban respondents compared to rural respondents.

Figure 1 shows the mean percentages of energy obtained from the intake of the three macronutrients, carbohydrate, protein and fat. In 2003, the mean carbohydrate intake was 288 g, which declined significantly to 273 g in 2014. This decline was observed among male, female, urban and rural populations. In 2003, carbohydrate intake contributed 57% to the total EI of the respondents, whereas in 2014 this value had decreased to 53%. Overall, mean carbohydrate intakes were higher among males

Table 1. Comparison of the mean daily nutrient intake of the adult population of Malaysia from MANS 2003 and 2014 (mean, standard error and statistical significance of the difference between the two periods)

Nutrient	*Total*			*Males*			*Females*			*Urban*			*Rural*		
	2003 (n=2964)	*2014 (n=1080)*		*2003 (n=1579)*	*2014 (n=571)*		*2003 (n=1385)*	*2014 (n=509)*		*2003 (n=1514)*	*2014 (n=601)*		*2003 (n=1450)*	*2014 (n=479)*	
	Mean (SE)	*Mean (SE)*	*p*	*Mean (SE)*	*Mean (SE)*	*p*	*Mean (SE)*	*Mean (SE)*	*p*	*Mean (SE)*	*Mean (SE)*	*p*	*Mean (SE)*	*Mean (SE)*	*p*
Energy, kcal	2032 (8)	2060 (13)	0.07	2190 (11)	2216 (19)	0.21	1852 (10)	1884 (15)	0.08	2030 (11.1)	2070 (17.9)	0.06	2034 (11.1)	2047 (19.6)	0.55
Protein, g	74.0 (0.4)	81.0 (0.9)	0.00	79.0 (0.6)	86.0 (1.4)	0.00	69.0 (0.6)	75.0 (1.3)	0.00	74 (0.6)	80 (1.2)	0.00	74 (0.6)	81 (1.5)	0.00
Carbohydrate, g	288.0 (1.4)	273.0 (2.3)	0.00	314.0 (1.8)	295.0 (3.3)	0.00	259.0 (1.8)	247.0 (2.8)	0.00	282 (1.9)	270 (3.0)	0.00	295 (1.9)	276 (3.5)	0.00
Fat, g	64.0 (0.4)	70.0 (0.7)	0.00	68.0 (0.6)	74.0 (1.1)	0.00	60 (0.6)	65 (0.9)	0.00	67 (0.6)	71 (0.9)	0.00	62 (0.6)	67 (1.1)	0.00
Sodium, mg	3260.0 (28.0)	2929.0 (48.5)	0.00	3463.0 (39.4)	3088.0 (67.3)	0.00	3029 (39.9)	2749 (69.1)	0.00	3314 (39.5)	2999 (61.6)	0.00	3205 (40.8)	2841 (77.2)	0.00
Calcium, mg	488.0 (4.2)	531.0 (9.0)	0.00	509.0 (5.7)	534.0 (11.0)	0.04	463 (6.3)	527 (14.7)	0.00	489 (5.9)	518 (11.2)	0.02	487 (6.2)	546 (14.7)	0.00
Iron, mg	14.0 (0.2)	16.0 (0.3)	0.00	15.0 (0.4)	17.0 (0.5)	0.00	12 (0.2)	15 (0.4)	0.00	14 (0.4)	16 (0.4)	0.01	13 (0.2)	16 (0.5)	0.00
Vitamin C, mg	72.0 (1.4)	75.0 (2.9)	0.46	70.6 (1.9)	70.3 (4.0)	0.92	74 (2.1)	79 (4.2)	0.22	70 (1.8)	73 (3.5)	0.48	74 (2.2)	77 (4.8)	0.63
Vitamin A, µg	645.0 (12.5)	870.0 (22.7)	0.00	698.0 (18.6)	909.0 (31.4)	0.00	583 (16.1)	825 (32.6)	0.00	628 (17.9)	801 (27.0)	0.00	662 (17.3)	956 (37.9)	0.00
Thiamine, mg	0.93 (0.01)	0.90 (0.01)	0.13	0.98 (0.01)	0.93 (0.02)	0.13	0.89 (0.01)	0.87 (0.03)	0.57	0.97 (0.01)	0.94 (0.03)	0.24	0.89 (0.01)	0.86 (0.02)	0.21

Table 2. Comparison of the mean daily nutrient intake as percentage of RNI 2017 in the adult population of Malaysia for the periods 2003 and 2014 (mean%, standard error and statistical significance of the difference between the two periods)

Nutrient (%RNI)	*Total*			*Males*			*Females*			*Urban*			*Rural*		
	2003 (n=2964)	*2014 (n=1080)*		*2003 (n=1579)*	*2014 (n=571)*		*2003 (n=1385)*	*2014 (n=509)*		*2003 (n=1514)*	*2014 (n=601)*		*2003 (n=1450)*	*2014 (n=479)*	
	Mean (SE)	*Mean (SE)*	*p*	*Mean (SE)*	*Mean (SE)*	*p*	*Mean (SE)*	*Mean (SE)*	*p*	*Mean (SE)*	*Mean (SE)*	*p*	*Mean (SE)*	*Mean (SE)*	*p*
Energy	99	100	0.04	99	100	0.14	99	100	0.13	99	101	0.03	99	100	0.47
	(0.4)	(0.6)		(0.5)	(0.8)		(0.5)	(0.8)		(0.5)	(0.8)		(0.5)	(0.9)	
Protein	130	142	0.00	129	140	0.00	132	144	0.00	130	142	0.00	130	142	0.00
	(0.8)	(1.6)		(1.0)	(2.2)		(1.2)	(2.4)		(1.1)	(2.1)		(1.1)	(2.6)	
Calcium	48	51	0.00	51	53	0.06	45	51	0.00	48	51	0.03	48	53	0.00
	(0.4)	(0.8)		(0.6)	(1.1)		(0.6)	(1.3)		(0.6)	(1.1)		(0.6)	(1.4)	
Iron	121	144	0.00	166	190	0.00	70	93	0.00	127	141	0.05	116	148	0.00
	(2.6)	(3.8)		(4.4)	(5.8)		(1.3)	(3.8)		(4.4)	(4.4)		(2.6)	(6.7)	
Vitamin C	103	107	0.46	101	101	0.92	105	113	0.21	100	104	0.49	107	110	0.63
	(2.0)	(4.2)		(2.7)	(5.7)		(3.1)	(6.1)		(2.6)	(5.0)		(3.1)	(6.9)	
Vitamin A	107	145	0.00	116	152	0.00	97	138	0.00	105	134	0.00	110	159	0.00
	(2.1)	(3.8)		(3.1)	(5.2)		(2.7)	(5.4)		(3.0)	(4.5)		(2.9)	(6.3)	
Thiamine	81	78	0.16	81	78	0.13	81	79	0.57	84	82	0.26	77	74	0.21
	(0.9)	(1.7)		(1.2)	(1.9)		(1.2)	(2.7)		(1.3)	(2.6)		(1.2)	(1.8)	

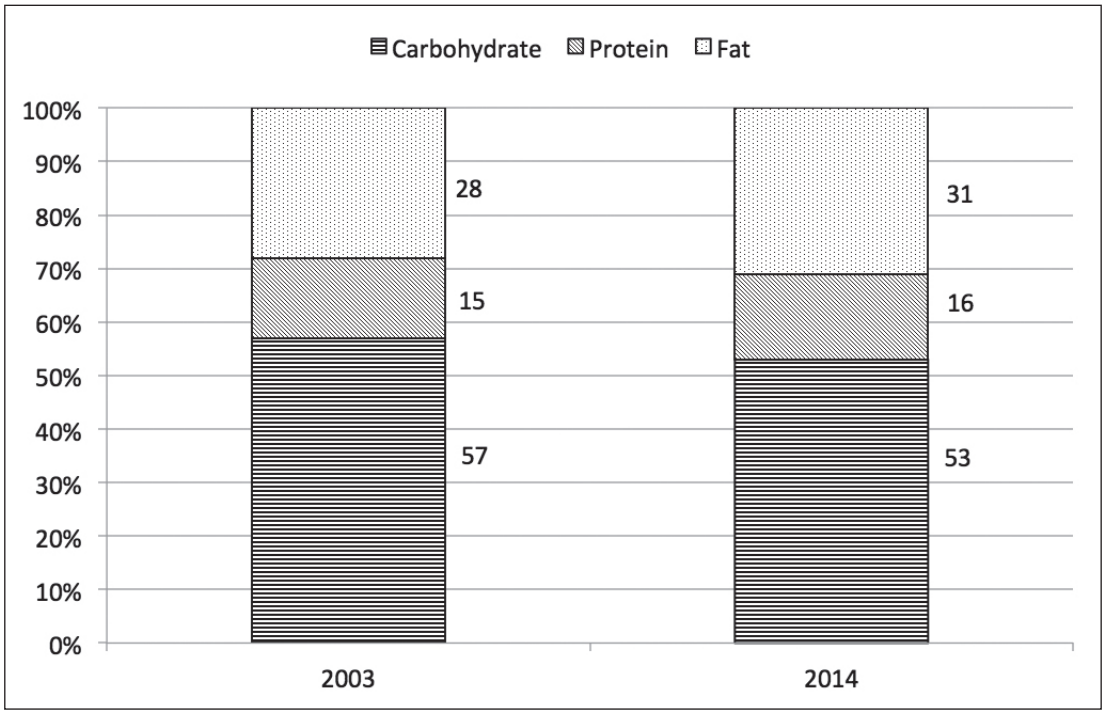

Figure 1: Mean percentages of energy obtained from macronutrient intake

than females. Rural adults had a higher carbohydrate intake than urban adults.

The mean protein intakes for 2003 and 2014 were 74 g (15% of total EI) and 81 g (16% of total EI), respectively. From 2003 to 2014, a significant increase in protein intake was observed among the male, female, rural, and urban populations. Both years showed high RNI achievements for protein intake (130% in 2003 and 142% in 2014).

The mean fat intake was significantly higher in 2014 (70 g or 31% of total EI) than in 2003 (64 g or 28% of total EI). From 2003 to 2014, the fat intakes among the male, female, urban, and rural populations all showed an increasing trend. It is worth noting that the percentage increase in total energy obtained from fat intake coincides with the decline in the percentage of energy obtained from carbohydrates, implying that a portion of carbohydrate sources had been replaced by fat sources.

Table 3 shows the percentages of total respondents for both survey years who had met the adequate ranges of energy contribution from carbohydrate, protein, and fat. These percentages are all based on 2005 RNI cut-off values. In 2003, the percentages of respondents meeting the adequate range of energy contribution from carbohydrate, protein and fat were 55.3%, 52.1% and 49.1% respectively, whereas in 2014, the corresponding values were 36.2%, 43.2% and 39.0% respectively. However, the percentages for protein and fat decreased when RNI 2017 cut-off values were used (Table 4). In MANS 2014, a decrease in carbohydrate contribution to total EI (57% to 53%) was observed, concomitant with a rise in protein (15% to 16%) and, fat (28% to 31%) contribution to total EI compared to 2003 (Figure 1). Based on these findings, it is clear that the results obtained using 2017 RNI cut-off values do not reflect the results obtained using 2005 RNI cut-off values.

Table 3. Adequacy of daily energy contribution from carbohydrate, protein and fat among Malaysian adult population based on RNI 2005 cut-off values

Characteristics (year)	*Carbohydrate adequacy*			*Protein adequacy*			*Fat adequacy*		
	Below (<55%)	*Adequate (55-70%)*	*Above (>70%)*	*Below (<10%)*	*Adequate (10-15%)*	*Above (>15%)*	*Below (<20%)*	*Adequate (20-30%)*	*Above (>30%)*
2003 (*n*=2964), %	39.5	55.3	5.2	7.6	52.1	40.4	11.1	49.1	39.8
2014 (*n*=1080), %	59.8	36.2	4.0	7.2	43.2	49.5	9.4	39.0	51.7

Table 4. Adequacy of daily energy contribution from carbohydrate, protein and fat among Malaysian adult population based on the 2017 RNI cut-off values

Characteristics (year)	*Carbohydrate adequacy*			*Protein adequacy*			*Fat adequacy*		
	Below (<50%)	*Adequate (50-65%)*	*Above (>65%)*	*Below (<10%)*	*Adequate (10-20%)*	*Above (>20%)*	*Below (<25%)*	*Adequate (25-30%)*	*Above (>30%)*
2003 (*n*=2964), %	21.5	62.3	16.2	7.6	83.5	8.9	33.5	26.7	39.8
2014 (*n*=1080), %	36.9	52.2	10.9	7.2	77.3	15.5	25.6	22.8	51.7

Table 5. Comparison of sodium intake by sociodemographic characteristics based on 2017 RNI and WHO 2012 cut-off

Sodium intake (mg/ day)	*Total (%)*		*Male (%)*		*Female (%)*		*Urban (%)*		*Rural (%)*	
	2003 (n=2694)	*2014 (n=1080)*	*2003 (n=1579)*	*2014 (n=571)*	*2003 (n=1385)*	*2014 (n=509)*	*2003 (n=1514)*	*2014 (n=601)*	*2003 (n=1450)*	*2014 (n=479)*
WHO 2012										
< 2000	21.0	29.1	16.0	24.3	26.6	34.4	19.0	27.1	23.0	31.5
≥ 2000	79.0	70.9	84.0	75.7	73.4	65.6	81.0	72.9	77.0	68.5
RNI 2017										
≤ 1500	9.9	17.4	6.9	14.2	13.2	21.0	9.4	15.8	10.3	19.4
> 1500	90.1	82.6	93.1	85.8	86.8	79.0	90.6	84.2	89.7	80.6

Changes in micronutrient intake

There is a significant increase (p = 0.00) in the mean intake of calcium from the year 2003 (488 mg; 48% of RNI) to 2014 (531 mg or 52% of RNI). Clearly, based on the 2017 RNI, calcium intake is inadequate. The mean intake of iron was 14 mg in the year 2003 (121% RNI) and 16 mg in 2014 (144% RNI). This showed that the mean intake of iron had significantly increased (p = 0.00) between 2003 and 2014. However, it should be pointed out that the increase in mean iron intake occurred largely among males. Even though there was an increase from 2003 to 2014, iron intake among females was still slightly below RNI in 2014. A significant increase (p = 0.00) was also found in the mean intake of vitamin A from 2003 (645 µg or 107% of RNI) to 2014 (870 µg or 145% of RNI). On the whole, the mean intakes and RNI achievements for calcium, iron, and vitamin A have all showed increasing trends between 2003 and 2014 among the male, female, urban and rural populations.

The mean intake of vitamin C was slightly higher (75 mg or 107% of RNI) in 2014 than in 2003 (72 mg or 103% of RNI). On the other hand, there was a decrease (p = 0.13) in the mean intake of thiamine from 2003 (0.93 mg or 81% RNI) to 2014 (0.90 mg or 78% RNI). When the respondents were categorised according to strata and gender, the mean intake and RNI achievement of vitamin C showed no significant changes from 2003 to 2014.

On average, Malaysian adults consumed less sodium in 2014 (2973 mg mean sodium intake) than they did in 2003 (3260 mg mean sodium intake). A decrease in sodium intake was also found when respondents were categorised according to gender and strata. However, the dietary intake of sodium among Malaysian adults was still almost double of the amount recommended by WHO (2012) and RNI 2017. Using the sodium intake level of <2000 mg/day (recommended for optimal blood pressure) as a guideline, only one-fifth of the Malaysian population had fulfilled this requirement in 2003 whereas in 2014, this number had increased to one-third. In the same vein, only one-tenth of the Malaysian population had a daily sodium consumption of ≤1500 mg in 2003. This figure had increased to almost one-fifth in 2014.

DISCUSSION

Our study augments the findings of previous studies concerning the nutrient intake in a population by accounting for the increase in overweight and obesity factors among the Malaysian population over a time period. Specifically, the prevalence of overweight and obesity had increased from 21% in year 1996 to 47.7% in year 2015 based on the National Health and Morbidity Surveys (IPH, 2015). In contrast, data from MANS surveys reported a median EI of 1,540 kcal per day in 2003 (Mirnalini *et al.*, 2008) which decreased slightly to 1,466 kcal per day in 2014 (IPH, 2014a). A possible reason for the discordance between the trends in diet and disease or disease risk factor in the population may be due to the analysis of data on the total survey population, without accounting for mis-reporting.

The presence of under- and over-reporting, however, was highlighted. Macdiarmid and Blundell (1998) in their review from previous surveys concluded that under-reporting in large nutritional surveys ranged from 18-54% of the whole sample, but could be as high as 70% in particular subgroups. In our case, the percentage of energy under-reporters in the MANS was 54% in 2003 and 61% in 2014. The detailed characteristics of under-reporting of EI in MANS have been described elsewhere (Ahmad Ali *et al.*, 2019). The previous reported daily EI of Malaysian adults ranged from 1500-2700 kcal (Chee *et al.*, 1997; Lee, Norimah & Ismail, 2010; Mirnalini

et al., 2008; Sharif *et al.*, 2016). After excluding the under- and over-reporting of EI, our analyses suggest that mean EI was around 2000 kcal per day, a figure which meets the recommended intake for the Malaysian adult population.

