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Insulin resistance, inflammation and metabolic syndrome in normal weight and overweight/obese primary school children in Kuala Lumpur

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ABSTRACT

Introduction: Studies on metabolic syndrome (MetS) of children are important in view of rising prevalence of childhood obesity worldwide. This study compares the risks of insulin resistance, inflammation and metabolic syndrome between overweight/obese (OW/OB) and normal weight (NW) children in Kuala Lumpur. **Methods:** A cross-sectional study was conducted in 12 primary schools selected using multi-stage stratified random sampling. Height and weight were taken of a total of 1971 children aged 10-11 years. Based on BMI-for-age, 235 OW/OB children matched for age, sex and ethnicity with 226 NW children were selected for the study. Overnight fasting blood samples were collected to determine insulin, high-sensitivity C-reactive protein (hsCRP), glucose and lipid profiles. Logistic regression analysis was conducted to estimate associations between weight status and metabolic risk factors. **Results:** Prevalence of MetS among OW/OB children was 3.8% compared to 0% in the NW. Prevalence of insulin resistance among OW/OB was 45.5% compared to 18.6% among NW children. High risk of inflammation was found in 28.1% of the OW/OB children compared to 12.4% in the NW. The odds ratio of having insulin resistance, inflammation and metabolic risk factors among OW/OB were 3.66 (95% CI: 2.40-5.59), 2.76 (95% CI: 1.69-4.50), 4.93 (95% CI: 3.42-7.10), respectively compared to the NW. **Conclusion:** The OW/OB children in this study showed higher risks of developing insulin resistance, inflammation and MetS compared to the NW counterparts. Further studies are suggested to better understand the relationships between insulin resistance, inflammation and MetS in children.

Keywords: Children, insulin resistance, hsCRP, metabolic syndrome, obesity

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INTRODUCTION

Childhood obesity is a serious public health condition due to its alarming increase in both developed and developing countries. In 2011, the South-East Asia Nutrition Survey (SEANUTS) revealed that the prevalence of overweight and obesity among children aged 6 months to 12 years was 21.6% (Poh *et al.*, 2013). The National Health and Morbidity Survey (NHMS) 2015 reported that the prevalence of obesity among children aged 10-14 years in Malaysia was 14.4% (IPH, 2015). Similarly, the MyBreakfast study revealed that the prevalence of overweight and obesity among Malaysian children age 6-12 years was 14.7% (Mohd Nasir *et al.*, 2017).

Metabolic syndrome (MetS) is defined as a clustering of risk factors of dyslipidaemia, hyperglycaemia and high blood pressure, which directly increases the chances of cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) (Agirbasli, Tanrikulu & Berenson, 2016). MetS in children is receiving attention due to the rise in the prevalence of childhood obesity worldwide. In Malaysia, the prevalence of metabolic syndrome among overweight and obese children was reported to range from 1.3% to 5.3% based on the International Diabetes Federation's paediatric definition (IDF) (Quah, Poh & Ismail, 2010; Wee *et al.*, 2011). Another metabolic complication observed among the overweight/obese children is insulin resistance (van der Aa *et al.*, 2015). Insulin resistance is defined as a decrease in the ability of insulin to stimulate glucose uptake by muscles and adipose tissues and to suppress hepatic glucose production (Matthaei *et al.*, 2000). Obesity is known to be a state of low-grade inflammation due to the rise in inflammatory factors (DeBoer, 2013).

Despite the increasing prevalence of childhood obesity in Malaysia, studies pertaining to the state of insulin resistance and levels of high-sensitivity C-reactive protein in Malaysian children are limited. As early detection of the risk of cardiovascular disease is important for early prevention strategies, this study aimed to determine the risk of insulin resistance, inflammation and MetS in overweight/obese (OW/OB) children compared to normal weight (NW) children in Kuala Lumpur.

MATERIALS AND METHODS

Study setting and subjects

A comparative cross-sectional study was conducted among primary school children aged 10-11 years. A multistage stratified random sampling was used whereby stratification was conducted according to the school type, namely National Type, National Type Cina and National Type Tamil primary schools in the Federal Territory of Kuala Lumpur. Out of the three education zones in the Kuala Lumpur, namely Bangsa-Pudu, Keramat and Sentul, Bangsar-Pudu Zone was randomly selected for the study. A total of 85 schools fulfilled the inclusion criteria of co-educational in composition.

The sample size for the study was calculated using the formula by Aday & Cornelius (2014). With the power of the study set at 80% and confidence level set at 95%, the estimated sample size was a minimum of 157 respondents for each group of NW and OW/OB children. The sample size was increased by approximately 30% to compensate for missing data. Hence a total of 205 children for each of the NW and OW/OB group.

A total of 1971 students from all Year 4 and Year 5 classes in the selected schools were screened for body mass index (BMI) based on height and weight measurements. The WHO growth reference 2007 (BMI-for-age) (de Onis *et al.*, 2012) was used to classify

the nutritional status of the children. There were 10% thinness (*n*=197); 57.5% normal weight (*n*=1136); 16.5% overweight (*n*=326); and 15.8% obesity (*n*=312). All the 638 OW/OB children were invited to participate. An equal number of NW children matched for age, sex and ethnicity with the OW/OB children was randomly selected. However, only 285 OW/OB and 299 NW children agreed to participate in the blood draw (response rate 46.9% OW/OB, 44.7% NW). During data collection, a total of 64 OW/OB and 59 NW children were excluded as they were unwell, afraid to have their blood drawn, did not fast for 10 hours or were absent. The final number of respondents were 235 OW/OB and 226 NW children, matched for age, sex and ethnicity.

The research protocol of this study was approved by the Ethics Committee for Research Involving Human Subjects, Universiti Putra Malaysia (FPSK(FR14) P017) and the Ministry of Education Malaysia (KP(BPPDP)603/5/JLD.10(17)) and Department of Education Federal Territory of Kuala Lumpur (JPNWP.900-6/1/7 Jld. 10(92)). Signed informed consent was obtained from the respondents and their parents prior to data collection between July 2014 and October 2015.

Anthropometric measurements

(i) Height and weight

Body weight was measured using OMRON Body Fat Analyzer model HBF-356 (Omron Matsusaka Co. Ltd, Matsusaka, Japan) to the nearest 0.1 kg. Height was measured using a SECA Body Tape Measure SE206 (SECA, Germany) to the nearest 0.1 cm. Both height and weight were measured twice, and the mean values were used for the calculation of BMI. The AnthroPlus software version 10.4 (WHO, Geneva, Switzerland) was used to assess the BMI-for-age of the respondents, which classified the nutritional status of the children based on BMI-for-age *z*-scores, according to the WHO Growth Reference 2007 (de Onis *et al.*, 2012).

(ii) Waist circumference

Waist circumference (WC) was measured over the skin midway between the tenth rib and the iliac crest at the end of a normal expiration, using a SECA Ergonomic Circumference Measuring Tape SE203 (SECA, Germany) to the nearest 0.1 cm. The 90th percentile was used as the cut-off point to define abdominal obesity for use among Malaysian children and adolescents (Poh *et al.*, 2011). Waist-to-height ratio was calculated by dividing waist circumference (cm) measurements with height (cm).

Blood pressure measurements

Arterial blood pressure was measured automatically using an OMRON Digital Automatic Blood Pressure Monitor HEM-907 (OMRON, Japan) with a suitable cuff size for each participant after a 5-minute rest. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded three times after an interval of 30 seconds each and the mean was calculated.

Biochemical measurements

A total of 5 ml venous blood sample was collected after 10-hour fast using standard venepuncture by a trained phlebotomist with an attendant nurse or physician. Fasting lipid profiles: triglycerides, total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C); high-sensitivity C-reactive protein (hsCRP) and fasting blood glucose were assessed using Roche Cobas E311 (Germany) whereas fasting blood insulin was assessed using Roche Cobas E411 Immunoassay Analyzer (Germany). All biochemical analyses were outsourced to a certified laboratory for analysis.

Metabolic syndrome criteria

Metabolic syndrome was defined based on the International Diabetes Federation's paediatric definition (Zimmet *et al.*, 2007). According to the definition, metabolic syndrome is defined as waist circumference ≥90th percentile plus two or more of the following indices for all boys and girls: triglycerides: ≥150mg/dL (1.7mmol/L); blood pressure: systolic ≥130 mmHg or diastolic ≥85 mmHg; fasting blood glucose: ≥100mg/dL (5.6mmol/L); high-density lipoprotein cholesterol: ≤40mg/dL (1.03mmol/L). Insulin resistance was determined according to the following formula fasting blood insulin (mU/L) x fasting blood glucose (nmol/L)/ 22.5, (Khoury, Manlhiot & McCrindle, 2013). A cut-off value of >2.8 as an indication of insulin resistance (Wee *et al.*, 2015). As for inflammation profile, hsCRP levels were categorised into low (<1.0mg/L), moderate (1.0-3.0 mg/L) and high (>3.0 mg/L) risk of inflammation or acute infection (Pearson *et al.*, 2003).

Statistical analysis

Data were analysed using IBM SPSS Statistics (Version 22.0). Pearson Chi-square test was used to estimate associations between categorical variables. Independent samples *t*-test and Mann Whitney U-test (where assumptions for the *t*-test could not be met) was used to analyse the differences in a continuous variable between two groups. Binary logistic regression analysis was performed to estimate the association between weight status (normal weight vs overweight/obese) and metabolic risk parameters. Observed associations were expressed as odds ratios (OR) with 95% confidence intervals (CI). Statistical significance level was set at $p<0.05$.

RESULTS

Socio-demographic factors, anthropometric characteristics, biochemical profiles and blood pressure of the children are shown in Table 1. Overweight/obese (OW/OB) children had significantly higher anthropometric measurements [height, weight, BMI and WC] compared to their normal weight (NW) counterparts ($p<0.001$). In terms of biochemical profiles, OW/OB children had significantly higher biochemical profiles [TG, LDL-C, glucose, insulin, HOMA-IR, hsCRP, SBP, DBP] compared to the NW ($p<0.05$). A significantly higher proportion of OW/OB children (45.5%) had insulin resistance compared to NW children (18.6%) ($\chi^2=38.246$; $p<0.001$). Similarly, a significantly higher proportion of OW/OB children had high (28.1%) level of hsCRP compared to the NW (12.4%) ($\chi^2=74.640$; $p<0.001$).

More than half of the OW/OB children (60.4%) had waist circumference ≥90th percentile compared to only 3.1% of the NW ($\chi^2=173.090$; $p=0.001$) (Table 2). High blood pressure was present in 5.1% of the OW/OB children compared to 0.9% of the NW ($\chi^2=6.972$; $p=0.008$). Prevalence of MetS was 3.8% among the OW/OB children while none of the NW had MetS ($\chi^2=9.830$; $p=0.002$).

Table 3 shows the binary logistic regression analysis assessing the relationship between body weight status with metabolic risk components such as fasting blood glucose, triglycerides, high-density lipoprotein, blood pressure, insulin resistance (HOMA-IR) and inflammation (hsCRP). OW/OB children had significantly higher odds of hypertension (OR: 6.01; 95% CI: 1.33-27.24; $p=0.020$), insulin resistance (OR: 3.66; 95% CI: 2.40-5.59; $p<0.001$), inflammation (OR: 2.76; 95% CI: 1.69-4.50; $p<0.001$) and metabolic risk factors (OR: 4.93; 95% CI: 3.42-7.10; $p<0.001$) compared to the NW.

Table 1. Mean values and distribution of sociodemographic factors, anthropometric measurements, biochemical indicators and blood pressure between OW/OB and NW children

Description	*Normal Weight (n=226)*	*Overweight/ Obese (n=235)*	*t/z/χ^2*	*p-value*
Age (years)§			0.433	0.510
10	106 (46.9)	117 (49.8)		
11	120 (53.1)	118 (50.2)		
Sex§			2.292	0.130
Male	112 (49.6)	133 (56.6)		
Female	114 (50.4)	102 (43.4)		
Ethnicity§			0.188	0.910
Malay	69 (30.5)	76 (32.3)		
Chinese	77 (34.1)	79 (33.6)		
Indian	80 (35.4)	80 (34.1)		
Anthropometric measurements				
Height (cm)†	138.89 ± 7.91	143.61±7.88	-6.408	<0.001**
Weight (kg)‡	31.65 ± 5.36	48.55±10.25	-16.492	<0.001**
BMI (kg/m^2)‡	16.32±1.54	23.32±3.19	-18.409	<0.001**
BMI-for-age *z*-score‡	-0.38±0.83	2.10±0.71	-18.571	<0.001**
Body fat percentage (BF %)†	19.71±6.16	30.41±3.59	-22.777	<0.001**
Waist circumference†	59.99±5.48	76.52±9.36	-15.885	<0.001**
Lipid				
Triglycerides (mmol/L)†	1.07 ± 0.35	1.22 ± 0.41	-4.251	<0.001**
HDL-cholesterol (mmol/L)†	1.60 ± 0.36	1.44 ± 0.37	4.718	<0.001**
LDL-cholesterol (mmol/L)†	2.66 ± 0.78	2.85 ± 0.79	-2.512	0.012*
Total cholesterol (mmol/L)†	4.47 ± 0.97	4.54 ± 0.93	-0.752	0.453
Total cholesterol/ HDL ratio†	2.86 ± 0.56	3.28 ± 0.83	-6.349	<0.001**
Insulin resistance				
Fasting blood glucose (mmol/L)†	5.01 ± 0.55	4.93 ± 0.52	1.657	0.098
Fasting blood Insulin (µmol/L)‡	8.27 ± 5.30	14.25 ± 9.74	-7.714	<0.001**
HOMA-IR‡	1.86 ± 1.24	3.15 ± 2.23	-7.153	<0.001**
No insulin resistance (<2.8)§	184 (81.4)	128 (54.5)	38.246	<0.001**
Insulin resistance (≥2.8)	42 (18.6)	107 (45.5)		
Inflammation				
HsCRP (mg/L)‡	1.04 ± 1.74	2.60 ± 3.15	-9.144	<0.001**
Low (<1.0 mg/L)§	170 (75.2)	83 (35.3)	74.640	<0.001**
Moderate (1.0-3.0 mg/L)	28 (12.4)	86 (36.6)		
High (>3.0 mg/L)	28 (12.4)	66 (28.1)		
Blood pressure				
Systolic blood pressure (mmHg)†	99.66 ± 8.94	109.43 ± 11.51	-10.181	0.001**
Diastolic blood pressure (mmHg)†	57.77 ± 7.67	65.23 ± 8.25	-10.053	0.001**

†Independent *t*-test; ‡Mann Whitney U-test; §Chi-square-test
*significant at $p<0.05$; **significant at $p<0.001$

Table 2. Comparison of metabolic syndrome indicators between OW/Ob and NW children

Biochemical indicators	*Normal Weight (n=226)*	*Overweight/ Obese (n=235)*	χ^2	*p-value*
Waist circumference ≥90th percentile†			173.090	<0.001**
No	219 (96.9)	93 (39.6)		
Yes	7 (3.1)	142 (60.4)		
Fasting blood glucose ≥5.6 mmol/L†			2.283	0.131
No	204 (90.3)	221 (94.0)		
Yes	22 (9.7)	14 (6.0)		
Triglycerides ≥1.7 mmol/L†			2.280	0.131
No	214 (94.7)	214 (91.1)		
Yes	12 (5.3)	21 (8.9)		
HDL-cholesterol ≤1.03 mmol/L†			3.161	0.075
No	216 (95.6)	215 (91.5)		
Yes	10 (4.4)	20 (8.5)		
Blood pressure (Systolic ≥130 mmHg or Diastolic ≥85 mmHg)†			6.972	0.008*
No	224 (99.1)	223 (94.9)		
Yes	2 (0.9)	12 (5.1)		
Metabolic syndrome†			9.830	0.002*
No	226 (100.0)	225 (96.2)		
Yes	0 (0.0)	10 (3.8)		

†Chi-square test
*significant at $p<0.05$; **significant at $p<0.001$

Table 3. Odds ratios for metabolic risk factors in overweight/obese children

Metabolic risk factors‡	*Odds ratio (95% CI)*	*p-value*
	Overweight/obese†	
Fasting blood glucose ≥5.6 mmol/L	0.59 (0.29-1.18)	0.134
Triglycerides ≥1.7mmol/L	1.75 (0.84-3.65)	0.135
HDL-cholesterol ≤1.03mmol/L	2.01 (0.92-4.40)	0.080
SBP/DBP (≥130/85mmHg)	6.01 (1.33-27.24)	0.020*
HOMA-IR (>2.8)	3.66 (2.40-5.59)	<0.001**
hsCRP (>3.0mg/L)	2.76 (1.69-4.50)	<0.001**
Metabolic risk factors	4.93 (3.42-7.10)	<0.001**

†Reference is normal weight children
‡Logistic Regression
*significant at $p<0.05$; **significant at $p<0.001$

DISCUSSION

Consistent with a previous study among Malaysian children (Wee *et al.*, 2011), significantly poorer anthropometric and biochemical parameters were observed among the OW/OB than in the NW except for fasting blood glucose. It was suggested that abnormal levels of blood glucose might be manifested only when other metabolic complications were present, as it takes years for blood glucose levels to be high in children (Misra *et al.*, 2007). In this study, despite the lack of difference observed in fasting blood glucose levels, the mean values and prevalence of insulin resistance measured through HOMA-IR were observed to be higher among the OW/OB compared to the NW.

The prevalence of insulin resistance of 45.5% among the OW/OB in this study is consistent with the findings among Japanese (46.8%) (Fujii & Sakakibara, 2012), Korean (47.1%) (Yi *et al.*, 2014) and Chinese children (44.3%) (Yin *et al.*, 2013). Insulin sensitivity in children has been attributed by the production of metabolites, hormones and adipocytokines, which in turn, is related to the pathogenesis of insulin resistance (Fujii & Sakakibara, 2012). As insulin resistance is more commonly observed among the OW/OB children, the measurement of HOMA-IR may be useful to assess undetected insulin resistance conditions in children (Barseem & Helwa, 2015).

The use of HOMA-IR index requires consideration of gender, ethnicity and pubertal stage (Andrade *et al.*, 2016). Although the HOMA-IR cut-offs used in this study provided high sensitivity and specificity, it is noteworthy that the cut-off was specifically developed for Malay children in Malaysia (Wee *et al.*, 2015). There could be a need to develop reference cut-offs for Chinese and Indian children in Malaysia.

The OW/OB had higher levels of hsCRP values and higher odds of developing inflammation compared to NW children. This is consistent with other findings whereby obesity was associated with elevated levels of hsCRP in various populations including children (Choi, Joseph & Pilote, 2013; El-shorbagy, 2010). The state of low-grade inflammation among the OW/OB is attributed by total adiposity through the production of inflammatory factors such as tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6), which in turn stimulate the production of high sensitivity C-reactive protein (hsCRP) (Calder *et al.*, 2011).

As inflammation is understood to be a key pathogenic mechanism in the initiation and progression of cardiovascular diseases (Bisoendial *et al.*, 2010; Calder et al., 2011), assessing levels of hsCRP may be an alternative for the screening of risk of MetS and cardiovascular diseases (DeBoer, 2013). Other benefits of hsCRP are that it is an easy tool to differentiate between the "healthy obese" children and those with higher risks of cardiovascular diseases without consideration of ethnicity (DeBoer *et al.*, 2013). Despite the benefits of the use of hsCRP as a screening tool, there is a lack of prospective studies that linked increased hsCRP levels to cardiovascular diseases specifically in children.

The prevalence of 3.8% among the OW/OB with MetS in the present study is much lower than that reported previously in Malaysia (5.3%) (Wee *et al.*, 2011) and Korea (7.3%) (Kang *et al.*, 2010). However, different definitions of MetS were owing to a lack of consensus on the definition for children. Hence, there is a need for a harmonized definition of MetS for children in the same way as has been agreed for adults.

In this current study, the International Diabetes Federation's (IDF)

paediatrics definition (Zimmet *et al.*, 2007) was used as it is age specific and the cut-offs for each risk factor was fixed for blood pressure, lipid profiles, glucose and waist circumference compared to the National Cholesterol Education Program for Children (NCEP/ATP III) and the World Health Organization (WHO) paediatrics definition. Also, the IDF definition was easier to apply as it does not use multiple tables to assess the metabolic criteria as proposed by other definitions (Mancini, 2009).

Although the overall prevalence of insulin resistance, inflammation and metabolic syndrome in the studied children is relatively low when compared to the prevalence in adult population (Lim & Cheah, 2016), it could pose a public health problem with the rising childhood obesity in Malaysia.

A major limitation of this study is that the association between insulin resistance, inflammation and metabolic syndrome was not examined due to the small percentage of children diagnosed with MetS. It is suggested that future studies include a larger sample size with a wider age range of children.

CONCLUSION

Overweight/obese children aged 10-11 years showed higher risks of insulin resistance, inflammation and metabolic risk factors than their normal weight counterparts. These findings suggest a need for further research and interventions to address obesity and associated metabolic problems among Malaysian children.

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Authors' contributions

All authors contributed to conception, design and interpretation of data. SEHT, MNMT, YSC, ZMS, ZJ, HSY contributed to the study concept and design. TSEH contributed to the data collection, data analysis and drafted the manuscript. MNMT, YSC, ZMS contributed to critical revisions of the manuscript. SEHT, SHY contributed by obtaining funding.

Conflict of interest

The authors declare no conflict of interest.

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Sugar intake and metabolic syndrome among older adults in Peninsular Malaysia

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ABSTRACT

Introduction: Sugar is widely consumed and excessive intake has been associated with increased risk of weight gain, diabetes mellitus and cardiovascular diseases, leading to metabolic syndrome (MetSyn). However, the association between sugar intake and MetSyn has seldom been studied among multi-ethnic Malaysian older adults. **Methods:** A total of 1,057 respondents aged ≥60 years were recruited through multistage random sampling from selected states. Anthropometric parameters, blood pressure, blood test for sugar and lipid profile were determined. Dietary intake was derived using a 7-day dietary history questionnaire (DHQ) and a semi-quantitative food frequency questionnaire (FFQ) for added sugar intake. **Results:** Prevalence of MetSyn was 39.9%, 30.9% and 42.2% using the harmonised definition, International Diabetes Federation (IDF) and National Cholesterol Education Program's Adult Treatment Panel III (NCEP-ATPIII) definitions respectively. Mean total sugar intake was 40.5±32.0 g (8 tsp) and added sugar intake was 33.0±31.0 g (6 tsp). Excessive added sugar consumption at 100th percentile increased risks of high total cholesterol by two-fold (p<0.001) and triglyceride by 1.8 fold (p<0.001). Total sugar intake at 50th percentile increased risk of high blood pressure by 0.68 fold (p<0.05) and total sugar intake at 50th, 75th and 100th percentile increased total cholesterol risk by 1.7 fold (p<0.01), 1.5 fold (p<0.05) and 2.3 fold (p<0.001) respectively. **Conclusion:** Excessive sugar consumption among older adults showed no association with MetSyn but revealed significant associations with blood pressure and lipid profiles. Effects of long term excessive consumption of sugar on health outcomes in older persons should be investigated.

Keywords: Metabolic syndrome, older adults, elderly, sugar intake, sugar consumption

INTRODUCTION

Metabolic syndrome (MetSyn) is defined as an existence of several risk factors, including abdominal obesity, dyslipidemia, high blood pressure, high blood sugar and insulin resistance (Gami *et al.,* 2007). While several criteria and definitions have been used to

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identify MetSyn, it is generally agreed that a combination of three or more of the following components must be present: large waist circumference, elevated triglycerides, low HDL-cholesterol, raised blood pressure, and elevated fasting blood glucose (Alberti *et al.,* 2009). MetSyn is categorised as a low grade chronic inflammation due to multiple complex interactions between genetic and environment factors (Kaur, 2014).

The prevalence of MetSyn among Malaysian adults was 37.1% (Mohamud *et al.,* 2011), with figures was notably high among the older adults (43.4%) (Johari & Shahar, 2014).

MetSyn was associated with inappropriate dietary pattern such as high fat and high carbohydrate induced metabolic syndrome and cardiovascular re-modeling in rats (Panchal *et al.,* 2011). In particular, Johari & Shahar (2014) showed that MetSyn was associated with higher intake of carbohydrates. Excess intake of carbohydrate would further increase blood sugar, blood pressure and metabolic effects. However, there was no description of which type of carbohydrates will actually affect the metabolic system. Sugar has been associated with increase the risk of weight gain, insulin resistance and dyslipidemia (Yang *et al.,* 2014). The latest recommendation from WHO (2015) and also Malaysian RNI (NCCFN, 2017) both suggested that consumption of additional sugar should be reduce, i.e. it should be limited to no more than 10% from total energy intake.

Daily total sugar intake of the adult population was 7 tsp which is equivalent to 37 g per day (Norimah *et al.*, 2008). A study among older adults in a rural area of Malaysia found that the sources of sugar intake were mainly from sweetened beverages (especially tea and coffee) and also traditional *kuih* (Shahar, Earland & Rahman, 2000). However, the amount of sugar intake and the effect towards health among older adults were not yet identified. Hence, the objective of this study was to identify the sugar intake and its association with the risk of MetSyn among older adults in Peninsular Malaysia.

MATERIALS AND METHODS

Study design and sampling

This was a cross-sectional study involving 1,336 individuals recruited from four states i.e. Johor, Perak, Kelantan and Selangor through a multistage random sampling between March to September 2016. This study was part of a large scale population-based study among older adults in Malaysia (LRGS TUA) (Shahar *et al.,* 2016). Inclusion criteria included individuals aged 60 years and above, able to communicate well either in Malay or English language with no known mental and terminal illness. A total of 1,057 candidates had completed the data that being included in the analysis. The formula used for sample size calculation for a cross-sectional study to relate between two parameters, namely P_1=0.3 (prevalence of MetSyn and high carbohydrate intake) and P_2=0.41 (prevalence of MetSyn and lower carbohydrate intake) (Mirmiran *et al.,* 2008).

Data collection

Respondents were interviewed at respective community centres to obtain socio-demographic data, health status and sugar intake using 7-day dietary history questionnaire (DHQ) (Shahar, Earland & Abdul Rahman, 2000) and supplemented with semi-quantitative food frequency questionnaire (FFQ) of added sugar intake (Nik Shanita *et al.,* 2012) which was used as a checklist of total sugar consumption. Anthropometric measurements including height, weight, waist and hip circumference were

taken. Clinical measurements including blood pressure test and biochemical measurement such as blood sugar and lipid profiles were also performed by trained interviewers.

Body weight was measured using a digital weighing scale (Tanita, HD-319 Digital Lithium Scale, Japan) to the nearest 0.1 kg. Height was measured using stadiometer (SECA, Seca 220 Portable, German) to the nearest 0.1 cm. Body mass index (BMI) was calculated using the formula [weight in kg/ (height in m)^2] and cut-off point of normal BMI as suggested by Nutrition Screening Initiative (NSI) for older adults of 22-27 kg/m^2 was used prior from WHO criteria however it should be taken note that there were no specific BMI criteria for diagnosis of obesity in the elderly until now (Vasconcelos *et al.,* 2010). Waist and hip circumferences were measured using Lufkin tape with ±0.1 cm. Waist-hip ratio was calculated using the formula (waist circumference in cm/hip circumference in cm).

Blood pressure was measured using automatic blood pressure instrument (Omron, HEM-907, Japan). Blood samples of 5 ml were taken and divided into two different colour tubes; 3 ml in red tube for lipid profile and 2 ml in grey tube for sugar profile. Those blood samples were immediately kept in portable refrigerator at 4°C before send to the medical lab for centrifuged and analysed on the same day as the blood was taken.

DHQ was used to obtain information on food, beverages and other nutrients consumption normally consumed by the respondents throughout the week (Shahar, Earland & Abdul Rahman, 2000). Portion sizes consumed by the individual were taken as an indication based on household measurement and the use of pictures from Food Exchanges and Portion Sizes Atlas in order to quantify the total intake and sugar intake (Shahar *et al.*, 2015). In addition, FFQ on added sugar intake among adults (Nik Shanita *et al.,* 2012) was used as a checklist to complete of high sugary food data and to identify missing details on normally dietary consumptions of other sources of sugar intake daily (Figure 1).

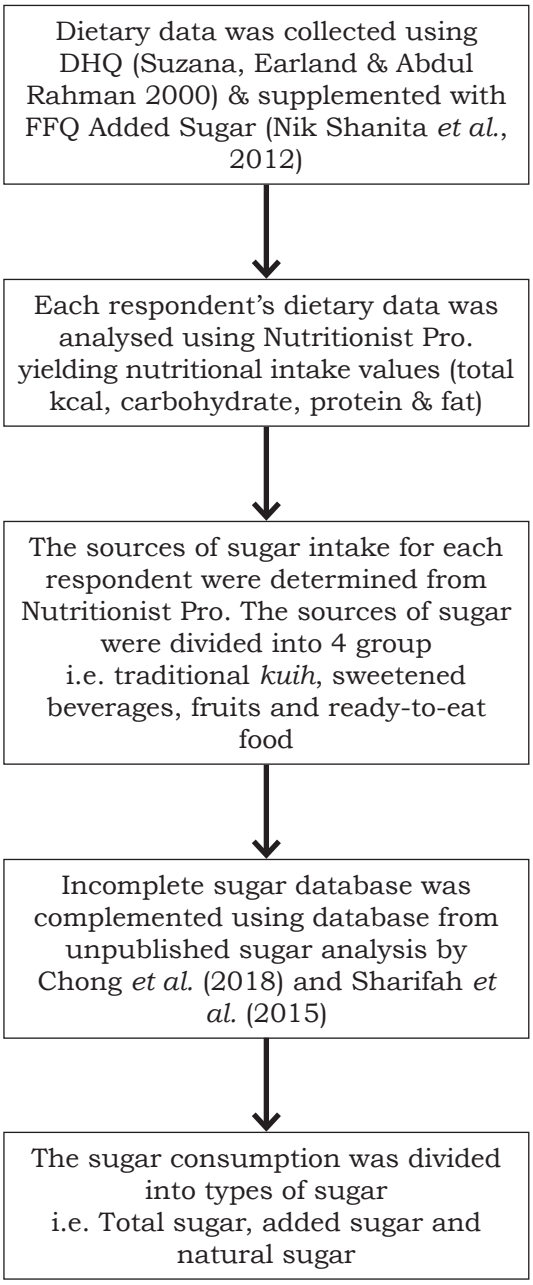

Figure 1. Flowchart of sugar data analysis

The MetSyn criteria suggested by Harmonised by Alberti *et al.* (2009) was used in this study. The criteria were at least any of three out of five risk factors: waist circumference (men: >90 cm; women: >80 cm), blood pressure (>130/85 mmHg), having diabetes mellitus or fasting blood sugar (>5.6 mmol/L), triglyceride (>1.7 mmol/L) or high-density lipoprotein (<1.0 mmol/L for men, <1.3 mmol/L for women). Meanwhile the definition of NCEP-ATP III differs from IDF and Harmonised with respect to the cut-off points of waist circumference used. While the NCEP-ATP continues to use the cut points for US (>102 cm male; >88 cm female), the Harmonised definition allows for national or regional cut points for waist circumference to be used (Table 1).

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 22.0 was used to analyse the data. Dietary sugar intakes were analysed using Nutritionist Pro version 4 and transferred to SPSS. Since the sugar databases were still unavailable, the sugar database from sugar analysis by Chong *et al.* (2018) and Sharifah *et al.* (2015) was used which providing total sugar (in grams) for each food that contain high amount of sugar (Figure 1). The added sugar was calculated by formula [(total sugar (grams) – natural sugar (grams)]. Descriptive data was used to obtain frequency and percentage of socio-demographic data, anthropometric data, total added sugar intake, clinical and biochemical parameters. The total added sugar intakes were further divided into four centiles i.e. 25th percentile [<10.3 g (2 tsp)], 50th percentile [10.3-23.8 g (2-5 tsp)], 75th percentile [23.8-47.0 g (5-9 tsp)] and 100th percentiles [>47 g (9 tsp)]. Independent *t*-test and one-way Anova test were performed to identify the significant differences of two or more than two groups of independent variables. Chi-square test was used to identify significant differences for two categorical data. Binary logistic regression test was used to obtain adjusted odds ratio for each parameters according to percentiles of added sugar.