Comparing the time periods 2003 and 2014, generally desirable changes in intake were found for almost all nutrients. Similar changes were observed in gender and strata groups. The average EI was satisfactory. The proportion of energy derived from macronutrients was within the dietary guidelines except for fat, which slightly exceeded for a healthy diet recommended by RNI Malaysia (NCCFN, 2017).

The accelerated pace of industrialisation and urbanisation of the recent years has generated marked changes in lifestyles, occupational patterns and dietary habits amongst Malaysians (Ismail, 2002; Sheng *et al.*, 2008) such that large numbers of the urban population habitually eat out (Ali & Abdullah, 2012). In both rural and urban areas, eating habits have shifted from traditional diets to the convenience of prepared and processed meals. The traditional diet is being replaced by diets higher in fats, salts and animal products and often with lower intakes of fresh fruits and vegetables (Soon & Tee, 2014). As Malaysia rapidly proceeds towards a developed economy status, the population's lifestyle will continue to change. The escalation of nutrition-related chronic degenerative diseases, once an urban phenomenon, has now spread to the rural population at an alarming rate (Ismail, 2002).

As shown earlier, only calcium intake did not meet RNI 2017. Asian populations, in general, have been reported to be calcium deficient, as evidenced by mean calcium intakes in Vietnam, Japan and Korea of approximately 500 mg/day or less (Danh Tuyen *et al.*, 2016; Ohta, Uenishi & Shiraki, 2016). This could be due to the Asian diet, including Malaysian, and may be one of the barriers to achieving an increase in calcium intake. According to Singh *et al.* (2015), calcium intake was highly positively correlated with milk consumption, with highest levels in Western and lowest levels in Eastern Sub-Saharan Africa. Across 21 world regions, Central Latin America was the region with highest milk intake. Milk consumption also exceeded three-quarters of a serving in Europe and Southern Sub-Saharan Africa. However, adults in East Asia and Oceania consumed the least milk, it being less than a quarter of a serving per day. In Malaysia, Norimah *et al.* (2008) found that food consumption patterns among Malaysian adults from MANS 2003 showed that the highest prevalence of daily consumption of full-cream milk was only 24% and this occurred among older, predominantly female adults, aged 50–59 years, whereas those aged 18-19 years had the lowest prevalence of daily consumption at only 15%.

Sodium intake has been a major concern with regard to the prevention of hypertension, and other related non-communicable diseases in Malaysia. Regular nationwide health campaigns and health education could be a contributing factor in the significant decrease in sodium intake over the two survey periods. While the mean daily sodium intake in 2014 was significantly lower than that in 2003, it is still >1.5 times higher than the recommended sodium intake limit of 2,000 mg to control blood pressure (WHO, 2012) and two times higher than the minimum recommended sodium intake limit of 1500 mg for Malaysia adults (NCCFN, 2017). These findings were lower than those obtained by assessing sodium intake using 24-hour urinary sodium excretion. It was reported that Malaysia adults aged 20-56 years excreted 142 mmol sodium per day which is equivalent to the intake of 3,429 mg sodium per day, and this is 1.7 times higher than the recommended sodium intake limit of 2,000 mg (Rashidah *et al.*, 2014).

In comparison with the global surveys of 2010, the mean sodium intake was 3,950 mg per day and nearly twice the WHO recommended limit of 2,000 mg per day. Intakes were highest in East Asia, Central Asia and Eastern Europe region (mean >4200 mg/day). However, contrary to the Asia regions, mean sodium intake in Malaysia was 3,570 mg/day and lower than the global mean sodium intake (Powles *et al.*, 2013). A systematic review and meta-analyses on the effect of sodium intake in non-acutely ill adults showed that reduced sodium intake reduces blood pressure and lower sodium intake is also associated with a reduced risk of stroke and fatal coronary heart disease (Aburto *et al.*, 2013).

The strength of this study was that it included a large representative sample of the Malaysian adult population, where the under-reporting and over-reporting of EI in dietary assessment analysis were excluded. We acknowledge that the limitation of our study was the use of a single 24-hour diet recall as measurement of dietary intake as may not provide good estimates of intake compared to multiple 24-hour diet recalls.

CONCLUSIONS

Our findings have shown that most changes in nutrient intakes have improved in accordance with Malaysian nutrient recommendations (NCCFN, 2017). There have been significant changes in sources of energy, calcium, iron, sodium and vitamin A over the study period. This study has also revealed areas of concern, namely, that the consumption of carbohydrate has declined while the intakes of protein and fat have increased. These trends are in tandem with increases in chronic disease risk factors in the country, namely obesity. The adequacy of protein from current RNI cut-off (NCCFN, 2017) is not consistent with RNI achievement and should be reviewed. There is considerable scientific evidence linking excessive dietary fat intake with various health problems such as cardiovascular diseases, cancer, elevated cholesterol levels and obesity.

The results of this study have highlighted important information. There is a need for the promotion of a healthy lifestyle, particularly in having a balanced diet among targeted Malaysian populations.

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Author's contributions

AAZ, wrote the manuscript with support and supervised from all authors; SMY and AINI, verified the analytical methods. All authors provided critical feedback, discussed the results and contributed to the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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Consumer awareness and understanding of front-of-pack (FOP) energy icon labelling in Negeri Sembilan, Malaysia

Fatimah Sulong[1,3*], Ruhaya Salleh[2] & Zainudin Mohd Ali[3]

[1]*Nutrition Division, Ministry of Health Malaysia;* [2]*Institute of Public Health, Ministry of Health Malaysia;* [3]*Negeri Sembilan State Health Department, Ministry of Health Malaysia*

ABSTRACT

Introduction: The implementation of front-of-pack (FOP) energy icon labelling helps consumers in making good food choices. This is the first study in Malaysia focusing on such labelling since it was launched in 2012. It was aimed at determining the awareness and understanding of the FOP energy icon on food labels in Malaysia. **Methods:** A total of 366 consumers aged 18-60 years old in the state of Negeri Sembilan participated in the study. A guided, self-administered survey was conducted using a convenient sampling method. **Results:** The results showed that 85% of consumers surveyed were aware of FOP energy icon. Among those who were aware of the icon, 50% (n=155) were categorised as 'excellent' and 41% (n=128) categorised as 'good', for understanding the FOP. **Conclusion:** This study indicated that the icon could be viewed as a potential tool to be used in conjunction with the nutrition information panel (NIP). Most of the respondents could extract nutrition information from the FOP (energy) icon. The study showed that those who had understood the icon were in the group categories of high education, youth and female. There was also no significant association between those who received nutrition labelling education and level of understanding nutrition information from the icon. Therefore, it is important to further explore the possibility the beneficial impact of FOP labelling system, including consumer education aspects.

Keywords: Label, nutrition labelling, front-of-pack (FOP), energy icon

INTRODUCTION

Simplified nutrition labelling has been identified as an important tool to help consumers make healtheir food choices. Recognising this potential, the nutrition labelling of all prepackaged foods was proposed as a policy measure in the non-communicable diseases (NCDs) action plan for 2013–2020, which was adopted by the 66th World Health Assembly in May 2013 (WHO, 2013).

Consumers are confronted with an increasing variety of foods, especially processed and packaged products. Consequently, it has become increasingly difficult for them to make healthy and informed choices. The Third National Health and Morbidity Survey (NHMS III) of Malaysia in 2006 reported that 78.2% of consumers read the nutrition label when buying or receiving food (IPH, 2008). However, the same study repeated in 2014 indicated that the prevalence

*Corresponding author: Fatimah Sulong
Nutrition Division, Ministry of Health Malaysia, Level 1, Block E3, Parcel E, Putrajaya, Malaysia
Tel: (603)88924405; Fax: (603)88924512; Email: fatimahsulong@moh.gov.my
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of reading nutritional information from food labels was only 45.0% (IPH, 2015).

Consumer testing by the Keystone Group has suggested that the simplicity of summarising the diverse nutritional information in the nutrition information panel (NIP) into a single indicator to classify products is highly desirable for consumers (Lupton *et al.*, 2010). Similarly, research in the European Union (EU) has indicated that consumers generally prefer simpler, "healthy choice tick" front-of-pack (FOP) icons (Feunekes *et al.*, 2008). Work by the Food Standards Agency in the United Kingdom also suggests that more complex FOP icons, such as Multiple Traffic Lights with percentages and levels that are based on the guideline daily amounts, may help with the evaluation of several nutrients for a given food (FSA, 2009).

When FOP systems first appeared in the late 1980s and early 1990s, they were largely developed by non-profit health organisations (IOM, 2010). Since then, food manufacturers have been adding summary nutritional information on the FOP in addition to that currently mandated, on the back or side of the NIP. These different approaches to communicate nutritional information through food labels have become important part of the strategy to assist consumers in adopting healthy dietary practices, as well as encourage food industries to produce healthier food options. The information may be a quick guide to inform consumers about the nutrition content of different products.

Malaysia has made nutrition labelling mandatory for most prepacked foods since 2003. In addition, one of the FOP systems introduced in Malaysia is energy icon that was launched by the Health Minister on 2nd April, 2012. It provides a description of the number of calories per serving contained in certain food and beverage products. It was reported that Federation of Malaysian Manufacturers Malaysian Food Manufacturing Group (FMM MAFMAG) had agreed to put FOP (energy) icon on food and beverage products to help consumers estimate their daily nutrient intake (The Star, 2012). As of September 2017, FMM MAFMAG reported that about 1,345 products had displayed the FOP (energy) icon (KKM, 2018). This has been part of the industry's commitment to tackle the issue of obesity and NCDs in the country.

Since the energy icon was launched more than five years ago, there has been no study published, to the best of our knowledge, in Malaysia, that has focused on the FOP for energy icon. There was a national study to review the proposal of using certain symbols or logos such as "healthier choice" that was carried out by Task Force Committee on Healthier Choice under Ministry of Health in 2008. However, the findings of the study of 1936 respondents from 15 states were not published. In 1992, a study by Schucker *et al.* suggested that consumers purchased more products which displayed FOP labelling than those which did not. Previous studies have found that FOP label formats could help consumers to differentiate between healthy and unhealthy products (Dunbar, 2010; Feunekes *et al.*, 2008). Among various FOP label formats, the consumers took the longest time to evaluate the products with the Guideline Daily Amount (GDA) format (Feunekes *et al.*, 2008).

This preliminary study in Negeri Sembilan was aimed at examining consumer awareness and understanding of the FOP for energy icon in the Malaysian context. It is important to gather this information to help relevant authorities to strengthen the consumer understanding of nutrition information displayed on food labels. Furthermore, the findings from this study may help the policy makers to design nutrition

labelling education programmes, and to undertake future research in this area.

MATERIALS AND METHODS

A total of 366 respondents were recruited using a convenient sampling method. These respondents were recruited from September to December 2016, prior to the department's activities of Healthy Community Kitchen, Healthy Supermarket, Nutrition Information Centre, Community KOSPEN (*Komuniti Sihat Pembina Negara*) and from participants of nutrition talks held in Negeri Sembilan. Respondents who met the inclusion criteria (i.e. age 18-60 years, Malaysian citizen and enrolled at any of those department's activities between September to December 2016) were recruited. The exclusion criteria for this study were the presence of illnesses such as dementia or mental disorders, special dietary needs and communication difficulties. Written consent from each respondent was obtained prior to data collection.

The questionnaire was designed to be self-administered with guidance by the interviewer. To establish the content validity, nine officials who were involved in the areas of food labelling, nutrition labelling and signposting were asked to review the questionnaire. Each reviewer independently rated the relevance of each section in the questionnaire using a four-point likert scale (1=not relevant, 2=quite relevant, 3=relevant, 4=very relevant). The questionnaire was piloted among 24 different subjects from the community and improved upon for intended purpose and usefulness. The average time taken to finish the questionnaire was about 20 minutes.

The questionnaire consisted of a few main sections. These sections included demographic information, awareness and understanding of the FOP (energy) icon. Section A involved eight questions on the general characteristics of the respondents namely, gender, race, age, education level, occupation, marital status and receipt of any nutrition labelling education. Section B contained two questions regarding awareness and availability of FOP (energy) icon. In Section C, the concept that was used in the previous studies (Byrd-Bredbenner, 2000; IGD, 2005) on understanding how to get the nutrition information from the food label, was adapted. Using the FOP (energy) icon on the food label, respondents were asked to answer ten questions about nutrition information from the shown FOP icon. Respondents were required to answer either "true" or "false and the responses were classified as correct or incorrect, based on the factual answers. A score was calculated by summing the number of correct responses which could range from 0-10, with higher the score, indicating a greater ability to understand the nutritional information. The scores were divided into four groups, namely, 'excellent' for score 9-10, 'good' for score 6-8, 'fair' for score 3-5 and 'weak' for score 0-2.

Ethical approval

Ethical approval was obtained from the Ministry of Health Research and Ethics Committee (MREC) (NMRR-16-1252-31661). The project was registered with the National Medical Research Register (NMRR) prior to implementation. All the information from the questionnaire including the personal information of the respondents' was kept confidential.

Data analysis

Data analysis was undertaken using the SPSS version 16.0 (IBM Corp., Armonk, New York). Pearson's chi-square test was used to test whether there was significant association between the awareness of the FOP (energy) icon and receiving nutrition labelling education. The level of significance used for the

data analysis was set at $p<0.05$. The correlation test was used to evaluate the association between category of understanding FOP (energy) icon with other factors including awareness of the icon, receiving nutrition labelling education and sociodemographic characteristics.

RESULTS

The demographic characteristics of the respondents are shown in Table 1. Female respondents constituted 71.0% (n=260) of the respondents and male 29.0% (n=106). The majority was Malays (84.0%), followed by Chinese (7.0%), Indians (6.0%) and the other ethnic groups such as Kadazans, Bajaus and Muruts constituted 3.0%. The age category with the highest percentage of respondents was 35–44 years, who made up 28.7% (n=105). The proportions and numbers for the other age categories were 18–24 (26.8%, n=98), 25–34 (15.0%, n=55), 45–54 (19.1%, n=70) and 55–60 (10.4%, n=38). Almost half (48.0%) had achieved a secondary level of education and the proportions who completed primary school, diploma/certificate, degree holders were 2.0%, 34.0% and 16.0%, respectively. The study also indicated that *Sijil Pelajaran Malaysia* (SPM)/Malaysia Certificate of Education (MCE) holders were the

Table 1. Sociodemographic characteristics of the respondents

Characteristics	*n*	*%*
Gender		
Male	106	29.0
Female	260	71.0
Ethnic group		
Malays	309	84.4
Chinese	25	6.8
Indians	23	6.3
Others	9	2.5
Age group		
18-24 years	98	26.8
25-34 years	55	15.0
35-44 years	105	28.7
45-54 years	70	19.1
55-60 years	38	10.4
Education level		
Primary school	8	2.2
Lower secondary	27	7.4
Upper secondary	147	40.2
Diploma/certificate	125	34.2
Degree	59	16.1
Occupational		
Public sector	116	31.7
Private sector	40	10.9
Self employed	52	14.2
Retiree	13	3.6
Housewife	55	15.0
Student	90	24.6
Marital status		
Married	228	62.3
Single	114	31.1
Divorced/widowed	24	6.6

Table 2. Association between awareness of FOP (energy) icon and receiving nutrition labelling education

Receiving nutrition labelling education	*Awareness of FOP, n (%)*			χ^2	*p-value*
	Yes	*No*	*Total*		
Yes	191 (52.2%)	23 (6.3%)	214 (58.5%)	8.242	0.004
No	119 (32.5%)	33 (9.0%)	152 (41.5%)		
Total	310 (84.7%)	56 (15.3%)	366 (100.0%)		

highest respondents (40.2%, n=147), followed by *Sijil Tinggi Persekolahan Malaysia* (STPM)/diploma/certificate holders (34.2%, n=125), degree holders (16.1%, n=59), *Penilaian Menengah Rendah* (PMR)/ *Sijil Rendah Pelajaran* (SRP)/Malaysia Lower Certificate of Education (LCE) holders (7.4%, n=27) and primary school leavers (2.2%, n=8). Most of the respondents worked in the public sector (31.7%, n=116). Others were students (24.6%, n=90), housewives (15.0%, n=55), self-employed (14.2%, n=52), private sector employees (10.9%, n=40) and retirees (3.6%, n=13). On marital status, married respondents showed the highest percentage (62.3%, n=228), followed by single respondents

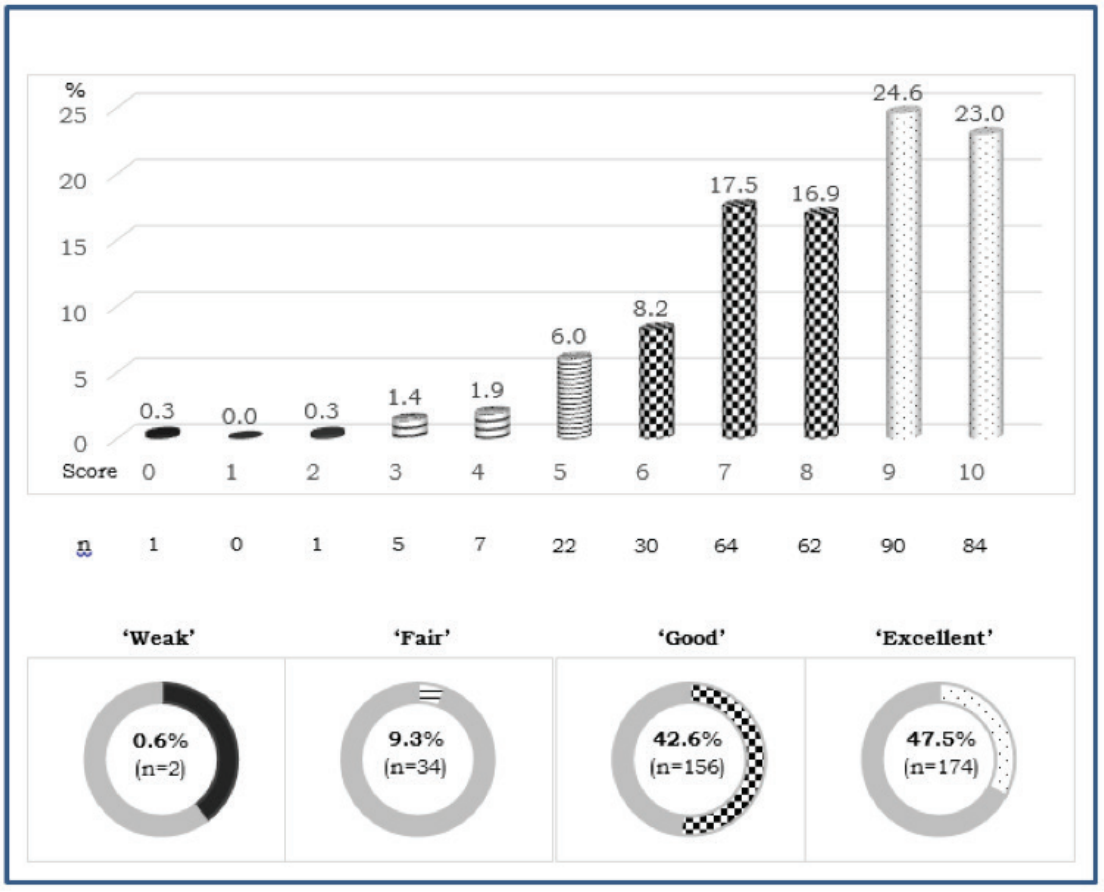

Figure 1. Category of understanding of the FOP (energy) icon

Table 3. Association between category of understanding FOP (energy) icon and those receiving nutrition labelling education and awareness

Status	*Category of understanding, n (%)*				*Total*	*r*	*p-value*
	Weak	*Fair*	*Good*	*Excellent*			
Receiving nutrition labelling education							
Yes	1 (0.5)	20 (9.3)	90 (42.1)	103 (48.1)	214	0.008	0.877
No	1 (0.7)	14 (9.2)	66 (43.4)	71 (46.7)	152		
Awareness of the icon							
Yes	2 (0.6)	25 (8.1)	128 (41.3)	155 (50.0)	310	-0.155	0.003
No	0 (0.0)	9 (16.1)	28 (50.0)	19 (33.9)	56		

(31.1%, n=114) and divorcees or widow/widower (6.6%, n=24).

The findings showed that 84.7% (n=310) aware of the FOP energy icon that had been printed on food label. Among these respondents, 66.1% (n=242) found that the energy icon was easy to recognise on the label, while 18.9% (n=69) claimed it was hard to find the icon and 15.0% (n=55) were not sure about the icon. Among the 366 respondents, 58.5% (n=214) had received nutrition labelling education mostly from health staff (65.4%, n=140). Other sources of nutrition labelling education were from advertisements (21.5%, n=46), industry or product promoters (3.3%, n=7) and others such as part of the education curriculum (9.8%, n=21) (Table 2). Among all the respondents, including those who had received nutrition labelling education, 52.2% (n=191) were aware of the icon. Among all the respondents and including those who had never received any nutrition labelling education, only 32.5% (n=119), were aware of the icon. Based on the chi-square tests, there was a significant association between receiving nutrition labelling education and the awareness of the FOP energy icon (p<0.05).

The mean score for understanding the FOP was 8.0±1.8 and 23.0% (n=84) of the respondents obtained full marks when they were asked to extract information from the FOP. A majority of the respondents (24.6%, n=90) scored 9 marks. Those respondents who scored 10 and 9 marks were grouped as 'excellent'. This group was the largest compared to other groups (47.5%, n=174). The second largest group was the respondents who scored 6-8 marks, who were categorized as 'good' (42.6%, n=156); and <10.0% (n=36) of the respondents scored <6 marks. They were categorized as 'fair' (scored 3-5 marks) and 'weak' (scored 0-2 marks), with the percentage of 9.3% (n=34) and 0.6% (n=2) respectively. Figure 1 shows the scores of the respondents for the understanding of the FOP (energy) icon.

The category of understanding by receiving nutrition labelling education and awareness of FOP is presented in Table 3. The findings showed that there was no relationship between those who had received nutrition labeling education and their understanding of FOP (energy) icon (p>0.05). However, there was an association between the awareness of FOP (energy) icon and understanding the icon (p<0.01).

Table 4 presents the category of understanding the icon by socio-demographic background. The results showed that among the males, 14.2% (n=52) were categorised as 'good',

Table 4. Association between understanding of FOP (energy) icon and socio demographic background

Characteristics	*Category of understanding, n (%)*				*Total*	*r*	*p-value*
	Weak	*Fair*	*Good*	*Excellent*			
Gender							
Male	1 (0.3)	14 (3.8)	52 (14.2)	39 (10.7)	106	0.134	0.010
Female	1 (0.3)	20 (5.5)	104 (28.4)	135 (36.9)	260		
Age							
18-24 years	0 (0.0)	4 (1.1)	38 (10.4)	56 (15.3)	98	-0.246	0.000
25-34 years	0 (0.0)	4 (1.1)	14 (3.8)	37 (10.1)	55		
35-44 years	0 (0.0)	14 (3.8)	44 (12.0)	47 (12.8)	105		
45-54 years	1 (0.3)	7 (1.9)	37 (10.1)	25 (6.8)	70		
55-60 years	1 (0.3)	5 (1.4)	23 (6.3)	9 (2.5)	38		
Education level							
Primary	0 (0.0)	1 (0.3)	7 (1.9)	0 (0.0)	8	0.284	0.000
Lower secondary	0 (0.0)	7 (1.9)	15 (4.1)	5 (1.4)	27		
Upper secondary	2 (0.6)	19 (5.2)	64 (17.5)	62 (16.9)	147		
Diploma/ Certificate	0 (0.0)	6 (1.6)	50 (13.7)	69 (18.8)	125		
Degree	0 (0.0)	1 (0.3)	20 (5.5)	38 (10.4)	59		

followed by 'excellent' (10.7%, n=39) and 'fair' (3.8%, n=14). As for the females, the majority (36.9%, n=135) were categorised as 'excellent', followed by 'good' (28.4%, n=104) and 'fair' (5.5%, n=20), respectively. For the age group of 18-24 years, 25-34 years and 35-44 years showed the same pattern where most of the respondents' understanding of FOP (energy) icon were categorized as 'excellent' (15.3%, n=56; 10.1%, n=37 and 12.8%, n=47 respectively). For the age group of 45-54 years and 55-60 years, both groups showed that the majority of the respondents' understanding of FOP (energy) icon was categorized as 'good' (10.1%, n=37 and 6.3%, n=27 respectively). Correlation tests showed that gender, age and level of education were significantly associated with the understanding of FOP (energy) icon (p<0.01).

DISCUSSION

Since the FOP energy icon was launched in 2012, about 85.0% of the consumers surveyed have become aware it. Grunert & Wills (2007) reported that consumers must be exposed or be aware of the label system in order for the label to have any effect. The results of this study also showed that majority of the respondents were able to understand nutritional information from the icon. This may be due to the fact that the nutritional information on the icon was self-explanatory and was compatible with the message that the industry had intended to communicate.

According to Grunert & Wills (2007), the indication that the FOP icon was helpful in assisting consumers to make informed food choices was when they could understand the nutritional information on the label. A year after the

implementation of FOP GDA labelling in Thailand, about 48% of consumers aware of GDA labels, and 52% were able to identify the information from the GDA labels when choosing products (Rimpeekool *et al.*, 2016). Based on unpublished data from the Singapore Health Promotion Board (HPB) in 2004, 67.4% of people were aware of Healthier Choice Symbol (HCS) labels on food products in the market, and 69.0% of these people had used this symbol to assist them in making healthier food choices (Soon *et al.*, 2008). A study in New Zealand also reported that the awareness of FOP "Tick" had increased from 71% in 1997 to 87% in 2000. The proportion of consumers claiming to use the "Tick" to guide food choices increased from 43% to 55% (Mhurchu, Eyles & Choi, 2017). These findings are an indication of how the awareness of FOP nutrition-related symbol can support healthier eating habits.

The findings also showed that there was no association between those who had ever received nutrition-labeling education and understanding of FOP (energy) icon. Education may support the use of nutrition information on food label by increasing the efficiency of label use. Previous studies on the relationship between nutrition labelling education and the use of the nutrition label revealed mixed findings. Kim, Nayga & Capps (2001) found that knowledge of health had a positive effect on label use, while Nayga (2000) could find no evidence to support this relationship. Findings in other studies suggested that the combination of nutrition labelling and education of consumers, can significantly influence consumer behaviour (Teisl, Bockstael & Levy, 2001; Teisl & Levy, 1997). The majority of a systematic review of 17 studies conducted in the United States, found that educational interventions could lead to a positive impact on the health of the population when they use and/or understand the nutrition information on food labels (Moore *et al.*, 2018).

The findings of understanding the FOP (energy) icon are consistent with previous studies in relation to the demographic characteristics of consumers. Our study indicated that those who understood the icon were in the categories of high education, youth and female. Ducrot *et al.* (2015) found that those who able to understand FOP labelling also tended to be female with higher education level. In a various review studies on nutrition labelling, several demographic differences such as being females and higher education level have been observed to be positively associated with food label use (Drichoutis, Lazaridis & Nayga, 2006; Cowburn & Stockley, 2005). The reason women would pay more attention to nutrition labels was that they were more concerned about the nutritional composition of food and the use of nutrition labelling would enable them to make a healthier choices (Nayga, 1999). However, the findings of some other studies showed contradictory results, in which men used nutrition labels more often than women (Aygen, 2012) or no significant differences were seen between men and women in the use of nutrition labels (Norazlanshah *et al.*, 2013). Consumers with higher education level may have a better chance of assessing nutrition information on labels as compared to those who are lesser educated. This is where the media can support in promoting the education of nutrition labelling to the less educated consumers, to attract their attention to become more interested in nutrition labelling (Hayati *et al.*, 2015). The previous studies in Malaysia found significant differences of age for the nutrition label literacy (Rashidah *et al.*, 2014; Cheong *et al.*, 2013). However, the study by Mohamad Rohieszan *et al.* (2016) was not able to support those

findings and showed that the difference was not significant by age. A review of past studies has also reported that the effects of age on label use were mixed (Drichoutis *et al.*, 2006).

CONCLUSION

The findings suggest that the majority of the respondents gave a correct interpretation of the nutritional information from the FOP (energy) icon. This study indicates that the icon is viewed as a potential tool to be used in conjunction with NIP. The icon also can give consumers useful information to help consumers in making food choices based on their daily requirements. Even though the findings showed no significant association between those who received nutrition labeling education and understanding of the icon, it is important to encourage better understanding to ensure the usage of the nutrition information on the label. Any nutrition labelling system including FOP (energy) icon needs to be accompanied by awareness and education programmes.

As a preliminary step in assessing understanding of Malaysian consumers towards FOP (energy) icon, this study has its limitation. The numbers of respondents according to ethnicity in Malaysia need to be taken into account to reflect the true features of Malaysian population structure. Studies focusing on consumer education aspects are also necessary. These scopes were beyond the objectives of the study. However, it is essential to address the knowledge gaps and future research would be needed to include these parameters in the context of Malaysian population.

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Authors' contributions

FS, principal investigator, conceptualised and designed the study, led the data collection in Negeri Sembilan, prepared the draft of the manuscript and reviewed the manuscript; RS, advised on the data analysis and interpretation and reviewed the manuscript; ZMA, assisted in conceptualised the study and reviewed the manuscript.

Conflict of interest

The authors have no conflict of interest regarding the publication of this article.

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Demographic factors, food security, health-related quality of life and body weight status of adolescents in rural area in Mentakab, Pahang, Malaysia

Susanti Alie, Norhasmah Sulaiman*, Fadilah Mohd Nor & Siti Farhana Mesbah

Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia

ABSTRACT

Introduction: Adequate, nutritive and safe foods are crucial for growth and healthy living. Adolescents are vulnerable to food insecurity. This study was aimed at determining the demographic factors, food security status, health-related quality of life (HRQOL) and body weight status of adolescents in Mentakab, Pahang, Malaysia. **Methods:** This study involved 160 households that comprised pairs of mothers and children aged 13-17 years. Face-to-face interviews were conducted with the mothers to assess their demographic and food security status (Radimer/Cornell Hunger and Food Insecurity Instrument). Meanwhile, the children answered a self-administered HRQOL questionnaire (Pediatric Quality of Life Inventory, PedsQL). Body weight and height were measured to obtain the body mass index (BMI). **Results:** About 48.8% of the adolescents were from households with food insecurity. The number of school-going siblings, occupation status of mother, occupation status of father, household income and house ownership status were predictors of food security status (p<0.05). After controlling for covariates, the HRQOL score and BMI were higher in adolescents from food-secure households than adolescents from food-insecure households (p<0.01). **Conclusion:** The prevalence of food insecurity was high and multifactorial. Food insecurity was further associated with HRQOL and BMI. Food assistance programmes are recommended to directly alleviate food insecurity. Concurrently, monetary and educational aids are advocated to reduce the economic burden, especially in low-income households.

Keywords: Adolescents, food insecurity, health-related quality of life, Malaysia

INTRODUCTION

Adolescence is the second most rapid phase of human growth. It is a period of life with specific health and developmental needs. Adolescent is defined as any person aged 10-19 years. However, the age range has been extended to between 10 and 24 years because it corresponds more closely to adolescent growth (Sawyer *et al.*, 2018). Nutrition plays an important role in fulfilling the energy and nutrient requirements for growth and bodily functions in adolescents (Das *et al.*, 2017). Therefore, food security is crucial to sustain active and healthy lives. According to the World Food Summit, food security occurs when all people at all times have physical and

*Corresponding author: Dr Norhasmah Sulaiman
Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia.
Tel: 03-89472461; Fax: 03-89426769; Email: norhasmah@upm.edu.my
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economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for active and healthy lives (WHO, 2012).

The prevalence of food insecurity varies among adolescents. Previous studies have found that about 70% of Mexican children and adolescents experienced some degree of food insecurity (Rodriguez *et al.*, 2017). The prevalence of food insecurity among Korean middle school adolescents (12-14 years old) and Korean high school adolescents (15-18 years old) were 11.1% and 16.8%, respectively (Nakitto *et al.*, 2017). A local study done by Roselawati *et al.* (2017) reported that the prevalence of food insecurity among adolescents aged 7-13 years in Kuantan, Pahang was 77.0%. The prevalence was higher among those from the low socio-economic status.

The literature has reported several demographic factors that affect food insecurity. Low income is the main predictor of food insecurity (Mohamadpour, Sharif & Keysami, 2012; Wang *et al.*, 2015; Roselawati *et al.*, 2017). Low income contributes to the inability to provide adequate basic needs (such as food) for the household members. Further, large numbers of school-going children in a household are associated with food insecurity because clothing, footwear, books, and pocket money for school-goers contribute significantly to the expenditure of the household (Norhasmah *et al.*, 2011). Likewise, residents of rented accommodation units are more likely to be food-insecure than those who live in their own houses. This is because rent payments limit the monetary resources (Sriram & Tarasuk, 2016). Demographic and socio-economic factors are risk factors of food insecurity.

Food insecurity has been associated with many undesirable consequences, including poor nutritional status (Norhasmah *et al.*, 2011; Nakitto *et al.*, 2017) and poor academic performance (Belachew *et al.*, 2011). Food-insecure children were three times more likely to be stunted and two times to be underweight as compared to food-secure children (Naser *et al.*, 2014). This might be due to the lower frequency of daily meal intake. Adolescents from poor were less likely to have breakfast than those from high and middle socio-economic backgrounds (Crawford *et al.*, 2015). School absenteeism rates were shown to be significantly higher in food-insecure primary school adolescents than those from food-secure in Ethiopia (Tamiru *et al.*, 2016). Food-insecure adolescents were probably unable attend school because of illness and/or lack of access to food.