RESULTS

As presented in Table 2, majority of the respondents were aged between 60-74 years old (73.1%), Malays (67.4%), married (85.5%), non-smokers (71.0%) and had hypertension (52.4%). Overall, respondents had normal body mass

Table 1. Definitions of Metabolic Syndrome

Risk Factors	*NCEP-ATP III (2001)*	*IDF (2005)*	*Harmonized (2009)*
Abdominal Obesity	Waist Circumference: ≥102 cm (M) ≥88 cm (W)	Waist Circumference: ≥90 cm (M) ≥80 cm (W)	Waist Circumference: ≥90cm (M) ≥80 cm (W)
High FBS	>6.1 mmol/L or DM	≥5.6 mmol/L or DM	≥5.6 mmol/L or DM
High BP	≥130/85 mmHg	≥130/85 mmHg	≥130/85 mmHg
High TG	≥1.7 mmol/L	>1.7 mmol/L	≥1.7 mmol/L
Low HDL-c	<1.03 mmol/L (M) <1.3 mmol/L (W)	<1.03 mmol/L (M) <1.3 mmol/L (W)	<1.03 mmol/L (M) <1.3 mmol/L (W)
Metabolic Syndrome	*At least 3 of the risk factors*	*Waist Circumference + 2 or more risk factors*	*At least 3 of the risk factors*

M – men; W- women; FBS – fasting blood sugar; DM – diabetes mellitus; BP – blood pressure; TG – triglyceride; HDL- high density lipoprotein

Table 2. Socio-demographic data and health status according to MetSyn (Harmonised, 2009) and added sugar intake of respondents (presented as *n* (%) or mean±SD)

Parameters	*Metabolic Syndrome*		*Added sugar intake (gram/day)*
	MetSyn (n=423, 40.0%)	*No MetSyn (n=634, 60.0%)*	
Gender[b]			
Men (*n*=525)	203 (38.7)	322 (61.3)	39.9±34.5
Women (*n*=532)	220 (41.4)	312 (58.6)	26.2±25.2
Age group (years)[ab]			
60-74 (*n*=773)	334 (43.2)	439 (56.8)	34.4±30.8
>75 (*n*=284)	89 (31.3)	195 (68.7)	29.9±30.9
Ethnicity[b]			
Malay (*n*=682)	277 (40.6)	405 (59.4)	38.7±32.7[αβ]
Chinese (*n*=324)	122 (37.7)	202 (62.3)	22.4±24.7[α]
Indian (*n*=51)	24 (47.1)	27 (52.9)	24.8±22.4[β]
State[b]			
Johor (*n*=167)	70 (41.2)	100 (58.8)	32.1±33.7
Perak (*n*=266)	108 (40.4)	159 (59.6)	28.1±27.3[α]
Kelantan (*n*=378)	135 (35.7)	243 (64.3)	39.5±32.3[αβ]
Selangor (*n*=242)	110 (45.5)	132 (54.5)	28.8±28.8[β]
Marital status[b]			
Single/separated (*n*=354)	146 (41.2)	208 (58.8)	27.8±26.7
Married (*n*=701)	277 (39.5)	424 (60.5)	35.7±32.5
Smoking status[b]			
Smokers (*n*=164)	66 (40.2)	98 (59.8)	48.1±37.2
Ex/non-smokers (*n*=893)	357 (40.0)	536 (60.0)	30.2±28.8
Diabetes mellitus[ab]			
Yes (*n*=289)	189 (65.4)	100 (34.6)	29.1±30.2
No (*n*=768)	234 (30.5)	534 (69.5)	34.5±31.1
Hypertension[a]			
Yes (*n*=583)	303 (52.0)	280 (48.0)	35.0±31.2
No (*n*=474)	120 (25.3)	354 (74.7)	31.3±30.6
High cholesterol[a]			
Yes (*n*=477)	224 (47.0)	253 (53.0)	31.2±30.1
No (*n*=580)	199 (34.3)	381 (65.7)	34.5±31.5
Heart disease			
Yes (*n*=86)	41 (47.7)	45 (52.3)	32.9±33.5
No (*n*=967)	382 (39.3)	589 (60.7)	33.0±30.7
Added sugar intake (gram/day)	32.3±30.3	34.1±31.9	33.0±30.9
Traditional *kuih* (gram/day)	3.9±6.1	3.8±7.0	3.9±6.7
Sweetened Beverages (gram/day)	28.9±29.8	26.8±27.6	27.7±28.5
Dairy beverages(gram/day)	1.1±4.1	1.5±4.3	1.3±4.2
Fruits (gram/day)	6.3±8.9	5.9±9.2	6.1±9.1
Ready-to-eat (gram/day)	1.3±3.0	1.6±3.6	1.5±3.4

[a] denoted for significant at cross-tab test for two categorical independent variable for MetSyn based on Harmonised definition

[b] denoted for significant at independent *t*-test for two continuous independent variable or two-tailed One Way Anova for more than two continuous independent variable for added sugar intake

[αβ] showed that significant at Schfee post-hoc test for more than two continuous independent variable

index (43.5%). However most of the women had a higher BMI (33.6%) and waist circumference (57.7%) compared to men 23.4%, 36.3% respectively) (p<0.0001) (data not shown).

Based on the harmonised criteria, 40.0% of the respondents had MetSyn, especially among respondents aged 60-74 years old (43.2%) and those reported having diabetes mellitus (65.4%), hypertension (52.0%) and high cholesterol (42.0%) (p<0.05) (Table 2). The prevalence was also higher among women, Indian, respondents from Selangor state, living as single or separated and smokers but these differences were not significant (Table 2).

The overall mean intake of total sugar was 40.5±32.0 g/day (≈8 tsp), natural sugar was 7.4±10.4 g/day (≈2 tsp) and added sugar was 33.0±30.9 g/day (≈6 tsp). The intake of habitual added intake was notably high in men, ages 60-74 years, Malays, respondents from Kelantan state, married couples, smokers and having diabetes mellitus (p<0.05) (Table 2). Intake of added sugar among MetSyn respondents were slightly higher (34.1±31.9 g/day) compared to those without MetSyn (32.3±30.3 g/day) but the difference was not significant (p>0.05) (Table 2). However, the highest prevalence of MetSyn (45.2%) was found at 100th percentile of added sugar intake (Figure 2). The highest sources of sugar consumption were sweetened beverages (27.7±28.5 g/day), followed by fruits (6.1±9.1 g/day), traditional *kuih* (3.9±6.7 g/day) and ready-to-eat food (i.e. sweets, honey, biscuits, cookies etc) (1.5±3.4 g/day) (Table 2).

There were significant mean differences for systolic reading (p<0.05), total cholesterol (p<0.05), LDL-c (p<0.01) between added sugar at 25th and 100th percentiles in men (Table 3). Meanwhile for women, there were also significant mean differences for diastolic (p<0.05), total cholesterol (p<0.001) and LDL-c (p<0.001) according to percentiles of added sugar intake (25th, 50th and 75th), with the highest level observed at the 100th percentile of added sugar intake (Table 3).

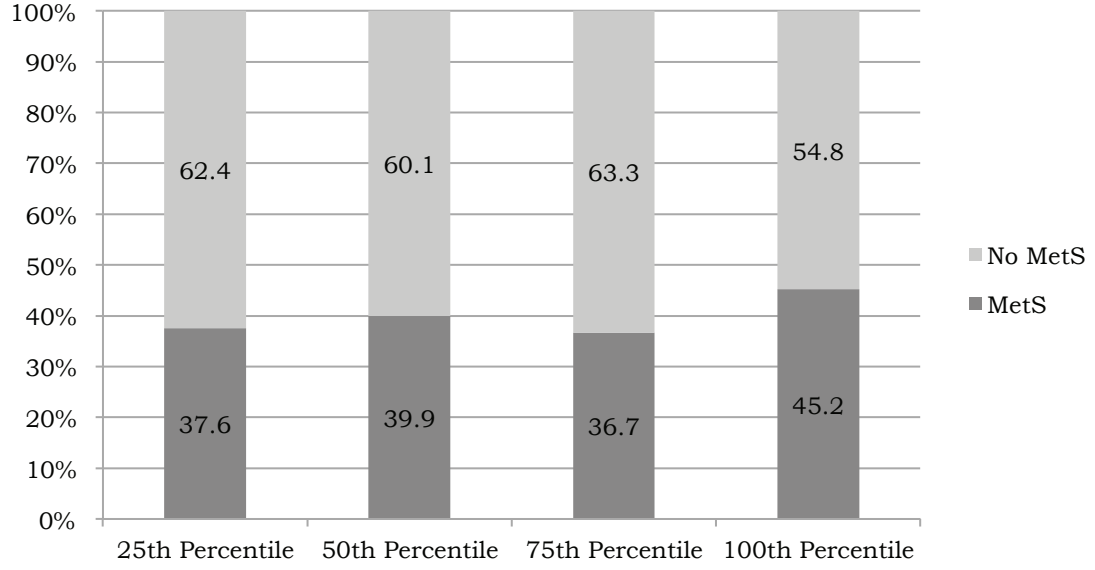

Figure 2. Prevalence of MetSyn based on percentile of added sugar intake

Table 3. Anthropometric, biochemical and clinical data according to percentile of added sugar intake and gender

Parameters	*25th percentile <2 tsp (<10.3 gram) (N=264)*	*50th Percentile 2-5 tsp (10.3-23.8 g) (N=265)*	*75th Percentile 5-9 tsp (23.8-47.1 g) (N=271)*	*100th Percentile >9 tsp (>47.1 g) (N=258)*
Men				
Body mass index	24.42±4.07	24.38±3.99	24.01±4.16	24.39±4.54
Waist circumference	87.32±10.77	86.90±12.66	84.93±10.99	85.81±12.68
Waist-hip ratio	0.94±0.180	0.94±0.157	0.92±0.07	0.91±0.08
Blood pressure				
Diastolic	139.74±22.17	138.08±20.37	139.04±20.57	142.89±23.68
Systolic [a]	72.55±12.07 [β]	74.47±12.81	74.18±12.19	77.40±13.8 [β]
Fasting blood sugar	6.43±2.30	6.46±2.70	6.22±2.29	6.24±2.22
Total cholesterol [a]	4.99±1.35 [β]	5.23±1.12	5.36±1.26	5.49±1.19 [β]
LDL-c [a]	2.95±1.20 [β]	3.15±1.01	3.32±1.14	3.44±1.06 [β]
HDL-c	1.33±0.36	1.33±0.44	1.31±0.35	1.27±0.30
Triglycerides	1.51±0.82	1.65±0.95	1.59±0.90	1.71±0.87
TC:HDL [a]	3.95±1.33 [β]	4.20±1.28	4.27±1.27	4.49±1.28 [β]
Women				
Body mass index	25.02±4.80	24.77±5.61	24.76±4.54	26.30±4.76
Waist circumference	82.27±11.76	83.43±14.57	82.54±11.82	83.05±13.38
Waist-hip ratio	0.87±0.102	0.88±0.09	0.87±0.08	0.87±0.10
Blood pressure				
Diastolic [b]	141.35±22.51	135.78±21.36	136.59±20.15[β]	142.53±22.86[β]
Systolic	72.07±13.24	71.30±12.13	72.86±13.78	73.61±10.36
Fasting blood sugar	6.28±2.36	6.15±2.26	5.99±1.98	6.24±2.73
Total cholesterol [b]	5.24±1.03 [βρ]	5.43±0.95	5.61±1.04 [β]	5.85±1.11 [ρ]
LDL-c [b]	3.06±0.95 [βρ]	3.28±0.91	3.43±0.99 [β]	3.57±1.00 [ρ]
HDL-c	1.47±0.33	1.49±0.34	1.53±0.36	1.51±0.39
Triglycerides	1.54±0.79	1.48±0.67	1.50±0.81	1.74±0.87
TC:HDL	3.70±0.94	3.86±1.18	3.85±1.06	4.06±1.05

[a] denoted significant at two-tailed One Way Anova for continuous independent variable for men
[b] denoted significant at two-tailed One Way Anova for continuous independent variable for women
[βρ] showed significant using Tukey post-hoc for more than two continuous independent variable
The unit used for BMI – kg/m^2, WC – cm, BP – mmHg, FBS, TC, LDL-c, HDL-c, TG – mmol/L

Binary logistic regression results in Table 4 showed that the risk of high cholesterol increased two-folds for added sugar intake at 100th percentile [adjOR 2.07 (95% CI 1.40-3.07) (p<0.001)]. Similarly, the risk of high triglyceride was increased by 1.8 fold for added sugar intake at 100th percentile [adjOR 1.80 (95% CI 1.21-2.68) (p<0.001)]. Further, high total sugar intake (added + natural sugar) at 50th percentile [adjOR 0.68 (95% CI 0.48-0.98) (p<0.05)] increased the blood pressure by 0.68 fold. The total sugar intake at 50th percentile [adjOR 1.69 (95% CI 1.17-2.44) (p<0.01)], at 75th percentile [adjOR 1.48 (95% CI 1.02-2.13) (p<0.05)] and at 100th percentile [adjOR 2.28 (95% CI 1.55-3.36) (p<0.001)] also increased the risk of high total cholesterol level.

Table 4. Health risk associated with percentile of added and total sugar intake

Parameters	*25th percentile (<2 tsp [a]/ <4 tsp [b])*	*50th Percentile (2-5 tsp [a]/ 4-6 tsp [b])*	*75th Percentile (5-9 tsp [a]/ 6-9 tsp [b])*	*100th Percentile (>9 tsp [ab])*
Added sugar				
Overweight	1.0	1.01 (0.63-1.61)	0.62 (0.38-1.01)	1.22 (0.71-2.11)
Abdominal obesity	1.0	1.08 (0.75-1.54)	1.07 (0.73-1.55)	0.97 (0.64-1.47)
High blood pressure	1.0	0.72 (0.46-1.12)	0.78 (0.49-1.25)	0.86 (0.53-1.41)
High fasting blood sugar	1.0	0.59 (0.25-1.34)	0.45 (0.17-1.10)	0.43 (0.17-1.12)
High total cholesterol	1.0	1.38 (0.96-1.99)	1.45 (0.99-2.10)	2.07 (1.40-3.07)***
High LDL-c	1.0	1.11 (0.74-1.66)	1.42 (0.92-2.19)	1.44 (0.92-2.24)
Low HDL-c	1.0	1.51 (0.88-2.60)	0.88 (0.49-1.60)	1.06 (0.60-1.87)
High triglyceride	1.0	1.44 (0.98-2.11)	1.45 (0.98-2.13)	1.80 (1.21-2.68)***
Total sugar				
Overweight	1.0	0.80 (0.50-1.29)	0.65 (0.40-1.08)	0.92 (0.53-1.60)
Abdominal obesity	1.0	1.18 (0.77-1.83)	1.25 (0.79-1.97)	1.05 (0.62-1.77)
High blood pressure	1.0	0.68 (0.48-0.98)*	0.78 (0.53-1.14)	0.98 (0.66-1.45)
High fasting blood sugar	1.0	1.04 (0.72-1.52)	0.88 (0.61-1.29)	0.85 (0.57-1.25)
High total cholesterol	1.0	1.69 (1.17-2.44)**	1.48 (1.02-2.13)*	2.28 (1.55-3.36)***
High LDL-c	1.0	1.21 (0.80-1.81)	1.26 (0.83-1.93)	1.45 (0.94-2.25)
Low HDL-c	1.0	1.02 (0.60-1.71)	0.77 (0.45-1.33)	0.73 (0.43-1.24)
High triglyceride	1.0	0.92 (0.63-1.33)	0.96 (0.66-1.40)	1.38 (0.95-2.01)

[a] denoted for percentile of added sugar intake
[b] denoted for percentile of total sugar intake
*$p<0.05$; **$p<0.01$; ***$p<0.001$ significant using binary logistic regression (adjusted for age, gender, ethnicity and medication – for biochemical and clinical parameters)

DISCUSSION

This study found that almost 40% of multi-ethnic Malaysian older adults had MetSyn as assessed using the harmonised criteria. The harmonised definition was used as it does not include abdominal obesity as a mandatory criterion, but instead it captures a wider scope of MetSyn by including three or more risk factor i.e. abdominal obesity, high fasting blood sugar, hypertension, low HDL-c or high triglyceride.