In Malaysia, little is known about food insecurity and the health-related quality of life (HRQOL) among adolescents. To the best of our knowledge, there has been no local study on the association between food security and HRQOL in adolescents; there was only one study conducted on food security and HRQOL in women (Ihab *et al.*, 2012). Only one local study on food-insecurity and nutritional status among children aged 7-13 years has been identified (Roselawati *et al.*, 2017). Although numerous studies have examined the contribution of food security to body weight status, the findings on the association between food insecurity and body weight status was inconsistent. Therefore, this study of adolescents was aimed at (i) determining if there was an association between demographic factors and food security status, and (ii) at examining the differences in HRQOL and body mass index (BMI) based on food security status.

MATERIALS AND METHODS

This cross-sectional study was carried out in Mentakab, which is one of the

sub-districts of the Temerloh district in the state of Pahang in Peninsular Malaysia. A list of residential areas in Mentakab was obtained from the Municipal Council of Temerloh, Pahang. Mentakab was chosen due to its larger population and household counts in the said list as compared to other sub-districts. Based on the list, *Kampung Penak* was chosen as the study location because it had the largest residential areas. There were three residential areas in *Kampung Penak*, namely the traditional village of *Kampung Penak*, *Taman Sri Penak*, and *Taman Penak Perdana*. Quota sampling was applied whereby the sampling ratio was 2:4:4. The village of *Kampung Penak* had 100 households while *Taman Sri Penak* and *Taman Penak Perdana* had around 300 households each; 32 households (20%) from the traditional village, as well as 64 households (40%) from each of *Taman Sri Penak* and *Taman Penak Perdana* were recruited into this study.

The total sample size was determined using the G*power 3.1.9.2 software (Faul *et al.*, 2007). The odds ratio (OR) was obtained from Tamiru *et al.* (2016), who had found that household food insecurity increased the odds of absenteeism by 2.81 times. The calculated minimum sample size was 133 respondents. After adding 20% to the minimum sample size to account for drop-outs, it was decided to increase the sample size to 160 respondents. The mothers with their children (the latter aged 13-17 years) were recruited in pairs. Mothers with hearing problems or mutism and physically disabled adolescents were excluded.

Ethical approval for this study was obtained from the Ethics Committee for Research Involving Human Subjects, Universiti Putra Malaysia (JKEUPM). Written consent was obtained from the respondents prior to data collection.

Independent variables

A questionnaire, which contained questions on demographic background, food security status and HRQOL, was used to collect data. The demographic background and food security status parts were answered by the mother, while the HRQOL of the adolescents part was answered by the adolescents themselves. Demographic background questions were on the child's age, child's sex, mother's age, ethnicity, marital status (of caregiver or parents), household size, number of siblings, number of school-going siblings, household income, per capita income, parental occupation status, parental education level and house ownership status.

Food security status was assessed using the Radimer/Cornell Hunger and Food Insecurity Instrument. This section contained ten items that measured the levels or severity of food insecurity, namely, food security, household food insecurity, individual or adult food insecurity and child hunger. The answers were considered to be positive if the responses were either "sometimes true" or "often true". Conversely, "not true" answers reflected negative responses. The households were categorised as food-secure when there were negative answers to all hunger and food insecurity items. Positive answers to one or more household item(s) (1-4) defined household food insecurity. Meanwhile, positive answers to one or more items concerning the adults (5-7) or items on the quality of children's diet (8) – in addition to negative answers to the items on the quantity of children's intake (9-10) – denoted individual food insecurity. Positive answers to the items on the children's quantity of food intake (9-10) indicated child hunger.

Dependent variables

The Pediatric Quality of Life Inventory (PedsQL) version 4.0 Generic Core

Scales was used to measure the HRQOL of adolescents. The PedsQL consisted of 23 items with four different subscales, namely physical, emotional, social, and school functions. The items were rated based on a five-point Likert scale that ranged from 0 (never) to 4 (almost always). In terms of scoring, the items were reverse-scored and could be linearly transformed into a 0-100 scale. Therefore, higher scores indicated better HRQOL. The reverse items were transformed to 0-100 as follows: 0=100, 1=75, 2=50, 3=25, and 4=0.

The BMI of the adolescents was assessed by measuring their body weight and height. Body weight was measured by using the calibrated TANITA weighing scale and height by using the stadiometer mobile height (Seca 206). All measurements were taken three times to obtain the average reading. The BMI was calculated by dividing the body weight with the height square.

Data analysis

Data were analysed by using IBM's Statistical Package for Social Sciences (SPSS) version 20 and the Anthroplus WHO software was used particularly to determine the category of the BMI among adolescents. Descriptive analysis included percentages and frequencies for categorical data. Means and standard deviations were used to describe the continuous data. Binary logistic regression was used to determine the demographic predictors of the food security status. After controlling the covariates, a general linear model (GLM) was used to determine the differences in the HRQOL scores and BMI based on the food security status. In the GLM analysis, food security status was grouped into two groups namely food secure and food insecure. The covariates were determined based on the presence of significant relationships between the demographic factors with HRQOL and BMI. The level of significance was set at $p<0.05$.

RESULTS

Demographic background, food security status, HRQOL and BMI

The mean±SD age of the adolescents was 14.9±1.4 years, with 61.2% of them aged 13-15 years (Table 1). The mean maternal age was 40.4±4.5 years, with half of the mothers (51.8%) in the range of 30-39 years. Over half (59.5%) of the mothers and 15.0% of the fathers were unemployed. The mean household size, number of children, and school-going children of the households were 5±1, 3±1, and 3±1, respectively. Over half of the respondents (53.1%) lived in rented units. The mean monthly household and per capita incomes were RM2363±1102 and RM467±305.66, respectively.

The prevalence of food insecurity in this study was 48.8%. This figure encompassed 20.0% who had household food insecurity, 13.8% individual food insecurity and 15.0% child hunger. Based on Radimer/Cornell hunger scale, food security status could be divided into four categories namely food secure, household food insecure, individual food insecure and child hunger. Household food insecurity was related to food supply management and acquisition issue, while individual food insecurity was related to food consumption issues and the physiological sensation of hunger. Child hunger was the most severe household food insecurity problem and it was characterised by a decrease in the quantity of food consumed by the children. Child hunger only occurs after adults in the household and quality of the children's diet had been affected by household food insufficiency. The total mean HRQOL score among the adolescents was 61.29±18.08, with the highest mean score noted in emotional functioning (64.03±22.55) and the lowest

Table 1. Characteristics of the adolescent participants in this study

Characteristics	*n*	*%*	*Mean±SD*
Age (Years)			14.9±1.4
13-15	98	61.2	
16-17	62	38.8	
Sex			
Male	85	53.1	
Female	75	46.9	
Ethnicity			
Bumiputera	124	77.4	
Non-Bumiputera	36	22.6	
Mother's Age (Years)			40.4±4.5
30-39	83	51.8	
40-49	70	43.8	
≥50	7	4.4	
Mother's educational level (Years)			10.19±3.67
No formal education	5	3.0	
Primary education	31	19.4	
Secondary education	56	35.0	
Tertiary education	68	42.6	
Father's educational level (Years)			12.96±2.83
No formal education	2	1.2	
Primary education	2	1.2	
Secondary education	50	31.3	
Tertiary education	106	66.3	
Mother's occupation status			
Housewife/unemployed	95	59.4	
Private	17	10.6	
Government	20	12.5	
Self-employed	28	17.5	
Father's occupation status			
Unemployed	24	15.0	
Private	39	24.3	
Government	30	18.8	
Self-employed	67	41.9	
Parents Marital Status			
Married	135	84.4	
Non-married	25	15.6	
Household size			5±1
1-5	79	49.4	
6-10	80	50.0	
≥11	1	0.6	
Number of siblings			3±1
1-3	70	43.8	
4-6	78	48.8	
≥7	12	7.4	
Number of school-going siblings			3±1
1-2	51	31.9	
3-4	96	60.0	
≥5	13	8.1	

Characteristics	*n*	*%*	*Mean±SD*
House ownership status			
Own	75	46.9	
Rented	85	53.1	
Household income (RM)			2363.13±1102.70
<1500	59	36.9	
≥1500	101	63.1	
Income per capita (RM)†			467.00±305.66
≤210	33	20.6	
>210	126	79.4	
Food Security Status			
Food secure	82	51.2	
Household food insecurity	32	20.0	
Individual food insecurity	22	13.8	
Child hunger	24	15.0	
Body Mass Index (BMI)			20.01±3.14
Severely thin	1	0.6	
Thin	6	3.8	
Normal	126	78.8	
Overweight	22	13.8	
Obese	5	3.1	
HRQOL			61.29±18.08
Physical functioning			58.81±21.13
Emotional functioning			64.03±22.55
Social functioning			61.69±21.50
School functioning			62.13±22.36

† Per capita income poverty line in Malaysia is RM210 and below

mean score in physical functioning (58.81±21.13). The total mean BMI was 20.01±3.14 kg/m^2 and 4.4% of them were categorised as thin and severely thin. The prevalence of the overweight and obesity among the adolescents was 16.9%.

Predictors of food security status

In the adjusted analysis, the factors that remained significantly associated with food security status were household income, number of school going children, occupation status of the parents and house ownership status (p<0.05)(Table 2). Adolescents from households with an income of below RM1500 were 12.6 times more likely to fall into the food insecure group than those who came from where the household income was RM1500 and above (*AOR*=12.626, 95% CI: 2.681, 59.458). Adolescents who had ≥4 school going siblings increased the odds of being food insecure by tenfold than adolescents who had <4 school going siblings (*AOR*=10.726, 95% CI: 1.241, 92.714). Furthermore, unemployed parents were 22 times more likely to experience food insecurity than parents who were in employment (p<0.05). Living in rented property was 18 times more likely to result in food insecurity compared to those living in their own (*AOR*=18.093, 95% CI: 3.770, 86.848). A household income of below than RM1500, with >3 school-goers in the household, unemployed parents and living in a rent house were the predictors of food insecurity and contributed 63.5% to 84.7% of the variance in food insecurity.

Table 2. Demographic factors and food security status (n=160)

Variables	Adjusted OR (95% CI)	p
Household income (RM)		0.001**
<RM1500	12.626 (2.681, 59.458)	
≥RM1500	Ref (1.00)	
Number of school-going children		0.031*
≤3	(1.00)	
≥4	10.726 (1.241, 92.714)	
Occupational status of mother		0.001**
Employed	Ref (1.00)	
Unemployed	22.221 (3.540, 139.473)	
Occupational status of father		0.012*
Employed	Ref (1.00)	
Unemployed	22.354 (1.969, 253.479)	
Household size		0.051
1-5	Ref (1.00)	
≥6	8.391 (0.995, 70.789)	
House ownership status		0.000**
Own	Ref (1.00)	
Rented	18.093 (3.770, 86.848)	

*Significant at p<0.05
**Significant at p<0.01
Cox & Snell R^2=0.635, Nagelkerke R^2=0.847

HRQOL scores based on food security status

The food-insecure group had significantly poorer HRQOL (42.13±10.51) than the food-secure group (69.95±7.50) after controlling for the covariates of maternal age, maternal and paternal years of schooling, household income, number of siblings, as well as number of school-going siblings (F=59.842, p<0.001) (Table 3). Similarly, the physical and psychosocial functioning scores were higher in the food-secure group than those in the food insecure group after controlling for the same covariates (p<0.001).

BMI based on food security status

Adolescents from food-insecure households had significant lower BMI (18.68±2.01 kg/m^2) than adolescents from food-secure households (21.28±3.50 kg/m^2), after controlling for demographic background factors, namely, monthly income, income per capita, number of children, years of schooling of mothers and fathers (F=18.141, p<0.001) (Table 3).

DISCUSSION

The prevalence of household food insecurity in this study was lower at 48.8% compared to the 77.0% shown in the earlier local study on children aged 7-13 years (Roselawati *et al.*, 2017). The study settings might explain the differences in the prevalence of food insecurity. The study by Roselawati *et al.* (2017) was conducted in Kuantan, Pahang which is categorised as urban area, while the current study took place in Mentakab, Pahang which is a rural area. Living in a rural area is associated with the low expenditure as the villagers cultivated home-grown vegetables and home-reared animals for food instead of buying from the markets (Roselawati

Table 3. HRQOL and BMI based on food security status after controlling the demographic factors

	Mean±SD		*F-value*	*p-value*
	Food security	*Food insecurity*		
HRQOL†	69.95±7.50	42.13±10.51	59.842	0.000*
Physical functioning	23.84±3.42	13.54±5.20	29.597	0.000*
Emotional functioning	15.32±3.30	10.17±4.10	12.543	0.001*
Social functioning	15.10±2.56	9.44±3.83	29.153	0.000*
School functioning	15.70±2.79	8.99±3.12	26.578	0.000*
BMI (kg/m^2)‡	21.28±3.50	18.68±2.01	18.141	0.000*

†GLM - adjusted for covariates - mother's age, years of schooling for mother, years of schooling for father, household income, number of sibling, and number of sibling going to school

‡GLM - adjusted for covariates - monthly income, income per capita, number of children, years of schooling of mother and father

* Significant at $p<0.001$

et al., 2017). Furthermore, the costs for items such as house rental in rural area were lower than in an urban area leaving less money for foods (Sriram & Tarasuk, 2016). This may explain the lower prevalence of food insecurity that we found in our study.

This study has revealed that unemployment among the parents of adolescents was associated with food insecurity (Etana & Tolossa, 2017). Furthermore, this study has confirmed the outcomes of previous studies where low monthly incomes were associated with food insecurity (Norhasmah *et al.*, 2011; Mohamadpour *et al.*, 2012; Wang *et al.*, 2015). Unemployment and low incomes result in poverty and thus food insecurity (Etana & Tolosa, 2017). This situation was exacerbated by the large household size, the large number of siblings and school-going children, and occupancy of rented units which increase household expenditures and cause problems in the fulfillment of the food and non-food needs (Norhasmah *et al.*, 2011).

The total mean HRQOL score in this study (61.29±18.08) was lower than the study by Husna *et al.* (2013) (78.50±13.48) that was done on secondary school adolescents in Kuala Lumpur. However, the setting of Husna *et al.* (2013) study was different from that of ours as it was conducted in the urban area of Kuala Lumpur. Living in rural settings negatively affects the physical HRQOL (Kurpas, Mroczek & Bielska, 2014). It might be due to the inadequate physical facilities in the rural areas and the consequent negative perception of their environment by the adolescents. Food insecurity was associated with physical functioning, as individuals in food-insecure households were more likely to report fair, poor, or very poor health statuses with activity limitations as compared to individuals in food-secure households (Ihab *et al.*, 2012; Chung *et al.*, 2016). It was previously shown that food insecure students were less likely to participate in strenuous physical activity and sport team (Shanafelt *et al.*, 2016). This may have been because food insecurity was associated with poor physical ability owing to poor nutritional status (i.e. deficiencies of essential nutrient and low-quality food intake) (Chung *et al.*, 2016). Therefore, physical functioning

among the adolescents in this study was lower among food-insecure group when compared with the food-secure group.

Food insecurity was associated with poor social and psychology functioning because the individuals from food insecure households were vulnerable to feelings of anxiety, helplessness, and loss of control besides having psychological impairment (Ihab *et al.*, 2012; Shanafelt *et al.*, 2016; Utter *et al.*, 2018). Furthermore, economic circumstances such as financial hardship could disrupt the emotional state of the mothers, which brought about the poor social and psychology functioning. In addition, food insecurity was found to be associated with poor functioning in school. This finding was consistent with that of a previous study (Shanafelt *et al.*, 2016) which documented that food-insecure students had lower grades or poor academic performance than food-secure students. Among the schooling children and adolescents, food insecurity was related with fewer family meals and skipping of breakfast. Breakfast has been reported to provide positive academic performance particularly in the memory and attention domains (Adolphus, Lawton & Dye, 2013). Inadequate nutrient intake among the food-insecure individuals could reduce normal brain function (Palar *et al.*, 2015).

In the context of the body weight status, the findings of this study contradicted that of the previous study of Roselawati *et al.* (2017), which found that there was no significant association between childhood obesity and food insecurity. The current study found that BMI was significantly lower in the food insecure group. Food insecurity was associated with inadequate food intake and low consumption of nutritious food (low intake of fruits and vegetables). Food insecure households might purchase low quality and quantity of food due to the limited income (Mohamadpour *et al.*, 2012). Food-insecure individuals usually experienced skipping meals and consumed smaller portion sizes of meals which contributed to their low overall energy intake.

There were several limitations in this study. This was a cross-sectional study, so the cause-and-effect relationships could not be determined. Only an association could be established between the independent variables and dependent variables. Furthermore, this study did not explore other factors that were related to food security status. Instead, only demographic factors such as sex, ethnicity, parental education levels, parental occupation status, house ownership status, household size, number of school-going siblings, number of siblings, parental marital status and household income were included in this study. Besides, as this study merely focused on the adolescent population, the HRQOL findings could not be extrapolated to other populations such as the elderly.

CONCLUSION

In conclusion, nearly half of the households (48.8%) suffered from some degree of food insecurity. Having ≥4 school going children, unemployed parents and a household income of <RM1500 and living in a rented property were the main contributors of food insecurity. Furthermore, food insecurity was associated with poor HRQOL and low BMI. Food assistance programmes are recommended to alleviate the high prevalence of food insecurity. Monetary and school aids are also advocated to reduce the economic burden in households with low monthly incomes. Future local studies pertaining to the factors and consequences of food insecurity in adolescents are recommended.