The prevalence of MetSyn using IDF in this study was 30.9%. This findings were lower compared to other studies on Malaysian older adults from low cost housing areas (Johari and Shahar 2014), and adults ≥40 years (Rampal *et al.*, 2012) of 43.4% and 44.6% respectively using the same definition. The differences may be due to the fact that IDF definitions used waist circumference as compulsory and the percentages of respondents having abdominal obesity in respective studies might be higher compared to this study. However, both studies did not provide the information on the percentage of respondents who had abdominal obesity hence; it was difficult to distinguish the main component that contributes to MetSyn.

The figure was also lower compared to prevalence of MetSyn among adults in Malaysia (37.1%) (Mohamud *et al.*, 2011) but higher than adults in other Asian countries i.e. China (18.2%) (Liu *et al.*, 2013), Nepal (22.5%) (Sharma *et al.*, 2011) and India (25.8%) (Deepa *et al.*, 2007) using IDF definition. Older adults have a higher risk of having

MetSyn compared to younger adults as aging increased risk of cardiovascular or coronary diseases (Lind *et al.,* 2018). However, there was still a paucity of studies regarding the prevalence of MetS in older person because it is known that different age groups have different body compositions and body fat is increasing while muscles decreasing at a certain age (Denys *et al.,* 2009). Using the IDF definition the prevalence of MetSyn among older adults in China was comparable (30.5%) (He *et al.*, 2006).

The prevalence of MetSyn in this current study was higher among women compared to men. This finding was similar from a study from Johari & Shahar (2014). Women were at higher risk of having abdominal obesity than men especially with increase in age (Wang *et al.*, 2010). Besides that, Indians showed the highest prevalence of MetSyn compared to other ethnic groups. This was postulated to be associated with environmental and genetic factors (Mohamud *et al.,* 2011).

The mean sugar intake by the older adults in this study was 40 g (8 tsp) which is comparable to MANS study among Malaysian adults (37 g or 7 tsp) (Norimah *et al.*, 2008). Men consumed higher amount of sugar (40 g) compared to women (26 g) probably due to bigger body size and higher daily energy requirements. The sources of sugar that were most consumed among respondents were sweetened beverages which included added sugar and sweetened condensed milk that were mixed in tea and coffee and also from traditional *kuih*.

The results indicated that sugar intake showed no association with body mass index, waist circumference, hip circumference and waist-hip ratio. This could be due to obesity is having multi-factorial etiology involving genetic and environment factors (Hu, 2013).

In this study, sugar consumption was found to be associated with blood pressures and lipid profiles. Blood pressure increased by 0.68 folds when the total sugar intake at 75th percentile (5-9 tsp). A direct association between intake of sugar-sweetened beverages or fructose and blood pressure was consistent which showed that from animal data indicate direct pressor effects of glucose, fructose, and sucrose on BP (Brown *et al.,* 2011). The relations between sugar consumption especially in fructose-form sugar may escalated blood pressure through few possible mechanisms (Cohen *et al.,* 2012) which were increase level of serum uric acid that further cause for smooth muscle to constrict; increase sodium absorption in gut making more salt retention in the body; activation of vasoconstrictor and deactivation of vasodilator of vascular; and stimulate the sympathetic nervous system that eventually increase the blood pressure. Also a prospective study by Te Morenga *et al.* (2014) reported an association between sugar consumption (for eight weeks) with blood pressure which possible association between adiposity (from extra caloric from sugar consumption) and both lipid and blood pressure. Excessive sugar consumption may lead to insulin resistance, impaired glucose tolerance and diabetes mellitus (Ferrier *et al.,* 2014).

Sugar intake appears to be associated with increased triglyceride levels, however, relative to the other effects towards high-density lipoprotein and low-density lipoprotein levels which remain unclear (Johnson *et al.,* 2009). This study demonstrated that the risk of TC was increased with increment of total sugar consumption at percentile 50th, 75th and 100th by 1.69 fold, 1.48 fold and 2.28 fold respectively. In addition, added sugar consumption at 100th percentile (>47 g/>9 tsp) also increased the risk of

high cholesterol and high triglyceride by 2.07 folds and 1.80 folds respectively. The metabolism of excessive sugar consumption are stored in liver and muscle as glycogen and when it is full it will be stored in adipocyte as fatty acids (Ferrier *et al.,* 2014). Lipogenesis is the process of synthesising fatty acids from other source than fat such as simple sugars from acetyl-coA metabolism. Further, a systematic review done by Te Morenga *et al*. (2014) proved that excessive intake of sugar can increased the level of TG, TC, LDL-c and reduce HDL-c significantly, was observed from studies conducted more than five years and involving large sample size.

This study found a lack of significant association between excessive intake of sugar and risk of MetSyn. This could be due to the cross-sectional study design and small sample size. Other studies conducted over a longer duration and using larger sample size had shown significant association between sugar intake and MetSyn (Palmer *et al.,* 2008). MetSyn is a complex interaction with a multifactorial combination involving biochemical, physiology, clinical, metabolic factors and environment factors (Kaur, 2014). Despite the limitation, this study provides information on sugar consumption among multi-ethnic Malaysian older adults and its effect on selected blood markers.

CONCLUSION

This study showed no significant association between excessive sugar consumption and MetSyn among older adults. However, a higher sugar intake was associated with high blood pressure and undesirable lipid profile. The effects of long term excessive consumption of sugar on health outcomes in older persons should be investigated.

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Authors' contributions

NurZetty Sofia Z, led the data collection, conducted the study, data analysis and interpretation, prepared the draft of the manuscript and reviewed the manuscript; Suzana S, principal investigator, conceptualized and design the study, advised on data analysis and interpretation and reviewed the manuscript; Nik Shanita S, advised on sugar intake data analysis and reviewed the manuscript; Hasnah H, advised on sugar analysis food lab and reviewed the manuscript; Mohd Azahadi O, advised on data analysis and interpretation.

Conflict of interest

The authors have no conflict of interest to disclose in this work.

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Contributions of socio-demographic and psychosocial characteristics, functional status and physical activity level on prevalence of depressive symptoms among rural elderly in Johor state

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ABSTRACT

Introduction: Depression and depressive symptom are common among the elderly. This study aimed to determine the influence of multiple factors and their correlations on the prevalence of depressive symptoms among elderly residents in selected FELDA schemes in Johor state. **Methods:** A total of 269 respondents were recruited through systematic sampling. Face-to-face interviews were conducted to obtain information on socio-demographic and psychosocial characteristics using pre-tested validated questionnaires; For functional status, the Lawton-IADL Scale was used to assess independent living skills; the Short Physical Performance Battery (SPPB) questionnaire was used to assess physical performance; cognitive function was assessed by the Hodkinson Abbreviated Mental Test (HAMT); physical activity level was determined using the Rapid Assessment of Physical Activity (RAPA); and depressive symptoms were assessed by the Geriatric Depression Scale-15. **Results:** Mean age of the respondents was 69.5±5.2 years. Prevalence of depressive symptoms was determined as 3.7%. Almost half (47.6%) were unable to perform one or more Lawton-IADL items, 30.9% had low physical performance, 15.6% had abnormal cognitive function and only 30.6% were physically active. There were significant correlations between the socio-demographic characteristics (age and monthly income; r=-0.135 and r_s=-0.133 respectively; p<0.05), functional status and physical performance; r=-0.171 and r_s=-0.194 respectively; p<0.01), and prevalence of depressive symptoms. Low physical performance contributed towards having depressive symptoms (β=-0.183; p<0.05). **Conclusion:** A relatively low prevalence of depressive symptoms was found among the elderly living in FELDA schemes in Johor. Low levels of physical performance was contributed towards prevalence of depressive symptoms among the elderly.

Keywords: Socio-demographic, psychosocial, functional, physical activity, GDS-15

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INTRODUCTION

Worldwide, a total of 322 million people live with depression, out of which 27.0% live in South-East Asia (WHO, 2017). Among the South East Asian countries, the prevalence of depressive symptoms in Malaysia is the lowest at 16.5% (Vanoh *et al.*, 2016), when compared to Thailand with 28.5% (Haseen & Prasartkul, 2011) and Indonesia with 43.8% (Gustryanti, Thongpat & Maneerat, 2017).

According to the American Psychiatric Association (2017), an individual is considered to have depressive symptoms when feeling sad or having depressed mood, loss of interest or pleasure in activities once enjoyed, changed in appetite, trouble sleeping or sleeping too much, loss of energy or increased fatigue, increase in purposeless physical activity, feeling worthless or guilty, difficulty thinking, concentrating or making decisions and thoughts of death or suicide. These symptoms can be varied from mild to severe. Segal, Qualls & Smyer (2011) stated that psychosocial factors can trigger the onset of depression among the elderly. The common psychosocial factors that become a depression stressor are related to marital status (death of spouse or get divorce), and social integration or social involvement (Segal *et al.*, 2011). In China, high levels of depression among empty-nest elderly in the rural area of Yong Zhou was not only associated with lower income and negative coping style, but also with less social support and an increasing feeling of isolation and loneliness (Xie *et al.*, 2010).

Physical activity level is also associated with depressive symptoms. A higher level of physical activity in the elderly is correlated with less prevalence of depressive symptoms (Salguero *et al.*, 2010). The authors also stated that people who exercise three or more times a week for 20 mins possess a better health-related quality of life. They found a reduction in depressive symptoms in more active institutionalised and community-dwelling elderly. Lee, Suzana & Chin (2011) also reported that elderly who exercise less have a higher risk of having depressive symptoms.

One of the urbanisation development programmes of the government of Malaysia is the Federal Land Development Authority (FELDA) schemes. There are 323 FELDA schemes in the country, most of which are located in Peninsular Malaysia. Urbanization is associated with economic, demographic, social and psychological effects (Noreen Noor, Wan Haslin Aziah & Nur Adilah, 2012). Chen, Chen & Pierre (2017) stated that urbanization might cause the psychological distress and mental disorders and will worsen the diseases. This study was conducted to determine the prevalence of depressive symptoms, and the contribution of socio-demographic and psychosocial factors, functional status and physical activity level as well as their correlations with depressive symptoms among elderly residents living in selected FELDA schemes in Johor state.

MATERIALS AND METHODS

This was a cross-sectional study in three FELDA schemes in Johor. Johor state was selected by simple random sampling, and three FELDA schemes namely, FELDA Bukit Batu, FELDA Air Tawar 4 and FELDA Air Tawar 5 were selected using the probability proportionate to size (PPS) sampling method (Aday & Cornelius, 2006). The number of subjects in each FELDA was calculated based on the proportion method. The name lists in each selected FELDA scheme were obtained from FELDA administrator and the subjects were chosen based on the systematic sampling method. A total of 269 respondents were recruited.

Malaysian elderly who were aged 60 years and above, and able to communicate in Malay were included in this study. Elderly who were bedridden, blind and had stayed in the scheme for less than six months were excluded. This study was approved by the Research Ethics Committee of Research Management Centre (RMC), Universiti Putra Malaysia and FELDA Head Quarters. Data collection was conducted from September 2014 to March 2015.

Instruments and data collection

Data were collected through face-to-face interviews based on a set of pre-tested questionnaires. The respondents were briefed about the study before written consent was obtained. Socio-demographic background (sex, age, education level, monthly income and financial dependency) and psychosocial characteristics (marital status and living arrangements) were adapted from the questionnaire used by Siti Nur 'Asyura *et al.* (2009). For functional status, the Lawton-IADL Scale was used to assess the independent living skills and to identify how the person functions at the present time. A score of 7 or less indicates that disability of respondents to do one or more items in the scale (Hesseberg *et al.*, 2013). The Cronbach's α value to test the reliability of the questionnaire was 0.80 (Tengku Aizan *et al.*, 2013).

The Short Physical Performance Battery (SPPB) (Guralnik *et al.*, 1994) questionnaire was used to assess the physical performance of the respondents. A total score of 0-6 indicates low physical performance, 7-9 (intermediate performance) and 10-12 (high performance) (Cruz-Jentloft *et al.*, 2010). The Cronbach's α value to test-retest reliability of the questionnaire was 0.89 (Freire *et al.*, 2012). The Hodkinson Abbreviated Mental Test (HAMT) was used to assess the cognitive function of the respondents. An abnormal cognitive function was set at scores of 7 or less (Swain & Nightingale, 1997). In this study, the Cronbach's α value to test the reliability of the questionnaire is 0.72. The Geriatric Depression Scale-15 (Sheikh & Yesavage, 1986) was used to assess the depressive symptoms with scores ranging from 5 to 15 suggesting the presence of depressive symptoms. Nyunt *et al.* (2009) stated that the Cronbach's α value to test the reliability of the questionnaire was 0.80.

The Rapid Assessment of Physical Activity (RAPA) was used to assess the physical activity level. This test consists of Part 1 (physical activity level) and Part 2 (flexibility). In this study, only Part 1 was assessed which refer to the objective in which to assess the physical activity level of the respondents. The scoring based on the number of questions in which 1 indicates that the subject is sedentary, 2 (under-active), 3 (under-active regular light activities), 4-5 (under-active regular) and 6-7 (active).

Pre-testing of the questionnaires was undertaken on 28 elderly in FELDA Taib Andak who fulfilled the inclusion and exclusion criteria. The instruments were modified based on the feedback from the pre-test.

Statistical analysis

The data obtained from the real data collection session were analysed using IBM SPSS Statistics version 22.0 (IBM Corp., USA). The categorical data of socio-demographic characteristics, functional status characteristics, physical activity level and depressive symptoms (using the GDS-15 score) were analysed for descriptive statistics. The Chi-square test was performed for determining associatiosn between two categorical data, while the Pearson product moment correlation and Spearman rank order correlation test was used to determine the correlation between continuous data. Multiple linear regression was used

to determine the factors contributing to depressive symptoms among the respondents. The significant level was set at $p<0.05$.

RESULTS

The mean age of the respondents comprising 130 men and 139 women, was 69.5±2 years. The majority of the respondents were married (77.0%). Almost all the respondents were living with their spouse or other family members. Most of them (86.2%) had formal education (primary/secondary school), with more men having received formal education, compared to women (Table 1). Overall, the mean monthly income was RM1673.99±870.95[†], with men having a higher income than women. About two-thirds of the respondents considered themselves as financially independent especially among the men. The female respondents reported receiving money from their spouse, children and other family members.

For the functional status characteristics, Table 1 shows that about half of the respondents were completely dependent on performing activities of daily living. More men were independent in performing items in the Lawton-IADL scale compared to women. Based on the score on the SPPB questionnaire for physical performance, a total of 30.9% of respondents had low performance and 23.4% had high performance. The same pattern is seen more noticeably among the women. For cognitive function status, 15.6% of the respondents were classified as having an abnormal cognitive function. In term of the physical activity level, a majority of the respondents were classified as underactive. Only 30.6% were classified as active based on the scoring in RAPA questionnaire. Overall, the prevalence of depressive symptoms among the respondents was 3.7%, with 3.1% and 4.3% in men and women, respectively.

Table 2 indicates the association between the socio-demographic characteristics (sex, educational level and financial dependency), psychosocial characteristics (marital status and living arrangement) and the presence of depressive symptoms. No significant associations were found between these variables.

The correlation between the socio-demographic characteristics (age and monthly income), functional status (daily living activity dependency, physical performance and cognitive function) and physical activity level with GDS-15 score are shown in Table 3. Age (r=-0.135) and monthly income (r=-0.133) were found to have a significant negative correlation with the GDS-15 score ($p<0.05$). The Lawton-IADL and SPPB score also indicated a significant correlation with the GDS-15 score in a negative direction with r=-0.171 and r_s=-0.194, respectively ($p<0.01$). This suggests that the higher the Lawton-IADL score, the lower the GDS-15 score and vice versa. This pattern was similar for the SPPB score. No significant correlation was recorded between the RAPA score and GDS-15 score (r=-0.120), and between HAMT score (r=-0.041) with GDS-15 score.

The model of factors contributing towards depressive symptoms among the respondents is shown in Table 4. Physical performance contributed 18.3% towards depressive symptoms while monthly income contributed 12.5% towards depressive symptoms. In general, the model is useful to predict the contributing factor towards depressive symtpoms by 5.2%.

DISCUSSION

A higher prevalence of the men possessed a formal education and were financially independent compared to the women. Norisma Aiza, Jariah & Zumilah (2015)

[†]1 MYR (RM) was equivalent to 0.25 US Dollar (USD) at the time of data collection

Table 1. Descriptive findings of the socio-demographic characteristics, psychosocial characteristics, functional status characteristics, physical activity level and the prevalence of depressive symptoms according to sex[†, ‡]

Characteristics	*Men (n=130)*	*Women (n=139)*	*Total (n=269)*
Socio-demographic characteristics			
Age group			
60-74 years old	96 (73.8)	123 (88.5)	219 (81.4)
≥75 years old	34 (26.2)	16 (11.5)	50 (18.6)
Education level			
No/informal education	7 (5.4)	30 (21.6)	37 (13.8)
Formal education	123 (94.6)[*]	109 (78.4)	232 (86.2)
Monthly income (RM); mean±SD	2063.11±894.55[*]	1310.07±670.12	1673.99±870.95
Financial dependency			
Dependent	4 (3.1)	65 (46.8)	69 (25.7)
Independent	126 (96.9)[*]	74 (53.2)	200 (74.3)
Psychosocial characteristics			
Marital status			
Unmarried	7 (5.4)	55 (39.6)	62 (23.0)
Married	123 (94.6)[*]	84 (60.4)	207 (77.0)
Living arrangement			
Alone	0 (0.0)	8 (5.8)	8 (3.0)
With others	130 (100.0)[*]	131 (94.2)	261 (97.0)
Functional status characteristics			
IADL			
Dependent	40 (30.8)	88 (63.3)	128 (47.6)
Independent	90 (69.2)[*]	51 (36.7)	141 (52.4)[*]
Physical performance			
Low performance	36 (27.7)	47 (33.8)	83 (30.9)
Intermediate performance	54 (41.5)	69 (49.6)	123 (45.7)
High performance	40 (30.8)	23 (16.6)	63 (23.4)
Cognitive function			
Abnormal	16 (12.3)	26 (18.7)	42 (15.6)
Normal	114 (87.7)	113 (81.3)	227 (84.4)
Physical activity level			
Sedentary	2 (1.5)	1 (0.7)	3 (1.1)
Underactive	15 (11.5)	9 (6.5)	24 (8.9)
Underactive-regular light	13 (10.0)	21 (15.1)	34 (12.6)
Underactive-regular	57 (43.9)	69 (49.6)	126 (46.8)
Active	43 (33.1)	39 (28.1)	82 (30.6)
Depressive symptoms			
Presence	4 (3.1)	6 (4.3)	10 (3.7)
Absence	126 (96.9)	133 (95.7)	259 (96.3)

[†]Pearson Chi-square test was used to determine the association between socio-demographic, psychosocial, functional status, physical activity level and depressive symptoms with sex
[‡]Independent sample *t*-test was used to determine differences in mean of monthly income with sex
[*]*p*-value is significant at the 0.05 level (2-tailed)

Table 2. Association between socio-demographic and psychosocial characteristics with depressive symptoms†

Characteristics	*Total (n=269)*		*Chi-square value (df)*	*p-value*
	Absence of DS (GDS <5; n=259)	*Presence of DS (GDS ≥5; n=10)*		
Socio-demographic characteristics				
Sex				
Men	126 (96.9)	4 (3.1)	0.591	0.42
Women	133 (95.7)	6 (4.3)		
Educational level				
No/informal education	36 (97.3)	1 (2.7)	0.123	0.59
Formal education	223 (96.1)	9 (3.9)		
Financial dependency				
Yes	194 (97.0)	6 (3.0)	1.120	0.24
No	65 (94.2)	4 (5.8)		
Psychosocial characteristics				
Marital status				
Unmarried	60 (96.8)	2 (3.2)	0.054	0.58
Married	199 (96.1)	8 (7.7)		
Living arrangement				
Alone	60 (96.8)	0 (0.0)	0.318	0.74
Live with others	199 (96.1)	10 (3.8)		

†Pearson Chi-square test was used to determine association between socio-demographic and psychosocial characteristics with depressive symptoms

reported that that as older women did not receive any income, they were more vulnerable to poverty in their old age.

More men were married compared to women and all of them were staying with others. This was in line with the study by Lim & Kua (2011) who found that elderly women were more likely to live alone compare to elderly men. Some of the respondents lived alone as their children had migrated to the city for work and they did not feel comfortable living with their children in the city.

The prevalence of depressive symptoms among the respondents in this study was lower compared to that reported by Rashid *et al.* (2012), Norhayati *et al.* (2013) and Vanoh *et al.* (2016), based on the same instrument (GDS-15). The lower prevalence of depressive symptoms in this study might be due to the absence of factors that can contribute towards the occurrence of depressive symptoms. Most of the respondents reported that they did not have any problems, they appeared happy, and not worried about life issues.

No associations between sex, education level, financial dependency, marital status and living arrangements with occurrence of depressive symptoms, and this is comparable to the result of Rajkumar *et al.* (2009). The negative correlation between age and depressive symptoms which indicates younger elderly were associated with the presence of depressive symptoms, may suggest that the younger age elderly may be more unsatisfied with their life conditions. The current economic issues such as an increase in living costs requires them to work, which in turn, might have an impact on their life as they feel stressed and pressured. More studies should be undertaken to confirm this finding.

Monthly income in this study was significantly correlated with having depressive symptoms. Some respondents

Table 3. Correlation between the socio-demographic characteristics, functional status and physical activity level with the GDS-15

Characteristics	*Total (n=269)*	
	r-value / rho-value	*p-value*
Socio-demographic characteristics		
Age	-0.135	0.027*
Monthly Income	-0.133	0.029*
Functional status characteristics		
Instrumental activity of daily living	-0.171	0.005**
Physical performance	-0.194	0.001**
Cognitive function	-0.041	0.499
Physical activity level		
Rapid assessment of physical activity	-0.120	0.050

†Pearson product moment correlation test was used to determine correlation between age, IADL score, HAMT score and RAPA score with GDS-15 score
‡Spearman rank order correlation test was used to determine the correlation between the monthly income and the SPPB score with the GDS-15 score
*p-value is significant at the 0.05 level (2-tailed)
**p-value is significant at the 0.01 level (2-tailed)

Table 4. Contributory factors of depressive symptoms among the respondents

Model	*F*	*Unstandardized Coefficients*		*Standardized Coefficients*	R^2	ΔR^2	*Sig. (p-value)*	*Durbin-Watson (d) value*	*Collinearity Statistics*	
		B	*Std. Error*	β					*Tolerance*	*VIF*
	7.234				0.052		0.001	2.069		
(Constant)		2.260	0.340				0.000			
Physical Performance		-0.116	0.038	-0.183		0.036	0.002		0.997	1.003
Monthly Income		0.00	0.000	-0.125		0.015	0.038		0.997	1.003

reportedly faced financial problems as the salary was not sufficient to support their family expenses, and they had to take up extra work, such as being factory security guards of taxi drivers. Studies by Rashid *et al.* (2012) and Yaka *et al.* (2014) also reported that elderly who were unemployed or had low income faced a higher risk of having depressive symptoms. .

Our study is in line with Garber *et al.* (2010) in finding significant association between physical function among community-dwelling elderly and several physical and mental health-related factors. The elderly with positive depressive symptoms had a significantly higher prevalence of functional limitations. Since falling is strongly associated with depressive symptoms, the respondents who move slower due to the fear of falling tend to have positive depressive symptoms (Santos *et al.*, 2012).

The study by Ciucurel & Iconaru (2012) who found that exercise reduced the reactivity to stress and optimise the respondents in coping with stress, while sedentarism acts as a depression risk factor. Endorphins hormones released during exercise act as analgesic and sedative that can alleviate the symptoms

of depression (Tan & Yadav, 2012). In the FELDA setting, there is an integrated weekly exercise programme organised by the FELDA management and the Ministry of Health Malaysia, called the 10,000 steps (*10,000 Langkah*). In this programme, the participants are required to walk 10,000 steps based on the route given. In addition, there are also '*gotong-royong*' activities involving the settlers in each block cleaning up their block own area. According to the respondents in this study, most of them joined these activities as it is a platform to meet friends besides getting physically active.

Our findings highlight the importance of physical performance as the contributing factor towards depressive symptoms. This result is in line with the study by Santos *et al.* (2012), reported that as an individual becomed older, they often experience a decrease in the activity related to motor performance, suc as balancing, mobility and gait, and also tend to move slower due to risk of falling and both of which are strongly associated with depressive symptoms.

CONCLUSION

This study found a relatively low prevalence (3.7%) of depressive symptoms among elderly living in selected FELDA schemes in Johor. Residents of the FELDA settings are supported by community social activities and with access to health care services. Only a few factors were found to impinge on the occurrence of depressive symptoms in the study population. These include socio-economic factors (lower income), functional status (with disabilities) and low physical performance.

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Authors' contributions

Nur Aqlili Riana H conceptualized and designed the study, led the data collection, data analysis, prepared and reviewed the manuscript; Siti Nur 'Asyura A conceptualized and designed the study, adviced on data analysis, data interpretation, assisted and reviewed manuscript; Mohd Nasir MT adviced on study methodology and adviced on data analysis; Chan YM conceptualized and designed the study, adviced on data collection, data analysis and interpretation and reviewed the manuscript; Zuriati I conceptualized and designed the study and Syafinas A assisted in data collection, data analysis and interpretation.

Conflict of interest

There is no conflict of interest to declare in this paper.

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Correlations between glycaemic control and serum chromium levels among type 2 diabetic patients in Denpasar, Bali

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ABSTRACT

Introduction: The National Basic Health Research (Riskesdas) in 2013 showed 6.9% diabetes prevalence in Indonesia with the highest among aged 55 years and above in urban areas. Poor glycaemic control is reported to be related to low chromium levels in type 2 diabetes mellitus (T2DM). This study aimed to determine the correlation between serum chromium and glycaemic control in T2DM patients. **Methods:** A cross-sectional study was conducted at six community health centres (Puskesmas) in Denpasar, Bali in July 2015-Jan 2016. A total of 165 T2DM patients who met the inclusion criteria were included. The subjects were aged 50-70 years, registered in the Chronic Diseases Management Programme (Prolanis), members of diabetic health clubs in the Puskesmas, and were taking oral hypoglycaemic medication. Anthropometric measurements were taken, including weight, height and waist circumference. Fasting blood samples were collected for determination of glycated haemoglobin (HbA1c) using HPLC, blood glucose (FBG) by tipyrine (GOD-PAP) enzymatic colorimetric method, and serum chromium using atomic absorption spectrophotometry (AAS). Correlations between HbA1c and FBG with serum chromium were determined using Spearman Correlation test (95% CI). **Results:** There was a significant negative correlation between FBG levels and serum chromium (r=-0.813; p<0.001); while no significant correlation was found between HbA1c and serum chromium (r=-0.059; p>0.05). **Conclusion:** Serum chromium levels of T2DM patients in this study were low, while their FBG levels correlated negatively with serum chromium status. Studies on a larger sample of T2DM patients should be undertaken to verify this finding for nutritional care of diabetic patients.

Keywords: Diabetes mellitus, fasting blood glucose, HbA1c, serum chromium

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is caused by a combination of genetic and lifestyle factors, such as sedentary lifestyle, high intake in carbohydrate and fat, and lack of physical activity (Hu, 2011). According to the International Diabetes Federation (IDF), Indonesia ranked seventh of the ten countries with the highest number of diabetes cases (8.5 million cases) in 2013 (IDF, 2013). The report also

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predicts that diabetes cases will reach 382 million cases globally by the year 2035. Within that figure, Indonesia will move into the sixth rank for the highest diabetes cases in the world (IDF, 2013). Based on Indonesian National Basic Health Research (RISKESDAS) in 2013, the prevalence of diabetes in Indonesia increased steadily from 5.7% in 2007 to 6.9% in 2013 with all provinces showing the same trend of increase (Balitbangkes, 2013). The prevalence was higher among women over the age of 55 years and in urban areas (Balitbangkes, 2007; Balitbangkes, 2013).

Based on the American Diabetes Association (ADA) and Indonesian Endocrinology Association (PERKENI) guidelines, there are four pillars of T2DM care; i.e. education, diet, physical activity and hypoglycaemic agent and/or insulin where necessary (ADA, 2011; PERKENI, 2015). Without proper management, patients with T2DM may suffer from repeated surge of blood glucose levels or having poor glycaemic control (Khunty*et al.*, 2012). Chronic poor glycaemic control may lead to various complications such as neuropathy, nephropathy, stroke, and retinopathy, which in turn affect the patient's quality of life (ADA, 2015).

Increasing evidence suggests that micronutrients (e.g. vitamin B3, vitamin D, magnesium, zinc, and chromium) play a role in glucose control and prevention of microvascular or macrovascular complications (Kelly & Dyson, 2011; Kaur & Henry, 2014). In the case of chromium, several studies have shown that T2DM patients have lower serum chromium compared to non-diabetic patients or the healthy population (Elabid & Ahmed, 2014; Hajra *et al.*, 2016; Nurohmi, 2017). Chromium is considered as a trace mineral that may help regulate carbohydrate metabolism, improve insulin action and help regulate blood glucose level (Bhanderi *et al.*, 2016; Bai *et al.*, 2015), as well as able to increase glucose uptake in diabetic patients by improving the regulation of glucose transporter 4 (GLUT4) (Hoffman *et al.*, 2014). However, there is limiting evidence associating diabetes and chromium status (Costello, Dwyer & Bailey, 2016).

This study was conducted to determine the correlation between serum chromium and glucose control in Indonesian T2DM patients in Bali.

MATERIALS AND METHODS

This cross-sectional study was undertaken in six community health centres (Puskesmas) in Denpasar, Bali. These sites were chosen purposively considering several factors, including that the Puskesmas has a diabetic club and agreed to provide its register of patients with T2DM. This registry was an integrated dataset of the Chronic Diseases Management Programme (Prolanis) and the National Health Insurance (BPJS Kesehatan). The study was conducted for six months in July 2015-January 2016.

The inclusion criteria for the study included patients aged 50-70 years, registered in the Prolanis registry in each Puskesmas, engaged in the diabetes club each week, taking diabetic medication (diet and anti-diabetic agent), and willing to participate in the study by signing the informed consent. The exclusion criteria were patients with complications (macrovascular and microvascular diseases) at the time of data collection based on medical diagnosis, and receiving insulin therapy. A total of 165 patients out of a total of 178 met the inclusion criteria. All were contacted by phone or door-to-door visits.

Body weight was measured with digital Camry step on weighing device with 0.1 kg precision, body height was measured with a microtoise tape with 0.1 cm precision, while waist circumference

was taken using a measuring tape with 0.1 cm precision.

Venous blood samples were taken after 10-12 hours fasting. HbA1c levels were measured by high performance liquid chromatography (HPLC) at the Prodia clinical laboratory, fasting blood glucose (FBG) was determined by GOD-PAP enzymatic colorimetric method in the Provincial Government Health Laboratory, while serum chromium was determined using atomic absorption spectrophotometer (AAS) in the Integrated Chemistry Laboratory of Udayana University in Denpasar.

Data were processed using Microsoft Excel and SPSS software. Correlations between glycaemic control and serum chromium were adetermined using Spearman correlation (95% CI, α=0.05). Analysis of covariance (ANCOVA) was performed to determine the association between serum chromium levels and glycaemic control variables (HbA1c and FBG). Chi-square test was employed to analyse the relationship between sex and the degree of glycaemic control.

Ethical clearance for the study was granted by Ethical Commission for Research of Faculty of Medicine, Udayana University/ Sanglah Hospital number 1439/UN.14.2/Litbang/2015.