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Authors' contributions

SA, conceptualised and designed the study, carried out the data collection and prepared the draft of the manuscript; NS, supervised the flow of the overall research and reviewed the manuscript; FMN and SFM assisted in the drafting and review of the manuscript.

Conflict of interest

The authors have no conflict of interest.

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Maternal diet and its association with human milk energy and macronutrient composition among exclusively breastfeeding Malaysian Malay mothers

Siti Munirah Abdul Basir[1], Radiah Abdul Ghani[2], Muhammad Ibrahim[1], Muhammad Muzaffar Ali Khan Khattak[1], Muhammad Nor Omar[3] & Nor Azwani Mohd Shukri[1]

[1]*Department of Nutrition Sciences, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia (IIUM), Pahang, Malaysia*; [2]*Department of Biomedical Sciences, Kulliyyah of Allied Health Sciences, IIUM, Pahang, Malaysia*; [3] *Department of Biotechnology, Kulliyyah of Science, IIUM, Pahang, Malaysia*

ABSTRACT

Introduction: This study aimed to determine the relationship of maternal dietary intake with human milk nutritional composition, among Malay mothers during the postpartum period of exclusive breastfeeding. **Methods**: Human milk samples (20-30ml) were collected from mothers (n=32) at least once monthly for six months postpartum. Macronutrients and fatty acids contents were determined using proximate analysis and gas chromatography methods, respectively. Maternal dietary intakes were recorded using the multiple-pass diet recall method prior to each milk sampling and were analysed using the Nutritionist Pro™ software. Associations between the milk composition and maternal diet were tested using Spearman correlation. **Results:** The energy content ranged between 49.6-59.2 kcal/100ml, protein 1.3-1.4 g/100ml, carbohydrate 6.5-9.7 g/100ml and total fat 6.5-9.7 g/100ml. The polyunsaturated, monounsaturated, and saturated fatty acids concentrations were 10.5-19.1 %, 40.6-43.5 %, and 38.0-49.7 %, respectively. During confinement (first month postpartum), total energy and total fat content of human milk were the highest whereas total carbohydrate was the lowest, compared to the rest of the exclusive breastfeeding period. In contrast, intakes of total calorie and total fat were the lowest, whereas protein was the highest during this period. However, no associations were detected between human milk nutritional contents and maternal dietary intake. **Conclusion:** In our study population, the composition of maternal diet and nutritional content of human milk differed between confinement and post-confinement periods. However, the association between maternal diet and human milk composition itself warrants further investigation.

Keywords: Breastfeeding, human milk, nutritional composition, maternal diet

INTRODUCTION

Breastfeeding has been shown to help in promoting survival and optimal development of infants by reducing the incidence of infection, sudden infant death syndrome, obesity, diabetes,

*Corresponding author: Dr. Nor Azwani Mohd Shukri
Department of Nutrition Sciences, Kulliyyah of Allied Health Sciences,
International Islamic University Malaysia, Jalan Sultan Ahmad Shah, 25200 Kuantan, Pahang, Malaysia
Tel: (6)09-5715307; Fax: (6)03-5716776; E-mail: norazwani@iium.edu.my
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childhood cancer and asthma (Mamun *et al.*, 2015; Horta, Loret De Mola & Victora, 2015). According to the World Health Organization (WHO), exclusive breastfeeding is defined as the consumption of human milk without supplementation, except for vitamins, minerals, medicines, water and the drops of syrup prescribed by health professionals or clinicians (WHO, 2011). Both the WHO and United Nations Children's Fund (UNICEF) strongly advocate exclusive breastfeeding for the first six months after birth as the optimal way of feeding infants. This is due to the beneficial effects on child health, growth and development as well as its positive implications on maternal wellbeing (WHO, 2009).

Human milk is categorised according to its production stages. Colostrum is a thick yellowish liquid secreted a few days before delivery up to approximately four days postpartum (Ballard & Morrow, 2013). The milk secreted between the 5^{th}-14^{th} days postpartum is known as transitional milk whereas mature milk is produced from the 15^{th} day onwards. The content of macronutrients in human milk differs within mothers and across lactation. As human milk changes from colostrum to mature milk, the nutrient composition shifts from low fat and high protein, to a concentration which is high in fat and low in protein (Ballard & Morrow, 2013).

Milk composition has been reported to be influenced by maternal diets (Maru, Birhanu & Tessema, 2013). This cross-sectional study investigated the association between cereal- and 'enset'-based dietary habits and the level of micronutrients (calcium, magnesium, iron, zinc, and copper) in the human milk samples. It was concluded that human milk calcium and copper content were influenced by dietary intake. Total protein, total fat, saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA), are among the nutrients in human milk which have been positively associated with maternal dietary intake of these nutrients (Bravi *et al.*, 2016). In this systematic review, it was reported that diet that was high in protein resulted in higher total protein content in the human milk. Higher fat and dairy food intakes were also found to increase the total fat content in the milk. However, these associations were considered weak. As for fatty acids, there were positive correlations of total SFA, MUFA, and PUFA intakes during breastfeeding with their content in human milk.

Maternal dietary intake may be affected by postpartum traditional dietary practices during confinement which are the norm among the Asian communities (Haron & Hamiz, 2014). Indeed, the majority of Malaysian mothers adhere to certain traditional postpartum regimen and practices which are believed to be helpful in restoring maternal health and well-being after delivery (Fok & Manager, 2016; Suraya & Jamaludin, 2014). Commonly, Malaysian women practise confinement for 30-44 days after childbirth, during which time there is an adherence to traditional food taboos is common. It is typical, for example, for some mothers to avoid the intake of some foods such as red meat, seafood, as well as certain fish, fruits and vegetables. These foods are perceived to have properties such as, "cold" (i.e. pumpkin, cucumber and watermelon), "windy" (i.e. cold rice, tapioca, and sweet potato), and "itchy" (i.e. seafood, chicken, and egg) that may hamper health recovery (Nor Azwani *et al.*, 2018). However, these traditional dietary restrictions may affect the quality of maternal diets. A Malaysian study demonstrated that the mean total energy consumed by ethnic Chinese women during confinement is 19% lower than the Malaysian Recommended

Nutrients Intake (Poh, Wong & Karim, 2005). In addition, >90% of the mothers surveyed did not meet the nutritional requirements for some micronutrients and minerals such as vitamins A and C, as well as calcium. There was also a reduced intake of fruits and vegetables which are sources of fibre, vitamins and minerals. This may not only affect the women's health and recovery, but also the production and nutritional quality of human milk.

Studies of the nutritional composition human milk of Malaysian mothers who were on traditional diets and who exclusively breastfed during the postpartum period, are few. This investigation of Malaysian Malay mothers aimed to address this gap in our knowledge.

MATERIALS AND METHODS

Design

This was a longitudinal study that assessed the nutritional composition of human milk during six months of exclusive breastfeeding period. It allowed the observation of the nutritional content of human milk at different stages of its production.

Setting

The study was conducted in the town of Kuantan, which is located on the east coast of Peninsular Malaysia. Data collection and follow-up sessions were carried out from May 2016 to July 2017.

Study population

The sample size required for this study as calculated using the single mean formula was 152, based on a previous study conducted by Chang *et al.* (2015). However, the low response rate from eligible study participants rendered random sampling nearly impossible to achieve within the designated study period. A total of 32 Malay mothers were conveniently recruited at several health facilities such as university, government, and private health clinics that were attended by pregnant women for antenatal check-ups in Kuantan area. The women normally lived or worked nearby (within 25 km radius) and this allowed for the regular data collection and follow up visits, either at the home or workplace of the participants. In addition, online advertisements were sent by email and placed on social media groups in order to increase the participation rate. The inclusion criteria consisted of Malay mothers aged 18-39 years who had delivered singleton infants at full-term (≥37 weeks of gestation) and had exclusively breastfed their infants for six months. The exclusion criteria were: diagnosis of pre-existing chronic diseases (such as diabetes mellitus and hypertension) and other complications during pregnancy, tandem nursing, and pre-term delivery. The International Islamic University Malaysia Research Ethics Committee approved the study (IREC 585). Each participant provided a written informed consent before the study commenced.

Data collection

The sociodemographic information of the participants including age, education level, occupation, number of pregnancies, method of delivery and smoking status was self-reported via face-to-face interview at the time of enrolment. Anthropometry measurements, namely height and weight of the mother, total gestational weight gain and baby birth weight, were also recorded. Maternal weight was measured to the nearest 0.1 kg using a portable weighing scale (Tanita, Japan), and height to the nearest 0.1 cm using a portable body meter (SECA, Germany). The total gestational weight gained was then calculated by subtracting the pre-pregnancy weight

from the current weight measured. The maternal body mass index (BMI) was computed as weight in kilograms divided by height in metres squared. For the infants, the weight was taken by using a portable infant weight scale (Tanita, Japan) and recorded to the nearest 0.1 kg. These measurements were done by a trained research assistant.

Human milk samples were collected on Day 3, 10, 30, 60, 90, 120, 150 and 180, postpartum. Participants were provided with capped tubes to store their human milk in their own refrigerator or freezer until the day of collection. The human milk was expressed either with an electric or manual breast pumps. The participants were asked to empty one breast (at any time of the day) and followed by collection of 20-30ml of human milk. This was to avoid the collection of only foremilk or hind milk. The frozen or refrigerated human milk was then transferred in a cool bag to the laboratory where it was immediately homogenized and stored at −40°C until analysis.

Maternal dietary intakes were recorded using the technique of multiple-pass diet recall for three consecutive days (two weekdays and one weekend day) prior to each milk sampling day and averaged for each time point (Day 3, 10, 30, 60, 90, 120, 150 and 180, postpartum). Participants were asked to recall all foods and beverages they had consumed during the said period. Common household utensils were used to aid the participants in estimating the amount of food they had consumed. For commercial or packed foods, information was obtained from the nutritional information on the package. Any intake of supplements was also recorded and analysed for the maternal dietary intake analysis. These included vitamins and minerals in the form of tablets, capsules, powder, or liquids, which may have contributed to the total calorie and other nutrients intake. The study participants were also asked if they were adhering to traditional confinement dietary practices during the postpartum period.

Data analyses

Human milk macronutrient analyses

The total protein of human milk was determined by the bicinchoninic acid (BCA) colorimetric assay (Pierce™ BCA Protein Assay, Thermo Scientific™, Illinois, USA), which is considered the most appropriate method for human milk-protein assay (Keller & Neville, 1986). The total carbohydrate content was determined by using the phenol-sulfuric acid method of DuBois *et al.* (1956). The creamatocrit method was used to determine the concentration of total fats in the human milk (Lucas *et al.*, 1978). This method involves separating the human milk samples into cream and aqueous layers. The total energy value of the human milk samples was computed as the sum of energy contributed by total protein, carbohydrate and fat.

There were two primary steps of fatty acids determination. These were lipid extraction by the Blight and Dyer method (with modifications) and transesterification of lipids. The composition of fatty acids methyl esters was analysed by a gas chromatograph (Agilent 7890A) that was equipped with a flame ionization detector (FID) and Agilent Chromatography Workstation software. The fatty acids methyl ester (FAME) was separated in a capillary column HP-5 (30m × 320µm and a film diameter of 0.25µm). The carrier gas used was helium which flowed at the rate of 1ml/min. The injector and detector's temperatures were set at 250°C and 270°C, respectively. The initial oven temperature was from 50-70°C at 10°C/min rate, then increased to 170-220°C at 2°C/min rate. The final

temperature of 220°C was maintained for 20 minutes. The total run time for the analysis was 57 minutes (Silva *et al.*, 2005). Samples were analysed within 12 hours of transesterification.

Dietary intake

The average intake of nutrients was calculated using Nutritionist Pro™ diet analysis software which is based on the Nutrient Composition of Malaysian Foods (Tee *et al.*, 1997) and the United States Department of Agriculture (USDA) Nutrient Database for Standard Reference (USDA Nutrient Data Laboratory, 2006).

Statistical analyses

The statistical analyses were performed using SPSS Statistics (Version 20). The confidence interval was set at 95%. The normal distribution of data was assessed visually using histogram and Q-Q plots. Descriptive analyses were used to describe the participant characteristics and human milk nutritional composition. The Spearman correlation was used to assess the relationship between the maternal intake and nutrient composition of human milk of each specific time point due to abnormally distributed data. The data were presented as mean± standard deviation (SD) or as otherwise stated.

To minimise the effect of measurement errors such as misreporting of energy intake (which could affect the absolute intakes of nutrients), nutrient intakes were statistically adjusted for total energy intake using the residual method. This was done by adding the residue of the difference between the observed nutrient values for each subject and the values predicted by regression equation to the nutrient intake that corresponded with the mean total energy intake of the study population (Willet & Stampfer, 1986).

RESULTS

General characteristics

The demographic data of the study participants are presented in Table 1. The mean age of the respondents was 31.1±4.7 years. About half (53%) of the study participants were government employees and a majority (88%) had tertiary education. About 84% had their infants via normal delivery and 72% had more than one child. Just over half (53%) of the mothers had normal BMI with a mean value of 23.5 kg/m^2. In addition, about two thirds of the participants (69%) had gestational weight gain within the values as recommended by Institute of Medicine (IOM) (2009). The mean birth weight of the infants was 3.1±0.3 kg which was also within the normal weight range. All the study participants (N=32) reported they had adhered to the traditional confinement dietary practices for the first 40-44 days of postpartum period.

Human milk energy and nutritional composition

Human milk samples collected in the study were analysed for macronutrients, as summarised in Table 2. The mean energy value of the human milk samples ranged from 49.6±4.4 kcal/100ml to 59.2±8.9 kcal/100ml across the study period. It was also shown that the energy content was slightly higher during Day 10 and Day 30. The human milk mean protein composition in this study remained relatively consistent (1.3-1.4 g/100ml) throughout the study period, except for Day 90 where the concentration was slightly higher at 1.6 g/100ml). In addition, the concentration of total fat was found to be higher during Day 10 and Day 30 (7.6 g/100ml and 7.5 g/100ml, respectively) compared to other study periods (3.5-6.4 g/100ml). The mean total carbohydrate concentration was 6.8±4.1 g/100ml on

Table 1. Characteristics of the study participants (N=32)

Characteristics	*n (%)*	*Mean±SD*	*Range*
Age (years)		30.6±4.4	22-39
Employment			
Government	17 (53)		
Private	6 (19)		
Unemployed (housewife)	6 (19)		
Studying	3 (9)		
Highest education level			
Secondary	4 (12)		
Tertiary	28 (88)		
Mode of infant delivery			
Normal vaginal delivery	27 (84)		
Caesarean section	5 (16)		
Parity			
1	9 (28)		
2 and more	23 (72)		
Pre-pregnancy BMI (kg/m^2)			
Underweight	8 (25)	23.5±4.8	16.4-36.4
Normal	17 (53)		
Overweight/obese	7 (22)		
Total gestational weight gain (GWG) (kg)*			
Less than recommended	3 (9)	11.9±4.4	5.0–24.0
Within recommendation	22 (69)		
More than recommended	7 (22)		
Infant's birth weight (kg)	32 (100)	3.1±0.3	2.6–3.8

*Determined based on IOM Recommended GWG (IOM, 2009)

Day 10, and 6.5±3.1 g/100ml on Day 30. The concentration increased after the first 30 days of breastfeeding period. As for fatty acids, it was found that the mean concentrations of PUFA, MUFA, and SFA varied from 10.5% to 19.1%, 40.6% to 43.5%, and 38.0% to 49.7% of total fatty acids, respectively.

The association between maternal dietary intake and human milk nutritional composition

Table 3 demonstrates the total energy and nutrient intakes of the participants in the current study. The total energy intakes during confinement period on Day 10 and Day 30 were found to be the lowest throughout the exclusive breastfeeding period (1479±441 kcal/day and 1532±418 kcal/day, respectively). In contrast to the human milk nutritional composition, the total fat intakes were lower on Day 10 (33.4±16.4 g/day) and Day 30 (32.7±15.7 g/day), whereas protein intakes on those days were higher (Day 10: 70.6±9.7 g/day and Day 30: 72.0±17.5 g/day), than at other time points of the exclusive breastfeeding period. In addition, fatty acids intake (SFA, MUFA, and PUFA) were also the lowest during the confinement period.