RESULTS

Just over half of the subjects were male (55.8%) and the average age was 60 years (Table 1). The median duration of being

Table 1. Characteristics of subjects and nutritional status based on sex

Variable	*Male (n=92)*	*Female (n=73)*	*p-value*[†]	*All (n=165)*
Age (year), mean±SD	60.98±6.3	60.49±5.1	0.585	60.76±5.8
Diabetes duration, median (range)	2.0 (0.5-31.0)	4.0 (0.5-19.0)	0.344	3.0 (0.5-31.0)
≤5.0 years, *n* (%)	74 (80.4)	57 (78.1)		131 (79.4)
5.1-10 years, *n* (%)	12 (13.0)	10 (13.7)		22 (13.3)
>10 years, *n* (%)	6 (6.6)	6 (8.2)		12 (7.3)
BMI (kg/m^2), mean±SD	23.8±3.5	25.0±4.1	0. 043[*]	24.33±3.8
WC (cm), mean±SD	89.7±9.8	91.2±9.4	0.321	90.4±9.7

SD: Standard Deviation; BMI: Body Mass Index; WC: Waist Circumference
[†]Based on Independent samples *t*-test between male and female
[*]Significant at $p<0.05$

Table 2. HbA1c, blood glucose and serum chromium levels based on the degree of glycaemic control

Variable	*Good glycaemic control (n=81)*	*Poor glycaemic control (n=84)*
Sex		
Female, *n* (%)	34 (46.6)	39 (53.4)
Male, *n* (%)	47 (51.1)	45 (48.9)
HbA1c (%), median (range)	6.4 (5.3-6.9)	8.5 (7.0-15.5)
FBG (mg/dL), median (range)	119 (75-404)	185 (76-493)
Chromium (µg/L), median (range)	45.0 (1.0-75.0)	43.0 (3.0-84.0)

FBG: Fasting Blood Glucose; Good glycaemic control: HbA1c <7.0%; Poor glycaemic control: HbA1c ≥7.0%

Table 3. Bivariate analysis on subjects' serum chromium and glycaemic control†

Variable	*r-value*	*95% Confidence Interval (mean)*		*p-value*
		Lower	*Upper*	
HbA1c (%)	-0.059	7.5	8.2	0.454
FBG (mg/dL)	-0.813	158.0	183.6	0.000

†Glycaemic control: HbA1c and FBG levels; FBG: Fasting Blood Glucose

diagnosed with T2DM was three years, and most of the subjects were diagnosed of diabetes for less than five years. Based on BMI, 40% were overweight or obese and both sexes also showed central obesity.

The median of HbA1c and FBG were 8.5% and 185 mg/dL, respectively (Table 2). The median chromium level of patients with poor glycaemic control was lower than those with better glycaemic control (43.0 µg/L vs 45.0 µg/L).

No significant findings were found between glycaemic control (HbA1c level and FBG) and serum chromium concentrations (Table 3). Based on ANCOVA performed to determine the correlations between serum chromium levels to HbA1c and FBG levels, controlling for age, sex, WC and BMI values, a significant association between serum chromium and FBG levels was found (p=0.032; p<0.05), while no significant association was found between serum chromium and HbA1c levels (p=0.369).

DISCUSSION

Most of the subjects in this study showed high fasting glucose level (a median of 140 mg/dL), indicating that they had poor glycaemic control. Patients were defined as having poor glycaemic control if their HbA1c levels were higher than 7% or their FBG levels were higher than 130 mg/dL (ADA, 2015). Inadequate insulin secretion and high level of glucagon contributed to the increase in blood glucose. Therefore, some patients with T2DM might have impairment in their glucagon level, thereby increasing the hepatic glucose production which caused an increase in blood glucose level (Hædersdal *et al.*, 2018). Blood glucose surge could also be linked to the duration of diabetes (Chacko, 2016). Leibowitz, Kaiser & Cerasi (2011) stated that the duration of DM might progressively affect insulin secretion and would eventually cause β cell failure. What happened to the β cell might impair the response to diet and oral hypoglycaemic drug and cause impairment in glycaemic control. However, T2DM care should not only focus on managing glycaemic control to lower cardiovascular risk but also to manage weight, blood pressure, lipid profile and prevent hypoglycaemia (Fox *et al.*, 2015; ADA, 2015).

Subjects in this study were 50-70 years with the mean age of 60.8 years. RISKESDAS (2013) reported that the highest prevalence of diabetes in Indonesia was among 55 years and above (Balitbangkes, 2013). Likewise in the United States, the prevalence of diabetes increased with age (Kirkman *et al.*, 2012). Other studies reported prevalence of diabetes increased after the age of 60 years (Kirkman *et al.*, 2012; Kamuhabwa & Charles, 2014; Mihardja *et al.*, 2014). Elderly population has a higher risk of glucose tolerance impairment and diabetes mellitus, due to the decline in pancreatic function and the reduction in insulin sensitivity (Kirkman *et al.*, 2012).

Most of the subjects were found to have central obesity despite having

normal BMIs (18.5-24.9 kg/m^2). Hu (2011) stated that the prevalence of obesity based on BMI in Asia was relatively lower compared to Western populations. The prevalence of diabetes in Asia is higher compared to that in the US, although obesity prevalence based on the BMI in Asia is lower compared to the US (Yoon *et al.*, 2006; Hu, 2011). A review by Misra *et al.* (2014) reported that diabetic population in South Asia significantly had poorer glycaemic control compared to Caucasians.

Half of the subjects in this study had poor glycaemic control (HbA1c >7.0%). This proportion was lower than the result of Khattab *et al.* (2010), who reported that 65.1% of T2DM patients had poor glycaemic control. This study also showed that there was a wide range of HbA1c levels among the subjects (5.3-15.5%), which meant that there were patients who had very high HbA1c levels. Female subjects tend to show poor glycaemic control and this finding was in line with the finding of Kamuhabwa & Charles (2014). The duration of diabetes also linked to poor glycaemic control. The longer someone suffers from DM, the faster the progression of the β cell destructions and impairment of insulin secretion, which are related to impairment in insulin action (Kamuhabwa & Charles, 2014).

The median value of serum chromium of the study subjects was 45.0 μg/L. The mean serum chromium level of patients with poor glycaemic control was slightly lower than that of those with good glycaemic control (43.0 μg/L vs. 45.0 μg/L). The serum chromium levels of the subjects were not much different from our previous study on serum chromium levels of T2DM patients and non-diabetic patients in Denpasar City. The study indicated that serum chromium levels of T2DM patients were lower than non-diabetic patients (42.0 μg/L vs 93.0 μg/L) (Sutiari *et al.*, 2017). This finding was in agreement with various other studies (Hasan, Ismail & Aziz, 2012; Elabid & Ahmed, 2014; Rajendran *et al.*, 2015; Hajra *et al.*, 2016). Each of these studies presented a different range of serum chromium levels in T2DM patients. The result variation might be affected by the method used to analyse the serum chromium level and by dietary chromium intake.

The low chromium status of the subjects might be caused by an inadequate intake, based on the recommended intake by age. There has not been any suitable or appropriate reference that we can use to determine the criteria for low, normal, and high serum chromium status of the subjects. Thus, the low chromium status of the subjects was assessed based on the ratio of subjects' serum chromium levels to serum chromium levels of non-diabetic patients. Most of the subjects were elderly, thus the low chromium status might be the result of low intake and absorption of chromium from diets. Therefore, it is recommended to have a high intake of chromium from food and take chromium supplement in order to fulfil the body's chromium requirement. The chromium concentration tends to decrease at the age of 40 (Rajendran *et al.*, 2015). However, there are still no studies confirming the correlation between age and the decrease in chromium levels through the metabolism (Wang & Cefalu, 2010).

The finding here of a negative correlation between serum chromium and the subjects' FBG levels was in line with result of Rajendran *et al.* (2015), who also reported that well-controlled T2DM patients had low serum chromium levels. Serum chromium levels of diabetic patients were lower than non-diabetic patients and healthy population. The negative correlation indicate that chromium might have a positive impact in improving insulin

resistance and glycaemic control in T2DM (Wang & Cefalu, 2010). There was no association found between HbA1c and serum chromium levels, but there was an association between FBG and chromium levels. It can be explained that HbA1c is a reflection of long-term glycaemic control; i.e. reflection of mean FBG levels 8-12 weeks before (Pujar *et al.*, 2014). HbA1c level does not depend on fasting condition. It is different from the FBG level, which is the short-term glycaemic control factor that can be accurately measured when examined under fasting condition.

Correlation analysis showed that patients with poor glycaemic control tended to have low BMI but suffered from central obesity. This tendency can be explained as follows: when the patients have poor glycaemic control, it is easy for them to lose weight; however, they will gain weight if their glycaemic control improve. As for central obesity, it may cause insulin resistant; thereby worsening the glycaemic control (Kamuhabwa & Charles, 2014).

CONCLUSION

The main finding of this study was the negative correlation between serum chromium concentration and FBG levels among T2DM patients with reportedly good glycaemic control. Further research on a larger sample size should be undertaken to verify these results.

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Authors' contributions

Sutiari NK was in charge of data analysis and writing the manuscript; Rimbawan R and Purwantyastuti A contributed in writing the Discussion and Recommendations; Kusharto CM contributed in writing the Results; Effendi AT contributed in making the Discussion.

Conflict of interest

All authors contribute equally to this work and declare that there is no conflict of interest in the study and its results.

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Regional differences in obesity prevalence and associated factors among adults: Indonesia Basic Health Research 2007 and 2013

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ABSTRACT

Background: Obesity prevalence has increased worldwide. Based on the Indonesia Basic Health Research (BHR), the prevalence of obesity among adults rose from 10.3% in 2007 to 15.4% in 2013. This study is aimed at examining selected obesity-related factors among adults aged 15 years and above from different regions of Indonesia. **Methods:** The BHR data comprising of 664,196 adults from 258,366 households in 440 districts in 2007, and 722,329 adults from 294,959 households 497 districts were included in this analysis. Frequency intake of fatty, sweet and salty foods, and status of physical activity were assessed using a validated questionnaire developed for IBHR. Mental health status was assessed using WHO Self Reporting Questionnaire. Logistic regression was performed to assess the risk factors of obesity. **Results:** Overall, obesity prevalence was 9.2% in 2007 and 14.2% in 2013. Obesity prevalence was comparatively higher in all regions in 2013, ranging from 14.1% to 15.5% in the western and eastern regions respectively. In 2007, the most likely risk factor contributing to obesity in the western and middle regions was frequent consumption of fatty food (OR=1.26 and OR=1.38, respectively), while physical inactivity (OR=1.27) was the highest odds for obesity risk in the eastern region. In 2013, frequent fatty food consumption showed the highest influence on obesity risk in all the regions. **Conclusion:** Risk factors for obesity in adults varied in different regions in Indonesia. Future research and interventions on obesity are recommended to focus on unhealthy dietary intake and lifestyles indifferent regions of Indonesia.

Keywords: Obesity, lifestyle trend, BMI, food consumption, physical activity

INTRODUCTION

According to WHO (2015), the prevalence of overweight and obesity globally have doubled since 1980, and it has reached epidemic levels. In United States, the prevalence of obesity increased dramatically from 22.6% (1996) to 40.2% (2014) and occurred dominantly in women (Ogden *et al.*, 2015). High BMI is associated with chronic diseases namely, cardiovascular diseases cancers, and chronic respiratory diseases. Besides, obesity also contributes to diabetes mellitus type 2. Roughly 50% of diabetic patients are obese (Abdelaal *et al.*, 2017). It is estimated that about 2.8 million mortality among adults occurs annually and is associated with overweight or

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obesity (Arojo & Osungbade, 2013).

Indonesia as a developing country also faces the obesity burden and this trend has been increasing annually. Based on the national survey of the Basic Health Research (BHR), the prevalence of obesity among Indonesian adults rose significantly from 10.3% in 2007 to 15.4% in 2013. Moreover, an increase on obesity prevalence significantly occurred in female adults, from 9.67% in 1993 to 19.64% in 2007 (Roemling & Qaim, 2012). The increase in obesity trend has resulted in high risk for morbidity and mortality among Indonesian adults. There has been a shift in the type of diseases that causes death among Indonesian adults, namely from infectious diseases to non-communicable diseases. Based on the five leading causes of mortality, four of five main causes of death are due to stroke, cancer, and diabetes mellitus (Moelok, 2017).

Fundamentally, obesity occurs due to an imbalance between intake and expenditure of calories. Excessive energy intake results in weight gain in the form of fat (Nestle & Nesheim, 2012). It is triggered by unhealthy behaviour, such as sedentary activity and unbalanced diet. The changes of lifestyle especially for people who live in urban areas are considered as an impact of westernisation in which people are encouraged to have unhealthy behaviours (Harrell *et al.*, 2015). Indeed, these behaviours might rise including in rural areas because the occurrence of inevitable nutrition transition (Khan & Talukder, 2013; Popkin, 2010).

Indonesia has three major regions with several provinces and hundreds of districts. There are main regions are the western, middle, and eastern part of Indonesia. The western part is mostly well developed because the capital city (Jakarta) is located in this region. While the middle part has been partially developed and others are growing, the eastern part is still largely left behind the other regions in terms of infrastructure and economic development. Because of its vastness, it leads to regional differences in characteristics (in terms of social, cultural and economic factors) which might result in different lifestyle patterns. It is hypothesised that obesity might be affected by the varying regional characteristics. This study aims to examine the differences of obesity prevalence and associated risk factors which might be varying among regions from 2007 to 2013.

MATERIALS AND METHODS

This study was a further analysis of Indonesia Basic Health Research (BHR) survey conducted in 2007 and 2013. Basic Health Research is a survey spearheaded by the Ministry of Health to describe and monitor the health condition of population in Indonesia. This survey included nutritional status component, a result of which is used in national policy-making.

The two datasets were obtained from the Research and Development of Health Agency, Ministry of Health. The samples of this study were individuals aged ≥15 years. The total number of samples in 2007 was 664,196 individuals from 258,366 households in 440 districts/cities. In 2013, a higher number of samples were shown, 722,329 individuals from 294,959 households spread over 497 districts. Data analysis was conducted from September 2016 to January 2017.

The characteristics of the respondent are presented by age, sex, education, occupation status, body mass index (BMI) value, waist circumference (WC), and smoking status. Education was divided into two categories, low (below secondary school) and high (secondary school and above). Occupation status was classified by employment and

unemployment (including student and housewife). Smoking status was categorised as smoking and not smoking. The dependent variable was obesity based on body mass index. According to Indonesian Ministry of Health, BMI ≥27 kg/m^2 was categorised as obese, while <27 kg/m^2 was normal weight (Hastuti *et al.*, 2017).

Weight, height, and WC measurements were collected with an anthropometric measurement kit from the Indonesian Ministry of Health. Independent variables including frequency consumption of fatty, sweet, and salty food, physical activity and mental health status were determined using the validated instrument of the BHR, 2013. Similar categories for these variables were also used in 2007, except for physical activity (PA) categorisation. For 2007, PA was categorised <150 minutes/day as low, ≥150 mins as high, while for 2013, PA was classified as low for sedentary activity of ≥five hours/day and as high PA for sedentary activity <five hours/day. The questions used to assess mental health status was translated from the Self-Reporting Questionnaire developed by WHO (Beusenberg *et al.*, 1994).

The two data sets were analysed separately. Univariate analyses were conducted to describe the participants' characteristics. Chi square test was used to determine the association between participants' characteristics and obesity status. Conditional logistic regression analysis was performed to examine the effect of single risk factor on obesity. Analysis is presented according to three regions of Indonesia namely, western (18 provinces), middle (11 provinces), and eastern (4 provinces). Data analysis was performed using SPSS software version 18 (IBM Corp., USA).

RESULTS

The characteristics of the respondents in 2007 and 2013 surveys are shown in Table 1. The mean of age of the respondents ranged between 38 to 39 years, with more than 51% females. A high proportion of the respondents in both years was from low socioeconomic status as characterised by occupational status (farmers 25.7% in 2007; 22.2% in 2013), unemployed (11.8%; 30.6%), and poor education level (27.8%; 29.0%). The percentage of unemployed was higher in 2013 (30.6%) compared to that in 2007 (11.8%).

Based on nutritional status, the mean BMI of the respondents were 22.00±3.69 kg/m^2 in 2007 and 22.77±4.18 kg/m^2 in 2013. However, their mean waist circumference was below normal at 76.42±11.28 cm in 2007 and 77.62±10.97 kg in 2013. The prevalence of obesity was higher in 2013 at 14.2% compared to 9.2% in 2007. Obesity prevalence was higher among the females and the unemployed respondents.

Figure 1 shows that in 2007, there were more areas /regions with prevalence of obesity considered as low namely, <9% and 10-19%. However, in 2013, there were less regions with obesity prevalence below 9%, while more regions were found with obesity prevalence >10-19%. The highest prevalence of obesity of 15.5% was in the eastern region namely, North Sulawesi and West Kalimantan. Overall, prevalence of adult obesity in all regions of Indonesia was higher in 2013 compared to that 2007.