Tables 4 and 5 show the associations between mean total energy and macronutrient composition in the maternal diet and in their human milk at each time point. In the current study, there was no correlation between maternal total calorie intake and the energy value of human milk in any of the time-points. The same could be observed with carbohydrate composition in the diet and in human milk. For

Table 2. Nutritional composition of human milk (mean±SD) during exclusive breastfeeding period (N=32)

Nutrients	*Day 10*	*Day 30*	*Day 60*	*Day 90*	*Day 120*	*Day 150*	*Day 180*
Total energy (kcal/100ml)	59.2±8.9	59.1±20.1	49.6±4.4	50.4±7.1	52.1±14.8	56.2±13.7	50.4±8.3
Protein (g/100ml)	1.4±0.3	1.4±0.4	1.3±0.3	1.6±0.7	1.4±0.4	1.4±0.3	1.4±0.2
Total carbohydrates (g/100ml)	6.8±4.1	6.5±3.1	9.8±1.7	9.7±2.3	8.8±2.5	8.4±2.6	9.4±3.3
Total fat (g/100ml)	7.6±3.4	7.5±7.8	3.7±1.7	3.5±2.1	5.9±5.4	6.4±5.3	4.1±3.2
Fatty acids (FA)							
Saturated fatty acids (% of total FA)	47.1±8.6	49.7±7.0	46.1±3.4	41.9±5.2	38.0±4.8	44.0±3.1	38.8±3.8
Polyunsaturated fatty acids (% of total FA)	10.7±0.4	10.5±0.5	15.6±3.6	16.1±3.2	19.1±3.1	14.5±2.9	18.2±2.8
Monounsaturated fatty acids (% of total FA)	43.5±5.6	40.6±5.5	40.6±0.5	42.1±3.3	42.9±2.9	41.5±2.6	43.0±3.1

Table 3. Maternal total energy and nutrient intakes (mean±SD) during exclusive breastfeeding period (N=32)

Time points	*Total energy (kcal/day)*	*Protein (g/day)*	*Carbohydrate (g/day)*	*Total fat (g/day)*	*Saturated fat (% of total energy)*	*Polyunsaturated fat (% of total energy)*	*Monounsaturated fat (% of total energy)*
Day 10	1479±441	70.6±9.7	217.0±81.7	33.4±16.4	2.0±0.9	1.1±0.8	1.3±0.9
Day 30	1532±418	72.0±17.5	229.5±51.2	32.7±15.7	1.7±0.7	1.4±1.5	1.8±1.8
Day 60	1845±338	62.2±19.3	231.5±64.9	59.5±27.3	3.9±1.1	4.2±2.6	8.9±4.6
Day 90	1742±474	64.8±23.0	272.0±76.9	61.1±35.2	4.7±1.9	4.1±2.3	8.6±2.4
Day 120	2022±498	66.9±19.1	243.1±67.6	70.2±20.6	5.5±1.5	3.7±1.8	7.9±1.7
Day 150	1701±126	58.3±21.9	197.1±83.1	51.3±23.5	3.6±1.0	3.3±2.7	7.9±2.5
Day 180	1878±398	61.5±25.1	188.8±59.2	56.9±39.4	4.6±1.1	3.8±1.8	8.5±2.1

Table 4. Relationship between maternal means of calorie and macronutrients intake and human milk energy and macronutrients composition of each time point (N=32)

Time points	*Energy*		*Protein*		*Carbohydrate*		*Fat*	
	r-coefficient	*p-value*†	*r-coefficient*	*p-value*†	*r-coefficient*	*p-value*†	*r-coefficient*	*p-value*†
Day 10	-0.305	0.493	-0.400	0.505	-0.564	0.322	0.400	0.505
Day 30	-0.063	0.845	-0.074	0.820	0. 413	0.183	0.119	0.712
Day 60	-0.217	0.498	-0.075	0.791	-0.049	0.880	-0.393	0.206
Day 90	-0.034	0.904	-0.075	0.791	-0.421	0.118	-0.136	0.629
Day 120	0.018	0.957	-0.687	0.014*	-0.119	0.713	0.536	0.072
Day 150	-0.557	0.060	0.116	0.721	-0.336	0.286	-0.175	0.586
Day 180	-0.033	0.932	0.293	0.444	-0.250	0.516	0.083	0.831

† Spearman correlation after adjustment of nutrient intakes
*p<0.05

Table 5. Relationship between maternal fatty acids intake and human milk fatty acids composition of each time point (N=32)

Time points	*Saturated fat*		*Polyunsaturated fat*		*Monounsaturated fat*	
	r-coefficient	*p-value*†	*r-coefficient*	*p-value*†	*r-coefficient*	*p-value*†
Day 10	-0.050	0.391	-0.200	0.747	-0.205	0.741
Day 30	0.000	1.000	0.678	0.015*	0.352	0.261
Day 60	-0.049	0.880	0.172	0.594	-0.194	0.546
Day 90	-0.186	0.508	-0.143	0.612	0.085	0.763
Day 120	0.527	0.117	-0.182	0.593	0.243	0.472
Day 150	0.336	0.312	0.082	0.811	0.102	0.766
Day 180	-0.050	0.898	-0.075	0.847	-0.293	0.444

†Spearman correlation after adjustment of nutrient intakes
*p<0.05

the protein, there was a significant negative correlation of maternal protein intake and its concentration in the human milk only at Day 120 (r=-0.687, p-value=0.014). In addition, there were no significant correlations between maternal dietary intake of total fat or fatty acids (SFA, PUFA, and MUFA) and their concentrations in human milk samples, except for PUFA at Day 30 (r =0.678, p-value=0.015).

DISCUSSION

The energy and nutrient composition of breastmilk showed some variation throughout the exclusive breastfeeding period. The mean energy content of their milk samples in the current study was 49.6±4.4 to 59.2±8.9 kcal/100ml. On the average, the energy content of mature milk (from Day 15 postpartum onwards) was 11.1-16.1 kcal/100ml lower compared to what was found by Jenness (1979) in a Western study (65-70 kcal/100 ml). Similarly, this value is also 11.1 kcal/100ml lower than the data from a longitudinal study of Egyptian women in which the mean energy concentration of their milk samples from one to four months of

breastfeeding were 66.0, 65.4, 64.7, and 63.9 kcal/100ml (Soliman, Soliman & Bakr, 2014). This may be generally due to the different diets among the Western, Middle Eastern, and Asian populations. However, a study of breastfeeding women from four different regions of Shanghai (Qian *et al.*, 2010), found that the mean energy content of their milk samples to be between 57.0 – 63.8 kcal/100ml, which was slightly higher than the value found in the current study.

Over the first four to six months, the protein level of human milk was found to be similarly decreasing as lactation progressed in mothers who had delivered their babies prematurely (at <28 weeks of gestation) as well as in those who delivered at term (Bauer & Gerss, 2011). It decreased rapidly during the first month of lactation and continued to decline but much more slowly after that (Lonnerdal, 2003). We found that the protein concentration in our samples were relatively consistent throughout the exclusive breastfeeding period, except for Day 90. Our finding of 1.4 g/100ml was similar to that of a South Korean study where protein concentration was also 1.4 g/100ml (Chang *et al.*, 2015) but higher than that of a study in China by Yang *et al.* (2014) which was 0.9 g/100ml.

The total carbohydrate concentration during the first 30 days was lower whereas the concentrations after 30 days were higher compared to the reference range of 7.2–7.7 g/100ml (Nommsen *et al.*, 1991). Human milk macronutrient studies specifically on carbohydrates, generally assessed lactose concentration instead of total carbohydrates contents. Lactose concentrations may vary from 6.3-8.1 g/100ml (Yang *et al.*, 2014). This may explain the results found in the present study. However, carbohydrate concentrations in breastfeeding women in Shanghai were between 7.2-8.1 g/100ml (Qian *et al.*, 2010). The difference in carbohydrates concentration from the present study may be because of the different method of analysis as Qian and colleagues calculated carbohydrates concentrations using a formula (carbohydrate content = Total solids - (ash + protein + fat), as opposed to using total carbohydrates proximate analysis.

The fat concentration was also higher than what some others have found (e.g. Nommsen, 1991 reported 2.2-5.0 g/100ml). There was a pattern of reducing of fat concentration in the milk samples as the lactation period progressed. A study by Soliman and colleagues (2014) also found that the fat concentrations reduced as the age of infants increased. On the contrary, a systematic review of 35 breastmilk studies in China revealed that human milk fat concentration was higher in mature milk (3.4±1.2 g/100ml) compared to colostrum (2.4±1.2 g/100ml) and transitional milk (3.1±1.2 g/100ml) (Yang *et al.*, 2018). Human milk fat concentration is the most variable constituent (Picciano, 2001). It may be affected by several factors such as breastmilk volume, parity and maternal diet.

The SFA level in the present study is comparatively lower than that reported in an older study by Kneebone, Kneebone and Gibson (1985) among breastfeeding women in Penang (38.0-49.7% vs. 52.7±5.4%). In addition, our PUFA levels were much lower (10.5-19.1 % vs. 47.7±5.4 %) but our MUFA concentrations were slightly higher (40.6-43.5 vs. 36.3±4.8 %) compared to the older study. Other studies have reported varied SFA concentrations between 26-51 % (Kresic *et al.*, 2013). This might have been because of the different diets among these populations. However, this could not be confirmed as none of the authors measured the dietary intakes of their subjects.

In the present study, maternal energy intake was not associated with the energy value of the human milk. This is in agreement with the findings of a systematic review study which concluded that maternal total calorie intake did not have any influence on the calorie concentration of the human milk (Bravi *et al.*, 2016). The authors, however, found that high fat and low carbohydrate diets resulted in higher caloric content of the milk. Their findings suggest that the energy content of the human milk may not be contributed by the total calorie intake *per se*, but more by the proportion of maternal carbohydrate and total fat intakes (Bravi *et al.*, 2016). At the fourth month of breastfeeding, protein intake showed a significant negative strong correlation with its concentration in the human milk. Even though the dietary protein intake at that time was lower than the first 30 days of postpartum, the level of protein in the milk remains consistent throughout the breastfeeding period, as discussed earlier. This shows that the protein content of the human milk may not be affected by dietary intake, as was also indicated by other studies (Ogechi & Irene, 2013; Nommsen *et al.*, 1991). This may be influenced by the different techniques used to analyse the macronutrients content of human milk. In addition, the human milk protein composition may also be influenced by other factors such as the endogenous production by mammary glands as well as maternal body stores (Emmet & Rogers, 1997).

This is a longitudinal study of the changes of the human milk nutritional composition throughout the period of exclusive breastfeeding. However, this study had only a small number of participants due to response rate from the target population within the stipulated time frame.

CONCLUSION

Based on the observations of the current study, the energy and nutrient composition of human milk within the study population showed some variation throughout the period of exclusive breastfeeding. Compared to the rest of the exclusive breastfeeding period, total fat content of human milk was the highest whereas total carbohydrate was the lowest during confinement (first month postpartum). The human milk nutritional content in the current study was also found to vary from the findings of other populations. This study has reported the concentrations of the nutritional constituents of human milk during exclusive breastfeeding period among the study population but it did not find any association of these with maternal diet. Future research should be conducted with a larger sample size to substantiate these findings.

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Authors' contributions

NAMS, principal investigator, conceptualized and designed the study, prepared the draft of the manuscript and reviewed the manuscript. SMAB, conducted the study, data analysis and interpretation, and assisted in drafting of the manuscript; MI, assisted in conceptualizing and designing the study, provided advice on data analysis and interpretation and reviewed the manuscript; MMAKK, MNO and RAG, provided advice on data analysis and interpretation and reviewed the manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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Validation of a semi-quantitative food frequency questionnaire for estimating dietary omega-3 fatty acids intake among urban Indonesian pregnant women

Dudung Angkasa[1,2], Rina Agustina[1,3*], Helda Khusun[4] & Erfi Prafiantini[1,3]

[1]*Department of Nutrition, Faculty of Medicine, Universitas Indonesia - Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia;* [2]*Department of Nutritional Science, Faculty of Health Sciences, Universitas Esa Unggul, Jakarta, Indonesia;* [3]*Human Nutrition Research Center, Indonesian Medical Education and Research Institute, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia;* [4]*Southeast Asian Ministers of Education Organization Regional Center for Food and Nutrition (SEAMEO RECFON), Jakarta, Indonesia*

ABSTRACT

Introduction: Studies on the development and validation of semi-quantitative food frequency questionnaires (SQ-FFQ) for assessing omega-3 (Ω-3) long-chain polyunsaturated fatty acids (LC-PUFAs) intake by pregnant women are few. This study aimed to determine the validity of a newly developed SQ-FFQ for assessing the LC-PUFA intake among Indonesian urban pregnant women. **Methods:** A cross-sectional study was carried out in 2015 on 100 Indonesian pregnant women who were in their late 3rd trimester, living in the urban setting of Jakarta. As a test tool, the SQ-FFQ was administered before the trained nutritionists executed the reference tool of non-consecutive two-day 24-hour dietary recalls (2DRs). The nutrients of interest were a total of Ω-3, eicosapentanoic acid (EPA), docosahexaenoic acid (DHA), alpha-linolenic acid (ALA), total Ω-6, linoleic acid (LA), arachidonic acid (AA), and LC-PUFAs. Statistical correlation, cross-classification and the Bland-Altman plot analysis were done to determine the agreement between tools. **Results:** Energy-adjusted correlation coefficients between SQ-FFQ and 2DRs were 0.385, 0.349, 0.352, 0.380, 0.338, 0.408, 0.409, 0.331, 0.341 and 0.341 for fat, total Ω-3, ALA, EPA, DHA, total Ω-6, LA, AA and LC-PUFAs, respectively and were statistically significant ($p<0.05$). Misclassification of these nutrients from SQ-FFQ and 2DRs was <6%. The Bland-Altman plots showed most of the points fell within the 95% limits of acceptable agreement for DHA, EPA, and LA. **Conclusion:** The newly developed SQ-FFQ of this study is a valid instrument for assessing of Ω-3 LC-PUFAs intake among Indonesian pregnant women living in urban area. Its further validation with relevant biomarkers is recommended.

Keywords: Semi-quantitative food frequency questionnaire, validation, fatty acids, pregnancy, omega-3, omega-6

*Corresponding author: Rina Agustina, MD, Ph.D
Department of Nutrition, Faculty of Medicine, Universitas Indonesia
Dr.Cipto Mangunkusumo General Hospital, Jakarta, Indonesia
Email: dr.rinaagustina@gmail.com; r.agustina@ui.ac.id
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INTRODUCTION

Omega-3 (Ω-3) long chain polyunsaturated fatty acids (LC-PUFAs) such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and alpha-linolenic acid (ALA), are important modifiable dietary factors associated with birth outcome (Koletzko *et al.*, 2014; Muthayya *et al.*, 2009). However, the dietary reference intakes (RDI) for these Ω-3 LC-PUFAs have not been reported in either developed (Colón-Ramos *et al.*, 2015) or developing countries (Koletzko *et al.*, 2014). Systematic reviews on the association between Ω-3 LC-PUFAs intake and birth outcome in developing countries are limited and there have been reports only from Indonesia, India and Bangladesh (Angkasa *et al.*, 2017; Koletzko *et al.*, 2014).

The Indonesian Central Bureau of Statistics has reported that the consumption of fish by the Indonesian people was low (Central Bureau of Statistic, 2014). Based on household survey statistics of the Food and Agriculture Organization (FAO), fish consumption of the Indonesian people when compared to that of eight countries in Southeast Asia region was lower than those in Lao, Myanmar, Thailand, Philippines, and Cambodia and lower than the average global consumption of fish (FAO, 2016). As fish and fish products are the main source of Ω-3 LC-PUFAs (Freeman *et al.*, 2006), most pregnant women in Indonesia were unlikely to have adequate intakes of DHA and EPA. This situation exists especially in women from the low-medium socioeconomic background, living in urban areas with limited access to fish that are rich in essential fatty acids. Such women have a poor knowledge of the benefits and sources of essential fatty acids and are unable to afford the relatively expensive fish that are high in Ω-3 LC-PUFAs. Therefore, the monitoring of regular maternal diet is required to assess the adequacy of Ω-3 LC-PUFAs intake, especially DHA and EPA during pregnancy. Tools for assessing dietary Ω-3 LC-PUFAs have been developed and validated for Indonesian children (Ansari *et al.*, 2016). Such validated tools for pregnant women are few, and are mainly available for women who are living in developed countries with a high consumption of fish such as Japan (Kobayashi *et al.*, 2017).

The semi-quantitative food frequency questionnaire (SQ-FFQ) is a method to assess usual intake and is widely used to study the relationship between maternal diet and birth outcome in developing and developed countries (Muthayya *et al.*, 2009; Willett, 2012; Zhang *et al.*, 2015). This tool is relatively convenient, inexpensive, and requires less time for data collection than other dietary assessment methods. SQ-FFQ is able to show the long-term dietary intake patterns. It is also a valid and reliable tool for detecting the changes in dietary intake during each trimester of pregnancy. Validation studies of SQ-FFQ have shown that nutrient estimates using this method agree closely with intake estimates using the 24-hour dietary recall method, particularly among pregnant women with low literacy levels or motivation (Brunst *et al.*, 2016; Gibson, 2005).

In this study, we developed and validated a SQ-FFQ for assessing dietary Ω-3 LC-PUFAs intake in Indonesian pregnant women living in the urban areas of Jakarta.

MATERIALS AND METHODS

Study design

This cross-sectional study was carried out between February and May 2015. It was part of the East Jakarta Cohort Study on "The Role of Nutrition, Maternal Factors, and Health Service

in Microbiota Composition and Birth Weight in Jakarta" that was initiated by Department of Nutrition, Faculty of Medicine, Universitas Indonesia (Angkasa *et al.*, 2017).