The risk factors of obesity among Indonesian adults examined were (i) occupational status, (ii) consumption of fatty foods, salty food, and sweet food, (iii) physical activity, and (iv) mental health. Table 2 shows the results of the conditional logistic regression for

Table 1. Characteristics of respondents, 2003 and 2007

Variable	*2007 (N=664,196)*		*2013 (N=722,329)*	
	Mean±SD	*%*	*Mean±SD*	*%*
Age (years)	38.26±16.21		39.92±16.20	
Weight (kg)	53.88±10.15		55.91±11.23	
Height (cm)	156.39±8.07		156.69±8.47	
Body mass index (kg/m^2)	22.00±3.69		22.77±4.18	
Waist circumference (cm)	76.42±11.28		77.62±10.97	
National prevalence of obesity		9.2		14.2
Sex, female		51.9		51.8
Obesity by gender*				
Female		12.3		18.8
Male		5.9		9.2
Occupation, farmer		25.7		22.2
Occupation, unemployment		11.8		30.6
Obesity by occupation*				
Unemployment		10.3		15.1
Employment		8.5		13.5
Education, elementary school		27.8		29.0
Obesity by education*				
Low		8.3		12.7
High		11.7		17.3

*Chi-square test, significant at $p<0.001$

the odds ratios of these risk factors. Overall, unemployed status, compared to employed as a referent, showed the highest odds of adult obesity (OR=1.226 in 2007 vs OR=1.215 in 2013), followed by high frequency intake ("always"), compared to "seldom intake" of fatty foods (OR=1.21 in 2007 vs OR=1.141 in 2013,) and low physical activity (OR=1.197 in 2007 vs OR=1.126 in 2013).

Regional differences were found for adult obesity risks. In 2007, unemployment status ranked the highest risk for obesity in the western region (Sumatra & western Java) and middle region (central and eastern parts of Java including Bali). In 2013, unemployment remained the highest risk factor only in the western region. High frequency intake of fatty foods ranked among the higher risk factors in all the regions for both years. Low physical activity was also shown to be a high-risk factor of adult obesity in all regions especially in 2013.

Intake of salty and sweet foods were not shown to be risk factors of adult obesity in nationally and by regions in 2007 and 2013. Mental status (low versus normal) was also not found to contribute to adult obesity in Indonesia based on the BHR studied.

DISCUSSION

In general, the mean BMI and waist circumference of Indonesian adults remained within the normal range in 2007 and 2013. While the overall prevalence of adult obesity can be considered as relatively low compared to other countries, the level was higher in

Table 2. Conditional logistic regression analyses of obesity risk factors in 2007 and 2013

Variable (Referent variable)	*Western Indonesia*		*Middle Indonesia*		*Eastern Indonesia*		*Nation*	
	2007	*2013*	*2007*	*2013*	*2007*	*2013*	*2007*	*2013*
Occupation								
Unemployed (Employed)	1.237 (1.212 – 1.262)	1.245 (1.244 – 1.246)	1.281 (1.240 – 1.323)	1.133 (1.131 – 1.136)	1.129 (1.046 – 1.219)	0.856 (0.851 – 0.860)	1.226 (1.197 – 1.255)	1.215 (1.214 – 1.216)
Fatty food intake								
Always (Seldom)	1.210 (1.185 – 1.235)	1.104 (1.103 – 1.105)	1.250 (1.205 – 1.297)	1.290 (1.288 – 1.293)	1.126 (1.035 – 1.226)	1.382 (1.375 – 1.389)	1.217 (1.196 – 1.239)	1.141 (1.140 – 1.142)
Salty food intake								
Always (Seldom)	0.889 (0.872 – 0.907)	0.861 (0.861 – 0.862)	0.851 (0.822 –0.881)	0.943 (0.940 – 0.945)	0.975 (0.899 – 1.059)[§]	1.002 (0.996 – 1.008)[§]	0.886 (0.872 – 0.901)	0.879 (0.878 – 0.880)
Sweet food intake								
Always (Seldom)	0.871 (0.850 – 0.892)	0.886 (0.865 – 0.867)	1.139 (1.095 – 1.184)	1.129 (1.126 – 1.132)	1.126 (1.015 – 1.248)	1.057 (1.051 – 1.063)	0.950 (0.931 – 0.969)	0.911 (0.910 – 0.912)
Physical activity[†]								
Low (High)	1.231 (1.196 – 1.267)	1.114 (1.112 – 1.115)	1.196 (1.142 – 1.252	1.213 (1.210 – 1.216)	1.298 (1.166 – 1.445)	1.064 (1.058 – 1.071)	1.197 (1.169 – 1.225)	1.126 (1.125 – 1.127)
Mental health[‡]								
Low (Normal)	1.112 (1.078 – 1.146)	1.032 (1.030 – 1.034)	0.898 (0.853 – 0.945)	0.772 (0.768 – 0.776)	0.883 (0.772 – 1.009)[§]	0.877 (0.866 – 0.889)	1.040 (1.014 – 1.067)	0.984 (0.982 – 0.986)

[†]Cut-off: ≤150 minutes
[‡]Score cut-off: ≤6
[§]Not significant (p>0.05)

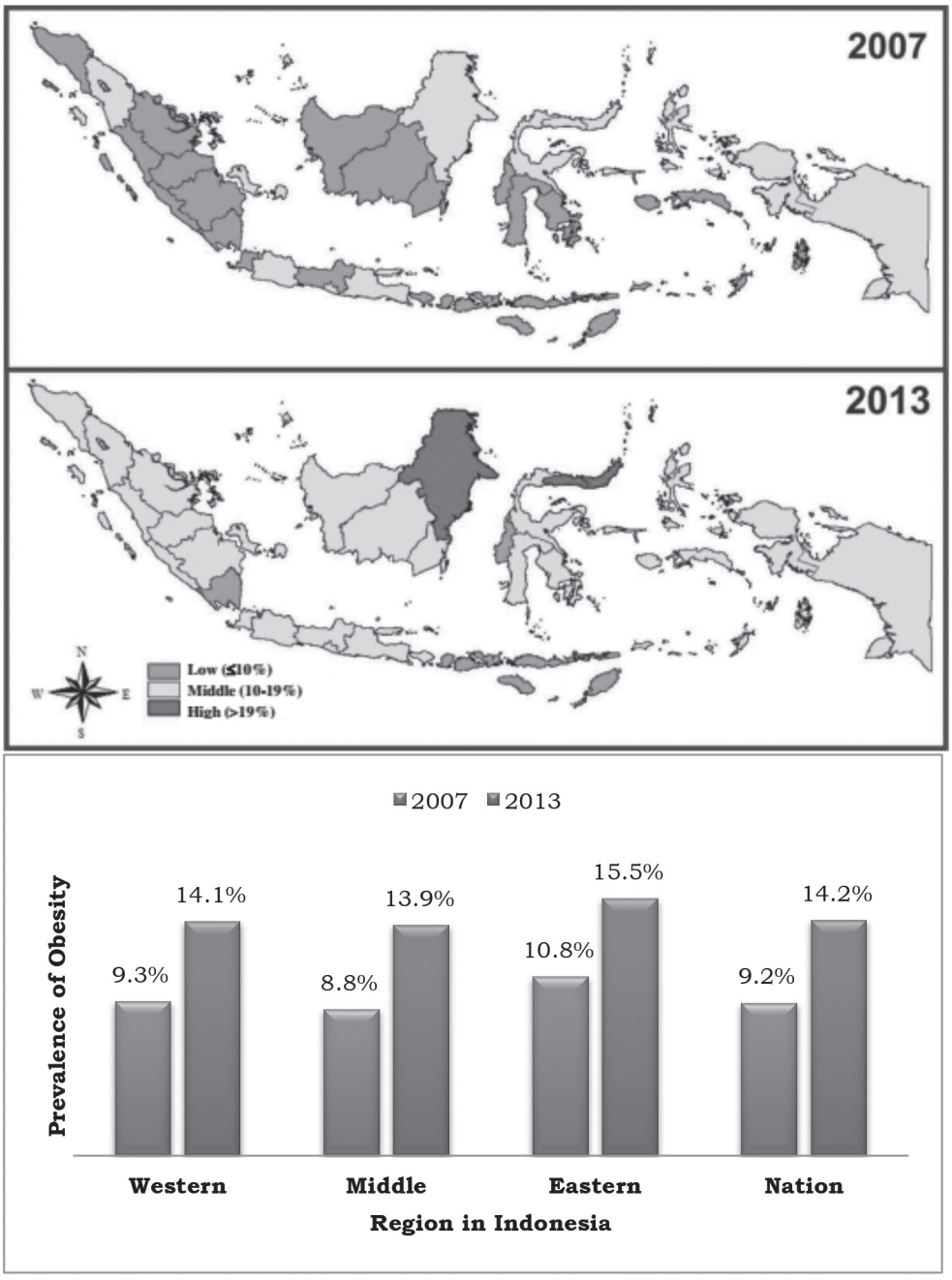

Figure 1. The trend of obesity prevalence of Indonesian adults from 2007 to 2013

2013 at 14.2%, compared to 9.2% in 2007. It is thus important to have an insight into the risk factors contributing to obesity among adults in Indonesia and according to the various regions.

Obesity prevalence in the western and middle regions was higher in 2013 compared to 2007, while that of the

eastern region remained at somewhat the same level. This might be due to the increasing number of towns and cities in the western and middle regions. The environment in city areas is described as obesogenic in exerting unhealthy influences leading to obesity (Lake & Townshend, 2006). Moreover, obesity risk was found to be high among the unemployed. The obesogenic environment tends to affect more the low income as they are not able to afford healthy choices e.g. in purchasing vegetables and fruits (Żukiewicz-Sobczak *et al.*, 2014).

Low physical activity was a leading risk factor of obesity in Indonesia with the middle region showing the highest risk in 2013. Physical activity levels could change within a few years with urbanisation (Downs *et al.*, 2012). Another important risk factor of obesity found is high frequency intake of fatty foods. A similar finding was reported previously in Bali and East Kalimantan Provinces (MoH, 2015).

Regional differences in adult obesity prevalence occur in other countries including United States (Myers *et al.*, 2015). Several attributing factors have been implicated, such as socio-economic, political, and cultural factors (Żukiewicz-Sobczak *et al.*, 2014, beside sedentary behaviour and excessive consumption of sugar-sweetened beverages (Chan *et al.*, 2014; Ottevaere *et al.*, 2011; Haning *et al.*, 2016). Dietary and physical activity changes are part of the nutrition transition that developing countries undergo (Kac & Perez-Escamilla, 2013).

While some studies reported that obesity and mental health are related (Kivimaki *et al.*, 2009; Taylor *et al.*, 2013). A study in Japan showed that high prevalence of adult obesity occurred particularly in those with mental health problems, leading to eating disorders, in both under- and over-consumption (Kivimaki *et al.*, 2009; Saiga *et al.*, 2013). This study did not find a conclusive association between adult obesity and mental health status.

Limitations

This study did not take into consideration the "weight variable" as reported in the Indonesian Basic Health Research, hence the findings here may be somewhat different from the national BHR report. We are not able to quantify the differences in terms of "increases' or "decreases" in significant terms for the prevalence of the obesity risk factors between 2007 and 2013, as statistical comparison was not undertaken between the BHR datasets.

CONCLUSION

This study revealed regional differences in the factors associated with obesity among Indonesian adults. It is recommended that more comprehensive studies be undertaken to investigate the contribution of socio-economic status and lifestyles, especially dietary intake and physical activity, to adult obesity in the different regions in Indonesia.

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Authors' contributions

Andi Imam Arundhana, did the study conception, performed data analysis and interpretation, wrote the manuscript, and provided revision for final version of the manuscript; Asry Dwi Muqni, drafted the manuscript and contributed to the final version; Aisya Putri Utama, contributed to the data preparation and analysis of the result; Maria Theresa Talavera, wrote the manuscript in consultation with Andi Imam Arundhana, performed results interpretation.

Conflict of interest

All authors declared no conflict of interest for this study.

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Effects of conjugated linoleic acid supplementation and exercise on body fat mass and blood lipid profiles among overweight Iranians

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ABSTRACT

Introduction: Conjugated linoleic acid (CLA) has been studied for its fat mass reduction effects. This study aimed to determine the effects of CLA supplementation on body fat mass (BFM) and selected blood lipid profiles among overweight Iranian. **Methods:** A total of 180 adults with BMI = 26-29 kg/m^2 and BFM exceeding 21% and 28% for men and women, respectively were recruited through voluntary participation from weight management clinics in Tehran. They were assigned randomly to three groups as follows: Group (1) (control group) receives weight loss diet only; Group (2) receives weight loss diet +3 gr/day CLA supplement (mixture of *cis*-9, *trans*-11 and *trans*-10, *cis*-12) twice a day and Group (3) weight loss diet +3 gr/day CLA supplement as Group (2) twice a day + regular exercise (walking at 5.5-6 km/h for at least 160 minutes/week). The trial was conducted for 12 weeks. Anthropometric measurements and blood lipid profiles were determined at weeks 0, 6 and 12. **Results:** Both Group 2 and Group 3 showed a significant between-group difference in reduction of BFM (1.3% and 2.6% respectively) compared to Group 1. Group 2 supplementation showed increased free fatty acid (FFA) (0.44 mM to 0.55 mM) and decreased HDL-chol (47.5 mg/dL to 42.0 mg/dL) between weeks 0 and 12. These results were not observed for Group 3. **Conclusion:** Combination of CLA supplementation with exercise showed BFM reduction in overweight Iranian adults. Further research is suggested to verify the findings of this study.

Keywords: Overweight, conjugated linoleic acid, body fat mass, lipid profiles, Iranians

INTRODUCTION

Prevalence of obesity is on the rise globally. The National Health and Nutrition Examination Survey in the United States reported that more than one-third of adults were obese, and this phenomenon is distributed equally between genders (Ogden *et al.*, 2014). In Malaysia the prevalence of overweight and obesity among adults were reported as 30% and 17.7%, respectively (IPH, 2015).

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In Iran, the prevalence of obesity is increasing. A recent review reported that the prevalence of obesity of Iranians aged below 18 years was 5.5%, and 21.5% for the older population (Mirzazadeh *et al.*, 2009). Obesity prevalence was considerably higher among women, and it was attributed to the effect of sedentary lifestyle among Iranian women.

Obesity is a risk factor for almost all chronic diseases (Finucane *et al.*, 2011). Obesity is preventable and manageable through a balanced, moderate, and varied diet along with regular exercising. Nevertheless, it is not easy for people to manage their diets and lifestyles; therefore, several weight management plans including the use of supplements have been studied to address this issue.

Conjugated linoleic acid (CLA) is an isomer of the essential fatty acid, linoleic acid, found in dairy products and meat. Research on CLA has increased as CLA has been found to have anti-carcinogenic effects in animals (Koronowicz & Banks, 2018). Common CLA isomers studied are *trans*-10, *cis*-12 and *cis*-9, *trans*-11 isomers. Previous studies used fatty acid or triglyceride form of CLA as a supplement added to drinks such as milk or as capsule or soft gel (Dilzer & Park, 2012). CLA seemed to have a body fat mass reducing effect some animals (Benjamin *et al.*, 2015), but findings from human studies varied due to differences in materials, methodology, and design of the studies (Chen *et al.*, 2012; Dilzer & Park, 2012).

In terms of lipid profiles, CLA has been shown to lower blood high density lipoprotein (HDL-chol) (Racine *et al.*, 2010). A study reported that the HDL-chol lowering effect of the CLA might be mostly related to *cis*-9, *trans*-11 isomer (Wanders *et al.*, 2010). Alterations of low density lipoprotein (LDL-chol), total cholesterol, triglyceride (TG), and free fatty acid (FFA) in blood by CLA remain unclear as study results are inconsistent (Dilzer & Park, 2012). It has been shown that increase in FFA levels is a risk factor among obese, diabetic, and those suffering from cardiovascular disease (Boden, 2011). While *trans*-10, *cis*-12 CLA is reported to influence the FFA levels and insulin resistance in a short term, *cis*-9, *trans*-11 isomer does not appear to have such an effect (Dilzer & Park, 2012).

This study aimed to determine the effects of supplementation of a mixture of two main isomers of CLA supplementation and exercise intervention on anthropometric indicators and blood lipid profiles (HDL-chol, LDL-chol, and FFA) in overweight Iranians. To the best of our knowledge, this is the first study which assesses the effects of CLA supplementation on overweight Iranians.

MATERIALS AND METHODS

Research design and study subjects

This study was a randomised controlled trial (RCT) in which volunteers were assigned randomly to three groups. The sample size of 180 participants were determined by considering previous studies and based on available guidelines (Machin & Campbell, 2005) with a possibility of 15% dropout during the study. The subjects (100 women and 80 men) were recruited through voluntary participation from three weight management clinics in Tehran. Duration of the study was 12 weeks.