Participants and recruitment

A total of 100 women were randomly selected from 315 pregnant women recruited at the third trimester. They fulfilled the inclusion criteria in being apparently healthy, pregnant women aged 19-40 years, at the gestational age of >32 weeks, who were registered for antenatal care in ten sub-district public health centres (PHCs) and one referral hospital in East Jakarta. The under- and over- reporting of energy intakes of the women were excluded from this study. Under-reporting was determined by Goldberg method (Goldberg *et al.*, 1991), in which basal metabolism rate (BMR) of each pregnant women was calculated by Schofield equation for women of their age (Schofield, 1984). The cut-off value for over-reporting of energy was set as >4700 kcal (Ambrosini *et al.*, 2011). Written informed consent was obtained from all respondents. The sample size of 100 was determined following a minimum sample size required for Bland-Altman analysis (Bland & Altman, 1999) and also in accordance to another validation study of pregnant women in developing countries (Muthayya *et al.*, 2009).

Data collection

Data on age, schooling, socioeconomic status and obstetric history were collected using a structured questionnaire based on the categories determined by the National Basic Health Survey (National Institute of Health Research and Development, 2013). The anthropometric status of the pregnant mothers was measured using calibrated tools namely the SECA® tape (SECA 201, UK) for middle upper-arm circumference (MUAC) measurement, the Shorr board® height measurement for mothers' height and the Tanita® calibrated weighing scale for mothers' weight. All measurements were conducted twice and the means of the measurements were inputted for further statistical analysis.

Before performing the validity test, the initial SQ-FFQ was developed by collecting potential food lists that contained high LC-PUFA from the Indonesian food composition database, and some previous studies among pregnant and urban women (Madanijah *et al.*, 2016). The draft was pre-tested among ten pregnant women in the same study area, but who were not included as participants. Using this prototype SQ-FFQ, participants were asked to report the foods they had consumed during the previous month. In addition, a single 24-hour food recall was administered to another twenty pregnant women in the same area for the completion of the food lists. Information on the common Ω-3 food sources and accessed food-market were emphasized during pre-testing phase. Commonly accessed food-markets were visited and some relevant foods were weighted for the food portion size. The prototype SQ-FFQ was subsequently revised based on the results of pretesting and market survey to its final form. Five trained nutritionists used the final questionnaire, which sought information of food items that were appropriate to describe the sources of fatty acids and adequate to assess the fatty acids content in individual, mixed or processed foods. The description of the portion sizes format of the SQ-FFQ were categorized as small, medium and large. The consumption frequency consisted of seven possible categories ranging from never or once a month to >1 a day. The final questionnaire included 53 items, and was grouped into seven food categories, as follows: staple food, animal protein rich-food, plant protein

rich-food, dairy product, certain fruits, ready-to-eat product (e.g. ice cream), and supplements.

The two-day, non-consecutive, 24-hour diet recalls (2DRs) method was chosen as a reference, as was also used by another study (Loy *et al.*, 2011). This 2DRs were administered twice representing one day in weekend and one day in weekday. Both SQ-FFQ and 2DRs were administered by trained nutritionists (Gibson, 2005). Conversion of household measures into grams of food consumed was carried out with the aid of an Indonesian food photograph from national total diet study (National Institute of Health Research and Development, 2014), which contained data on recipes and household measures. In cases where the food was not available in this manual, the research team conducted a market survey, bought and weighed the same portions of the foods. Conversion of processed foods from wet to dry or the reverse was calculated following the national guidelines on raw-cook food conversion (National Institute of Health Research and Development, 2014).

The nutrients of interest in the present study were the total Ω-3 LC-PUFA, EPA, DHA, ALA, total Ω-6, LA and AA. The estimation of nutrient composition of the FFQ was analysed using the Nutrisurvey software version 2007 (Erhardt, 2014). Until this study was done, an Indonesian Food Composition Table (FCT) for Ω-3 FAs content was not available. Therefore, the current study used FCTs from other countries. Calculation of Indonesian Ω-3 FAs food was estimated by using values from different but similar food items from several ASEAN countries (Berger *et al.*, 2013). In order of priority, we used FCTs from the Association of Southeast Asian Nations (ASEAN) countries (Puwastien *et al.*, 2000) such as Vietnam and Malaysia and the United States Department of Agriculture (USDA) (Schakel, Buzzard & Gebhardt, 1997).

Data analyses

The general characteristics of the participants are presented as frequency and percentage for categorical variables. Continuous variables with normal distribution, were tested by Kolmogorov-Smirnov, and expressed as mean and standard deviation (SD), and those with violated distribution are presented as median and interquartile range. For validation testing, Pearson's correlation and paired t-test were used for normally distributed data. The Spearman and Wilcoxon signed rank test was used for non-normally distributed data. Cross-classification was presented as correctly classified, correctly and adjacent classified, and extremely misclassified quintile. All nutrient intakes were log transformed (x+1). Energy-adjusted nutrient intakes were calculated as the residuals from the regression of nutrient intake as the dependent variable while energy was the independent variables (Willett, 2012). The Bland-Altman scatter plot was also produced from the mean (bias) of SQ-FFQ and 2DRs against difference of SQ-FFQ and 2DRs. The Limits of Agreement (LoA) was calculated by mean+1.96 SD for upper LoA and mean-1.96 SD for lower LoA (Bland & Altman, 1999). Antilog of the mean bias and LoA were multiplied by 100, in which value close to one or 100% represented perfect agreement. The regression coefficient was also produced to estimate under or overestimate of average intake between two methods. All significant values were set at $p<0.05$. All statistical tests were calculated using the SPSS version 21.0 for Windows software. The Bland-Altman scatter plot or graph was drawn by Prism 6 for Windows version 6.05 software.

Ethical approval

The study was approved by the ethical committee of the Faculty of Medicine of Universitas Indonesia and the Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia under the serial number 859/UN2.F1/ETIK/2014. The local authority of East Jakarta District, District Health office of East Jakarta, and sub-districts Public Health Center also gave their approval for the study.

RESULTS

Table 1 presents the baseline characteristics of the pregnant women. Their median age was 28 years. More than half of the mothers had at least 12 years of formal education and most of them were housewives with a median household income of 2.6 million rupiahs (~196 US Dollars) per month. More than half of pregnant women were exposed to smoking in their daily lives. More than a third of women (39%) were nulliparous. Only small number of pregnant mothers had a history of premature births (n=7), abortions (n=8) and low birth weight infants (n=11). At recruitment, the mean gestational age of the pregnant mothers was 35.5 weeks. Mean MUAC and body height; and median body weight of pregnant women were 27.2 cm, 153.4 cm, and 61.6 kg, respectively.

Table 2 shows the mean daily intakes of Ω-3 fatty acids as assessed by the SQ-FFQ and 2DRs. Except for energy, fat, total Ω-6 and LA, the mean intake of the measured nutrients as estimated by

Table 1. Sociodemographic characteristics and nutritional status of the100 pregnant women of this study

Variables	*n (%)*	*Mean±SD*
Sociodemographic		
Mother's age, years		28 (25-31.8)†
Education, years		
< 12	25 (20)	
≥ 12	75 (80)	
Working status		
Working	27 (27)	
Housewife	73 (73)	
Household income in million‡		2.6 (2.0-3.5)†
Daily smoking exposure, yes	53 (53)	
Obstetric profiles		
History of		
Nulliparous	39 (39)	
Premature birth (n=58)	7 (12.1)	
Abortion (n=61)	8 (13.1)	
Low birth weight (n=58)	11 (19)	
Gestational age, weeks		35.5 (34.0-36.9)†
Maternal antrophometric		
MUAC, cm		27.2±2.9
Height, cm		153.4±5.1
Weight, kg		61.6 (57.1-69.5)†

†Median (quartile 25th-75th)

‡Rupiah/month, 1 US Dollars = 13.000 Rupiahs

Table 2. Validation study: comparison, Spearman correlation (SC) and cross classification of mean daily intakes of fat and fatty acids between SQ-FFQ and the 2DRs

Energy/ Nutrients	*SQ-FFQ*		*2DRs*		*p-value*[†a]	*SC (r)*		*Cross C*		
	Mean	*SD*	*Mean*	*SD*		*Crude*[†]	*Adjusted*[‡]	*CC*	*CC/ AC*	*IC*
Energy (kcal)	2025	635	2186	527	0.001*	0.386*	-	25	87	3
Fat (g)	64.2	31.5	75.5	26.3	0.000*	0.348*	0.385*[§]	36	88	3
Total Ω-3 (g)	11.30	7.83	6.82	4.72	0.000*[¶]	0.309*[§]	0.349*	22	88	5
ALA (g)	11.00	7.83	6.57	4.70	0.000*[¶]	0.310*[§]	0.352*	26	88	4
EPA (g)	0.14	0.16	0.13	0.21	0.014*	0.308*	0.380*	26	88	5
DHA (g)	0.17	0.14	0.12	0.15	0.000*	0.201*	0.338*	26	80	4
Total Ω-6 (g)	1.35	1.40	4.47	11.30	0.062	0.118	0.408*	24	80	6
LA (g)	1.17	1.37	4.38	11.40	0.091	0.118	0.409*	20	79	4
AA (g)	0.17	0.12	0.10	0.08	0.000*	0.211*	0.331*	29	84	6
LC-PUFA (g)	12.60	8.62	11.30	12.40	0.002*[¶]	0.213*[§]	0.341*	22	85	3
Average						0.25	0.37	25.6	84.7	4.30

N=100
[†]based on log-transformed values
[‡]energy-adjusted, residual of linear regression, energy as dependent while nutrients as independent (17)
[§]Pearson's correlation r
[a]Wilcox son signed rank test
[¶]paired t-test
*significantly correlated, $p<0.05$
CC= correctly classified; AC= adjacent classified; IC = incorrectly classified. ALA= α-linolenic acid; EPA= eicosapentaenoic acid; DHA= docosahexaenoic acid; LA=linoleic acid; AA= arachidonic acid; LC-PUFA= long chain polyunsaturated fatty acids; SQ-FFQ = semi-quantitative food frequency questionnaires; 2DRs = two-day 24-hour dietary recalls

Table 3. Validation study: mean differences between SQ-FFQ and the 2DRs, the limits of agreement and the slope with 95% confidence intervals for a linear regression of the difference against the means of the two methods

Fatty Acids[†]	*Mean*	*SD*	*LoA lower, upper*	*Slope*[‡]	*95% CI*	*Mean*[§]	*SD*	*LoA*
Fat	-0.09	0.21	-0.51, 0.32	0.52	0.25, 0.79	0.81	1.63	0.31, 2.10
Total Ω-3	0.20	0.29	-0.38, 0.77	-0.07	-0.36, 0.22	1.58	1.96	0.42, 5.93
ALA	0.21	0.30	-0.39, 0.79	-0.12	-0.41, 0.17	1.60	2.00	0.41, 6.23
EPA	0.01	0.07	-0.13, 0.15	-0.39	-0.65, 0.12	1.01	1.18	0.74, 1.40
DHA	0.02	0.06	-0.10, 0.14	-0.09	-0.41, 0.22	1.05	1.15	0.80, 1.37
Total Ω-6	-0.05	0.42	-0.87, 0.78	-1.32	-1.54, -1.1	0.89	2.63	0.13, 5.96
LA	-0.06	0.43	-0.90, 0.78	-1.34	-1.56, -1.12	0.87	2.69	0.12, 6.07
AA	0.03	0.05	-0.06, 0.12	0.48	0.16, 0.79	1.07	1.12	0.86, 1.33
LC-PUFA	0.12	0.37	-0.61, 0.84	-0.50	-0.81, -0.19	1.31	2.33	0.25, 6.87

[†]All variables were transformed by log (x+1)
[‡]Linear regression
[§]antilog (10^y), agreement if the value close to 1
ALA= α-linolenic acid; EPA= eicosapentaenoic acid; DHA= docosahexaenoic acid; LA=linoleic acid; AA= arachidonic acid; LC-PUFA= long chain polyunsaturated fatty acids

Table 4. Food items, portion and content of Ω-3 fatty acids per 100 gram consumed

No	*Food Items*	*Gram*	*Household measures*	*SFA (g)*	*MUFA (g)*	*LA (g)*	*ALA (g)*	*ARA (g)*	*EPA (g)*	*DHA (g)*	*PUFA (g)*
A	Carbohydrate sources										
1	Nasi putih (white rice)	300	1 large size	0.04	0.05	0.06	0.00	0.00	0.00	0.00	0.07
2	Nasi goreng (fried rice)	200	1 medium size	2.30	3.08	0.91	0.06	0.03	0.01	0.06	1.09
3	Roti (white bread)	30	1 small size	0.91	1.78	0.87	0.06	0.00	0.00	0.00	0.92
4	Biskuit (biscuit)	135	1 large pack	11.10	5.52	0.68	0.25	0.02	0.01	0.01	0.98
5	Biskuit with cream (biscuit with cream)	190	1 large pack	2.97	8.43	7.01	0.52	0.00	0.00	0.00	7.55
6	Mie instan (instant noodles)	69	1 medium size	0.12	0.10	0.00	0.17	0.00	0.00	0.00	0.19
B	Plant-protein source										
7	Tahu (boiled white or yellow tofu)	30	1 small size	2.91	4.45	10.00	1.34	0.00	0.00	0.00	11.40
8	Tahu goreng (fried Tofu)	30	1 small size	2.15	3.29	7.41	0.99	0.00	0.00	0.00	8.41
9	Tempe (tempeh)	30	1 small cut	3.76	4.13	2.77	0.13	0.00	0.00	0.00	2.91
10	Kacang ijo (mungbeans)	10	1 medium spoon	0.35	0.16	0.36	0.03	0.00	0.00	0.00	0.38
11	Susu kacang ijo (mungbean extract)	250	1 medium pack	0.06	0.03	0.06	0.01	0.00	0.00	0.00	0.06
12	Bubur kacang ijo (mungbean porridge)	200	1 medium bowl	0.35	0.16	0.36	0.03	0.00	0.00	0.00	0.38
13	Kacang mete (almonds)	10	1 medium spoon	10.60	31.30	3.66	0.18	0.00	0.00	0.00	3.84
14	Kacang kedele (soybeans)	10	1 medium spoon	2.88	4.40	1.33	9.93	0.00	0.00	0.00	11.30
15	Kacang tanah (peanuts/groundnuts)	10	1 medium spoon	6.91	24.70	15.80	0.00	0.00	0.00	0.00	15.80
16	Kacang atom (deep-fired peanuts wrapped with flour)	17	1 medium pack	6.84	31.20	10.50	0.19	0.00	0.00	0.00	10.70
17	Kacang telur (peanut wrapped with flour)	30	1 medium pack	6.84	31.20	10.50	0.19	0.00	0.00	0.00	10.70
18	Kacang kapri (greenpeas)	10	1 medium spoon	0.06	0.03	0.12	0.03	0.00	0.00	0.00	0.15

No	Food Items	Gram	Household measures	SFA (g)	MUFA (g)	LA (g)	ALA (g)	ARA (g)	EPA (g)	DHA (g)	PUFA (g)
C	Animal-protein source										
19	Hati ayam (chicken liver)	30	1 medium size	2.91	2.33	0.90	0.02	0.62	0.00	0.00	1.53
20	Ampela goreng (fried chicken gizzard)	15	1 medium size	2.07	1.99	0.98	0.00	0.35	0.00	0.00	1.41
21	Daging Ayam (cooked chicken, non-fried)	50	1 medium cut	5.53	8.00	3.69	0.18	0.00	0.01	0.04	4.14
22	Ayam goreng (fried chicken)	40	1 medium cut	2.55	3.48	1.85	0.09	0.13	0.01	0.05	1.76
23	Ayam goreng dada (fried chicken breast-fast food)	100	1 medium cut	3.89	5.97	6.47	0.32	0.04	0.00	0.00	6.91
24	Ayam goreng paha (fried chicken thigh-fast food)	70	1 medium cut	4.10	6.38	6.23	0.31	0.07	0.00	0.00	6.71
25	Usus ayam (chicken intestine satay)	20	1 small stab	5.17	4.26	2.85	0.11	1.03	0.04	0.08	4.14
26	Ceker ayam (chicken feet)	15	1 piece	3.92	5.50	2.57	0.10	0.00	0.01	0.04	2.98
27	Daging kambing (goat meat)	50	1 medium cut	0.82	1.20	0.12	0.02	0.07	0.00	0.00	0.20
28	Daging sapi (cow meat)	50	1 medium cut	0.12	0.01	0.59	0.17	0.00	0.00	0.00	0.76
29	Bakso sapi (meatball)	15	1 medium pieces	1.43	2.19	4.15	0.52	0.00	0.00	0.00	4.66
30	Sosis sapi (meat sausage)	30	1 medium pieces	10.10	12.60	2.57	0.22	0.06	0.03	0.00	2.96
31	Sosis ayam (chicken sausage)	30	1 medium pieces	7.16	10.20	4.34	0.35	0.00	0.00	0.00	4.72
32	Kikil sapi	60	1 medium cut	4.40	7.00	0.00	0.00	0.00	0.00	0.00	17.50
33	Ikan lele (catfish)	40	1 small fish	1.77	3.59	0.88	0.10	0.09	0.07	0.21	1.57
34	Ikan (fish) mas/gurame/sepat	100	1 small fish	1.03	2.14	0.50	0.26	0.14	0.23	0.11	1.37
35	Ikan (fish) mujaer	40	1 small fish	0.21	0.44	0.10	0.05	0.03	0.05	0.02	0.28
36	Ikan (fish) tongkol/kembung/ kakap	80	1 medium cut body fish	2.32	3.89	0.15	0.12	0.13	0.64	0.15	2.38
37	Ikan (fish) bandeng/bawal/sarden (sardines)	40	1 medium cut body fish	1.48	2.30	0.00	0.00	0.00	0.00	0.00	1.64

No	Food Items	Gram	Household measures	SFA (g)	MUFA (g)	LA (g)	ALA (g)	ARA (g)	EPA (g)	DHA (g)	PUFA (g)
38	Ikan asin teri (salted anchovy)	15	1 medium spoon	0.40	0.30	0.00	0.00	0.00	0.00	0.00	0.60
39	Telur ayam ras/kampong (mentah, utuh) (chicken egg whole edible part)	55	1 item	2.85	3.50	1.06	0.03	0.13	0.00	0.04	1.25
40	Telur ayam (chicken egg)(yellow part)	30	1 item	10.50	12.90	3.89	0.11	0.00	11.00	0.12	4.62
41	Telur puyuh (quail egg)	10	1 item	2.81	3.41	0.00	0.03	0.10	0.00	0.00	1.04
42	Telur bebek (raw, whole, duck egg)	60	1 item	3.96	7.03	0.60	0.11	0.35	0.00	0.00	1.31
D	Milk and milk products										
43	Instant cereal	30	1 sachet	2.12	1.54	5.04	0.37	0.01	0.00	0.00	5.41
44	Susu hamil (pregnancy commercial milk)	30	1 spoon	3.36	1.68	0.13	0.07	0.00	0.00	0.00	0.20
45	Susu kental manis (sweetened condensed milk)	42	1 sachet	1.67	5.95	3.79	0.00	0.00	0.00	0.00	3.79
46	Susu cair (others) (UHT milk)	250	1 medium pack (ml)	2.40	1.10	0.00	0.00	0.00	0.00	0.00	0.10
E	Fruits										
47	Alpukat (avocado)	270	1 medium size	0.11	0.03	0.04	0.02	0.00	0.00	0.00	0.07
48	Pisang ambon (ambon banana)	100	1 medium size	0.18	0.05	0.07	0.03	0.00	0.00	0.00	0.12
49	Pisang raja/kepok (kepok banana)	100	1 medium size	0.07	0.02	0.03	0.01	0.00	0.00	0.00	0.05
F	Snacks										
50	Es Krim (ice cream)	65	1 small pack	1.22	1.84	3.07	0.18	0.00	0.00	0.00	3.26
G	Supplements										
51	Fish oil 1 (containing EPA/DHA 373 mg)		1 item	0.00	0.00	0.00	0.00	0.00	0.03	0.34	0.00
52	Fish oil 1 (tuna oil 179 mg, Ω-3 64.5 mg, DHA 48.5 mg, EPA 12.5 mg, Promavit)		1 item	0.00	0.00	0.00	0.00	0.00	0.25	0.97	0.00
53	Fish oil 3 (Prolacta)		1 item	0.00	0.00	0.00	0.00	0.00	0.02	0.21	0.00

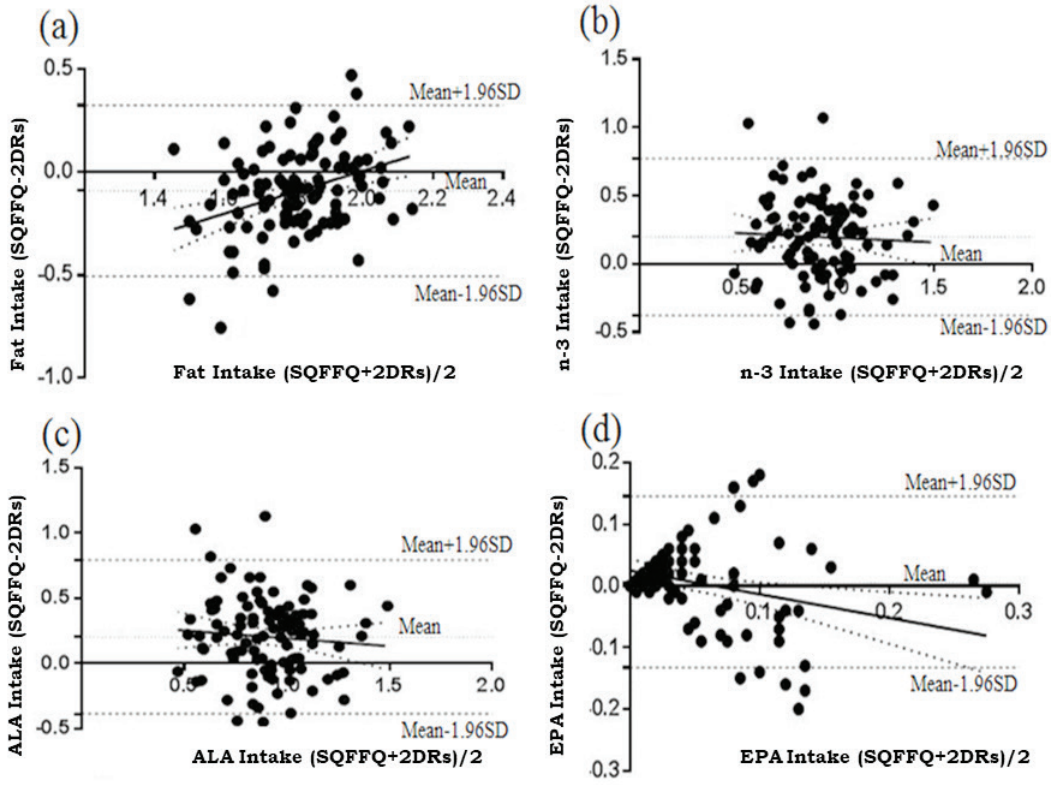

Figure 1. Bland–Altman plot showing agreement between the average of SQ-FFQ and the 2DRs in estimating the intakes of (a) fat, (b) n-3, (c) ALA, alpha linolenic acid and (d) EPA, eicosapentaenoic acid. After natural log transformation. SQ-FFQ = semi-quantitative food frequency questionnaires; 2DRs = two-day 24-hour dietary recalls

SQ-FFQ were significantly higher (4.44, 4.38, 0.01, 0.05, 0.07 and 1.31 g/d for total Ω-3, ALA, EPA, DHA, AA and LC-PUFA, respectively) than the mean intake estimated by 2DRs. Table 2 also presents the unadjusted Spearman correlation coefficients between both methods ranging from 0.118 for total Ω-6 and LA to 0.386 for energy intake. The Spearman correlation coefficients increased after energy adjustment for all nutrients. The most obvious changes compared with the unadjusted values occurred in the total Ω-6 (from 0.118 to 0.408) and LA (from 0.118 to 0.409). Overall, the average correlation coefficient of all nutrients was r=0.25 and after energy adjustment, the average of correlation coefficients improved for 0.12 points (r=0.37). All the energy-adjusted coefficients for fat and fatty acids were statistically significant (p<0.05) between two methods. A high proportion of the pregnant women (>79 %) were categorised into the same or adjacent quintiles by the SQ-FFQ vs quintiles of 2DRs for energy and all fatty acids intakes. On the average, about 4.3% of pregnant women were in extreme misclassifications. Bland Altman plots showed acceptable agreement between the SQ-FFQ and 2DRs for DHA, EPA and AA, as indicated in Table 3. The values of the antilog mean for DHA (1.05 or 105%), EPA (1.01 or 101%) and AA (1.07 or 107%) were close to one (or 100%) and the values of LoA were narrow

(DHA 80-137%; EPA 74-140%; AA 86%; 133%) which indicated good agreement between both methods.

Table 4 shows the food groups, food items, standard portion sizes and content of LC-PUFA. Plant protein-sources from legumes family contained the highest amount of LA and ALA in the new developed SQ-FFQ and were also the most frequent consumed food source for both LA and ALA intake among pregnant women. Soy and its products such as tofu and tempeh contained almost 10 g ALA/100g while peanut and its products contained about 0.15 g per gram of peanuts. The highest amount of EPA was in chicken egg (11 g/100g) and fish (0.64 g/100g) while chicken intestine satay (1.02 g/100g) and chicken liver (0.61 g/100 g) contained the highest amount of ARA. Except from the supplement (0.97 g/1 capsule), a high amount of DHA intake was derived from catfish (0.21 g/100g), mackerel tuna (0.15 g/100g) and yellow egg (0.12 g/100 g). Figure 1 shows the Bland–Altman plots for fat, total Ω-3, DHA, and EPA intakes. Most of the points fell within the 95% limits of agreement (LoA) for total Ω-3, DHA, EPA, and ALA intakes. Except from total fat, Ω-6 and LA, the SQ-FFQ overestimated all fatty acids intake from 2DRs.

DISCUSSION

The current study presents a newly developed SQ-FFQ, with 53 food items, that we believe is valid for estimating dietary DHA, EPA and AA intake among Indonesian pregnant women in the third trimester. The Bland-Altman plots imply acceptable agreement between the SQ-FFQ and reference method (two-repeated 24-h recalls) for these nutrients as the mean values of the antilog for these nutrients were close to one (or 100%) and the values of LoA were narrow. The analyses of correlation coefficient and a cross-classification also indicated a good relative validity of the SQ-FFQ in assessing intake of fat and fatty acids. Agreement based on the comparison test was found for the total Ω-6 and LA.

In a validation study, several tests including Cohen's kappa coefficient (κ) might be used to find agreement between methods. However, not all tests were appropriate for use in this study. We prioritised the results of the Bland-Altman test for generating the conclusions of this study because this approach was appropriate for quantitative variables (Zhang *et al.*, 2015) as shown by some other studies (Kobayashi *et al.*, 2017; Zhang *et al.*, 2015). In the Bland-Altman Test, the agreement of both methods was met when antilog of mean (bias) was close to one and the interval of LoA was narrow. We assumed that the antilog of mean (bias) for DHA, EPA and AA were sufficiently close to one and the SD of those nutrients were intuitively narrow. The study of Ansari *et al.* (2016) reported almost similar antilog means and SD of DHA between SQ-FFQ and three-day, 24-hour food recalls as in the Indonesian children's study. Therefore, the Bland Altman test could support the use of the SQ-FFQ as a valid instrument for assessing LC-PUFA Ω-3 a in larger study. A comparison of both methods, indicated that there was a tendency for SQ-FFQ to overestimate the dietary recalls as reported by Ansari *et al.* (2016). On the contrary, other tests, such as the *t*-test and correlation coefficient were not appropriate to show agreement. The *t*-test informed us very little about the accuracy of the methods (Bland & Altman, 1999) while the correlation test evaluated only the linear association of two sets of observations (Bland & Altman, 1999; Zhang *et al.*, 2015). These approaches were inadequate and could be very misleading when assessing agreement.

The correlation coefficients of most validation studies that compared the two dietary methods ranged between 0.30 and 0.49 (Streppel *et al.*, 2013; Zhang *et al.*, 2015). However, in most of the studies, energy-adjustment or de-attenuation increased the correlation of the methods assessed (Barbieri *et al.*, 2013; Bizjak, Jenko-Pražnikar & Seljak, 2014; Streppel *et al.*, 2013). The observed correlation coefficients (*r)* between SQ-FFQ and 2DRs were 0.38, 0.34, 0.35 and 0.35, for EPA, DHA, ALA and total Ω-3, respectively, in this study. These figures were slightly higher than that of the studies that performed unadjusted energy intake, such as a study comparing SQ-FFQ and three-day diet records (3DRs) among Japanese women in the late pregnancies where the correlation coefficients (*r*) were 0.37, 0.34, 0.32 and 0.32 for EPA, DHA, ALA and total Ω-3, respectively (Kobayashi *et al.*, 2017), and another study that compared 2DRs and SQ-FFQ among pregnant women in Malaysia (*r*=0.24 for fat) (Loy *et al.*, 2011). However, in another study where energy-adjustment was done, the correlation coefficients were *r*=0.39 for total Ω-3 and *r*=0.42 for LC-PUFA (Bizjak *et al.*, 2014) were higher than the coefficient found in the present study. As the correlations in this study are comparable with those reported among other groups of pregnant women, the Ω-3 SQ-FFQ that has been developed may be considered valid for use in further studies.

The cross-classification of quintiles is different from correlation as the former is more informative when reporting the capacity of an assessment method to rank persons in relation to their intakes (Zhang *et al.*, 2015). One study reported that FFQ can be an effective instrument if the result of cross-classification could categorise >70% of respondents into the same or adjacent quintile (Barbieri *et al.*, 2013). The present study has fulfilled this criterion. Interestingly, the result of the correlation test in this study is similar to that of the cross-classification test in that it could rank fat and all fatty acids >80% into correctly and adjacent quintile, indicating good validity for the methods. This finding is similar with the results of study of 41 pregnant women that found higher agreement based on cross validation (quartiles) from 3DRs and FFQ for ALA, total Ω-3, and LA (Zhang *et al.*, 2015). Although agreement was found only for the total Ω-6 and LA, for both correlation and cross-classification tests, it suggested good validity for the newly developed SQ-FFQ for total fat and fatty acids.

Some validation studies have used blood biomarkers for dietary evaluation. However, the use of biomarkers may be challenging because of its reliability and sensitivity for pregnant women who tend to have different food patterns (Sartorelli *et al.*, 2012; Zhang *et al.*, 2015) and an increment of maternal plasma volume (Parker *et al.*, 2015). Therefore, dietary assessment methods (e.g. food recall, SQ-FFQ) may be more reliable since it can reflect the changing of dietary patterns during pregnancy and thus can be used for dietary evaluation. Nevertheless, caution should be exercised when using dietary evaluation since it is based on selected methods and the use of food composition tables as well as calculation when the nutrients of interested are not available. The use of food recall as a reference may be subjective and prone to error because it relies on memory (Gibson, 2005). Another study reported that food recall reflected the different kinds of memory and was suitable for use in specific populations and among individuals of low literacy level and motivation (Brunst *et al.*, 2016; Gibson, 2005). Compared to the FFQ which related to generic memory, the food recall relies on episodic memory (Vioque *et al.*, 2016). In the current study, we

used 2DRs, not a minimal of 3DRs as used by many studies, to compare the women's intake with SQ-FFQ for a validation study. Arguably, it may be inadequate to capture the habitual intake of pregnant women. However, Loy *et al.* (2011) used 2DRs as a comparison and found the SQ-FFQ to be a valid tool to collect and rank individual dietary intake for prospective study. In the current study, the unavailability of existing Indonesian FCTs for Ω-3 fatty acids food source may hinder the accuracy of calculation of Indonesian food and may under- or over-estimate the intake. To control this possible bias, we selected the closest or similar foods in the FCT of other countries to estimate fatty acid content in Indonesian foods. As a result, the same reference food item was used for several different foods (e.g. fish group) although the research team had tried to find the closest genus by fish classification (Schakel *et al.*, 1997). In addition, to have representative results, this validation study included randomly selected subjects with similar characteristics with respect to age, social-economic, education level, pregnancy history, smoking exposure and nutritional status in The Project of Role of Nutrition, Maternal Factors, and Health Service in Microbiota Composition and Birth Weight in Jakarta.

The newly developed SQ-FFQ can be used to estimate the intakes of Ω-3 LC-PUFA intakes among pregnant women in large urban settings. Since reported intakes of Ω-3 LC-PUFA are only available from Bangladesh and India (Koletzko *et al.*, 2014), this study provides new information intake in another developing countries, by using a simple, easy and valid tool. Nutritional education of pregnant women can also be improved with the new information on Ω-3 LC-PUFA that this study has generated to help them identify and choose main sources of this important nutrient.

CONCLUSION

The present study has shown that there is agreement between the two dietary methods, SQ-FFQ and 24-h recall, based on Bland Altman approach. The newly developed SQ-FFQ is reasonably valid for assessing the DHA, EPA and AA intake among pregnant women. Absolute validation and reproducibility studies of pregnant women in each trimester using biomarkers are recommended.

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Authors' contributions

DA, designed this present study, conducted data collection, analysed and interpreted data, prepared the draft manuscript; RA, coordinated the design of the overall umbrella study entitled 'Role of Nutrition, Maternal Factors, and Health Service in Microbiota Composition and Birth Weight in Jakarta', designed this present study, conducted data collection, analysed and interpreted data, contributed to developing the study design, writing the manuscript, coordinated and decided on the final draft of manuscript for submission; HK and EP, contributed to the development of the study design and manuscript preparation; all the authors have read the whole manuscript and have approved its publication.

Conflict of interest

The authors declare they have no competing interests.

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