The inclusion criteria were apparently healthy Iranian volunteers aged between 20-50 years old, and with a BMI between 26-29 kg/m^2. These subjects must have a body fat mass (BFM) of more than 28% for women and 21% for men, and were not taking any medication or supplement. Pregnancy, lactating, history of hospitalisation, or previous or current health condition were exclusion criteria.

Subjects (*n*=180) were randomly assigned into three different groups namely Group 1 (control), Group 2 (CLA), and Group 3 (CLA + Exercise). All subjects were on a balanced weight loss diet which means their diet had been adjusted to provide 50-55% of calories from carbohydrates (CHO), 15-20% from protein and not more than 30% calories from fat. Group 1 received only the weight loss diet; Group 2 received the weight loss diet plus conjugated linoleic acid supplement (a mixture of the two bioactive isomers in the form of a 1500 mg soft gel -50% *cis*-9, *trans*-11 and 50% *trans*-10, *cis*-12- containing 78-84% CLA twice a day); Group 3 received the weight loss diet plus the same CLA supplement as Group 2 plus performing moderate intensity exercise (walking at 5.5-6 km/h for at least 160 minutes per week).

Ethics, consent and permissions

The present study was performed following the ethical guidelines of the Declaration of Helsinki, and the Good Clinical Practice rules. The study was approved by The Human Research Ethics Committee of the University Putra Malaysia (JKEUPM) as FPSK Mei (13)01 and all subjects signed an informed written consent form. The trial was registered in UMIN-CTR as UMIN000020284.

Clinical assessments

During the first session, general information pertaining to characteristics, demographic background and medical history of the subjects was collected. Data related to anthropometric measurements including body weight, height, body mass index (BMI), waist to hip ratio (WHR), and body fat mass (BFM) percentage and dietary assessment (24-hour recall) were collected at each visit. Blood samples were drawn for the analysis of total triglycerides (TG), low density lipoprotein (LDL-chol), high density lipoprotein (HDL-chol), fasting blood sugar (FBS), and free fatty acid content of blood (FFA).

Adverse effects

Adverse effects (AEs) were self-recorded by the subjects and defined as any unexplainable unfavourable effect. Subjects were instructed to record symptoms, frequency, severity, and duration of each AE. During each visit, the investigator reviewed recorded AEs, and subjects could visit physicians for treatment with the study covering their treatment. Subjects would be excluded from the study if they were concerned about the AE or if the physician considered them ineligible for the study.

Diet

A 3-day, 24-hour dietary recall was used to analyse the caloric intake of the subjects. This was done at weeks 0, 6 and 12 of the study. Subjects were interviewed by a dietitian to recall food consumed during the previous 24 hours. Nutritionist Pro software (Axxya Systems, 2006) were used to analyse the dietary information.

Exercise

For the Group 3, the exercise was defined as walking at 5.5-6 km/h for at least 160 minutes per week. The subjects had the choice to conduct the 160 mins exercise three or four times a week. This was set to meet the criteria for a moderate intensity exercise defined by Ainsworth *et al.* (2000). Subjects kept track of walking speed and distance using treadmill or software installed on their cell phone. Their exercise activity was provided to the dietitian at each visit.

Anthropometric measurements

Body weight was reported in kg. Height was measured with a standing scale

in meters with accuracy of 0.005 meters and body mass index was computed using those measurements. Waist circumference was measured with a measuring tape recorded in cm Jackson-Pollock four-site formula (from abdomen, suprailiac, triceps, and thigh) was used to calculate the body fat mass percentage (BFM). For the calculation of the skin fold thickness a Harpenden caliper was used.

Biochemical analyses

Participants were asked to fast 12 hours before each blood collection. At weeks 0, 6, and 12, 10 ml blood was collected from each subject. Lipid profiles (TG, LDL-chol, HDL-chol), FBS and FFA contents were determined. Detergent Solubilisation/ Enzymatic Analytical method was used to determine blood HDL-chol, and LDL-chol, and the Quantitative Enzymatic method was used for determination of TG contents. FFA content was analysed using quantitative spectrophotometry, while FBS level was determined using a quantitative enzymatic method.

Statistical analyses

All of the analyses were done using SPSS 22 software (IBM Corp. Released 2013. Armonk, NY, USA). Chi-square test was used to test categorical variables for significant differences between groups. Shapiro-Wilk test was used for test of normality. For analysing within-group differences, repeated measures ANOVA and Friedman test for parametric and non-parametric data, respectively were applied. Between-group comparisons were performed with one-way ANOVA and Kruskal-Wallis test for parametric and non-parametric data, respectively, and post hoc analysis were performed with a Bonferroni adjustment. A significance level of 0.05 was indicated for all tests.

RESULTS

Study subjects

After 12 weeks of follow up, 171

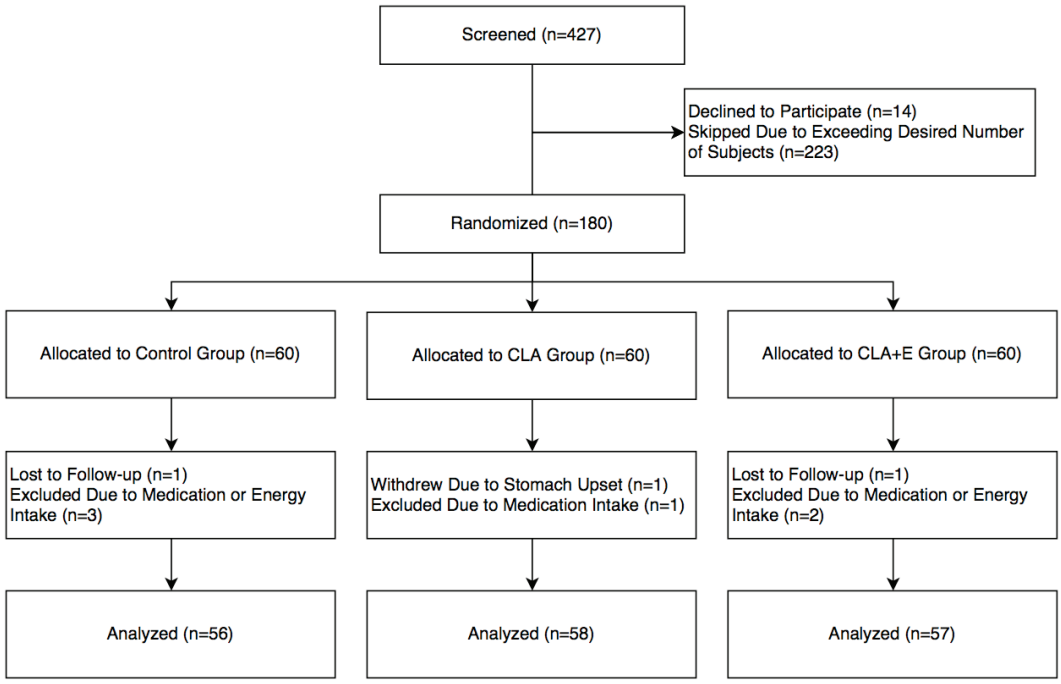

Figure 1. Flowchart of the total number of subjects recruited and analysed

participants completed the study (95%) (Figure 1). Five participants were excluded because having to take medication (Group 1, *n*=2; Group 2, *n*=1; Group 3, *n*=1), one because of stomach upset as an adverse effect (Group 2, *n*=1), two did not show up for follow up sessions (Group 1, *n*=1; Group 3, *n*=1), and two participants did not follow the prescribed calorie diet (Group 1, *n*=1; Group 3, *n*=1).

Among all the participants who started the study only one (*n*=1) reported an AE which was related to an upset stomach. While the AE was not considered serious by the subject and physician, the subject decided to quit the study. The completed sample size of 171 was higher than the calculated minimum number needed for this study (153).

Table 1 summarised the baseline characteristic of the subjects in three study groups. Majority of subjects were female (55%, *n*=94). No significant difference existed between the groups for gender, age, marital status, income, and education level. All participants reported no alcohol use as alcohol consumption is prohibited in the country. Only ten subjects (all male) reported tobacco use. The daily caloric intake within different groups was not significantly different during the 12 weeks of study (Table

Table 1. Baseline characteristic of the subjects

Variables	*Group*†			*Total*	*p*	x^2
	Group 1 n=56	*Group 2 n=58*	*Group 3 n=171*			
Gender‡					0.94	0.135
Female	30 (31.9%)	32 (34.0%)	32 (34.0%)	94		
Male	26 (33.8%)	26 (33.8%)	25 (32.5%)	77		
Education level‡					0.51	5.231
Primary	7 (46.7%)	5 (33.3%)	3 (20.0%)	15		
Secondary	7 (46.7%)	5 (33.3%)	3 (20.0%)	15		
Diploma	14 (38.9%)	11 (30.6%)	11 (30.6%)	36		
University	28 (26.7%)	37 (35.2%)	40 (38.1%)	105		
Income‡					0.66	4.090
≤500 USD	10 (34.5%)	11 (37.9%)	8 (27.6%)	29		
500<X<≤1000	15 (38.5%)	9 (23.1%)	15 (38.5%)	39		
1000<X≤1500	14 (27.5%)	18 (35.3%)	19 (37.3%)	51		
>1500	17 (32.7%)	20 (38.5%)	15 (28.8%)	52		
Marital status‡					0.55	4.958
Single	14 (31.8%)	15 (34.1%)	15 (34.1%)	44		
Married	34 (29.7%)	39 (35.1%)	39 (35.1%)	112		
Divorced	4 (50.0%)	2 (25.0%)	2 (25.0%)	8		
Widowed	5 (62.5%)	2 (25.0%)	1 (12.5%)	8		
Age§	35 (29)	36.5 (30)	35 (30)		0.66	
BFM§						
Female	29.0 (0.79)	29.0 (1.76)	29.3 (1.1)	–	0.80	
Male	23.4 (1.24)	22.9 (1.07)	22.9 (1.17)	–	0.25	

†Group 1, diet only; Group 2, diet + conjugated linoleic acid (CLA) supplement; Group 3, diet + CLA supplement + moderate-intense exercise; Values are represented as number of subject (percentage) or median (IQR)

‡Chi-square test

§Non-parametric test, Kruskal-Wallis, α=0.05

Table 2. Dietary intake of the subjects

Variable (kcal)	*Group*†					
	1		*2*		*3*	
	Median	*IQR*	*Median*	*IQR*	*Median*	*IQR*
Calories, week 0	1575	350	1650	950	1650	1000
Calories, week 6	1600	350	1625	1000	1650	900
Calories, week 12	1575	413	1650	850	1650	950
p-value‡	0.950		0.937		0.122	

†Group 1, diet only; Group 2, diet + conjugated linoleic acid (CLA) supplement; Group 3, diet + CLA supplement + moderate-intense exercise
Unit: Calories (kcal)
‡Non-parametric test, Kruskal-Wallis, α=0.05

Table 3. Anthropometric measurements of the subjects

Variables	*Group*†			*p**
	1	*2*	*3*	
BW‡				
Week 0	80.30±10.5	82.95±10.16	83.11±9.78	0.27
Week 6	78.80±10.4	81.13±10.03	80.96±9.63	0.38
Week 12	76.90±10.3	79.26±9.91	78.80±9.65	0.40
BMI§				
Week 0	27.70 (2.98)	27.6 (2.90)	27.6 (2.74)	0.73
Week 6	27.10 (3.01)	27.0 (3.12)	26.8 (2.76)	0.14
Week 12	26.50 (2.97)	26.4 (2.76)	26.1 (3.15)	0.12
WHR‡				
Week 0	0.89±0.11	0.89±0.09	0.88±0.09	0.85
Week 6	0.88±0.11	0.88±0.09	0.87±0.08	0.67
Week 12	0.87±0.10	0.87±0.08	0.85±0.08	0.37
BFM§				
Week 0	28.00 (9.80)	27.70 (9.80)	27.30 (8.6)	0.99
Week 6	28.10 (10.60)	26.60 (9.70)	25.20 (9.5)	0.004
Week 12	28.10 (10.80)	26.40 (10.40)	24.70 (9.8)	<0.0005
ΔBaseline¶	–	2.09	2.54	–
ΔCLA¶	–	–	0.51	–

†Group 1, diet only; Group 2, diet + conjugated linoleic acid (CLA) supplement; Group 3, diet + CLA supplement + moderate-intense exercise
‡Parametric test is one-way ANOVA (α=0.05)
§Non-parametric test is Kruskal-Wallis (α=0.05)
¶Difference in differences (DID) as a measure of intervention effect for variables with significant between group differences. ΔBaseline: intervention effect compared to baseline. ΔCLA: intervention effect compared to CLA group
*Between-group Differences (comparing three groups)

Notes:
1. BW, body weight; BMI, body mass index; WHR, waist-to-hip ratio; BFM, body fat mass; Values are represented as mean±SD or median (IQR).
2. BW (kg); BMI (kg/m^2); WHR is a ratio; BFM (%).
3. All within-group Differences (comparing three weeks of 0, 6 and 12) are significant across AM variables with p<0.0005.
4. Bonferroni corrected α=0.0167 (for tests based on each variable), α=0.0028 (when considering all AM variables).

Table 4. Blood profiles of the subjects

Variables	*Group*†			*p**
	1	*2*	*3*	
FBS‡				
Week 0	81.5 (35)	82.0 (32)	81.0 (31)	0.99
Week 6	82.0 (37)	82.0 (24)	79.0 (28)	0.16
Week 12	83.0 (30)	79.0 (28)	78.0 (24)	0.003
*p***	0.98	0.94	0.002	
TG§				
Week 0	90.9±20.51	87.7±19.02	86.9±16.69	0.48
Week 6	89.2±17.19	86.5±17.34	84.8±12.07	0.33
Week 12	88.8±19.08	84.5±16.99	81.9±10.62	0.07
*p***	0.21	0.02	0.002	
HDL-chol‡				
Week 0	48.0 (36)	47.5 (40)	46.0 (38)	0.46
Week 6	47.0 (33)	46.0 (27)	50.0 (31)	0.003
Week 12	49.0 (35)	42.0 (33)	52.0 (28)	<0.0005
*p***	0.43	0.006	<0.0005	
ΔBaseline¶	–	2.09	2.54	–
ΔCLA¶	–	–	0.51	–
LDL-chol§				
Week 0	85.6±15.20	85.0±17.97	85.1±17.19	0.98
Week 6	84.8±14.16	84.2±16.63	81.3±11.79	0.39
Week 12	84.5±13.25	82.8±14.62	76.5±11.30	0.004
*p***	0.67	0.16	<0.0005	
FFA‡				
Week 0	0.42 (0.73)	0.44 (0.81)	0.42 (0.86)	0.94
Week 6	0.40 (0.71)	0.51 (0.95)	0.32 (0.75)	<0.0005
Week 12	0.35 (0.66)	0.55 (1.03)	0.31 (0.83)	<0.0005
*p***	<0.0005	<0.0005	0.003	
ΔBaseline¶	–	2.09	2.54	–
ΔCLA¶	–	–	0.51	–

†Group 1, diet only; Group 2, diet + conjugated linoleic acid (CLA) supplement; Group 3, diet + CLA supplement + moderate-intense exercise
‡Non-parametric test is Kruskal-Wallis (α=0.05)
§Parametric test is one-way ANOVA (α=0.05)
¶Difference in differences (DID) as a measure of intervention effect for variables with significant between group differences. ΔBaseline: intervention effect compared to baseline. ΔCLA: intervention effect compared to CLA group
*Between-group Differences (comparing three groups)
**Within-group Differences (comparing three weeks of 0, 6 and 12)

Notes:

1. FBS, fasting blood sugary; TG, triglyceride; HLD, High Density Lipoprotein; LDL-chol, Low Density Lipoprotein; FFA, Free Fatty Acid; Values are represented as mean±SD or median (IQR).
2. FBS, TG, LDL-chol, and HDL-chol are reported in mg/dl; FFA is reported in mmol/l.
3. Bonferroni corrected α=0.0167 (for tests based on each variable), α=0.0033 (considering all blood profile variables).

2). Furthermore, no between-group differences were found at baseline for anthropometric status (Table 3) and blood profiles (Table 4).

Effects of CLA on anthropometric measurements

Summary of changes in anthropometric status is presented in Table 3. Compared to body weight at baseline, all groups experienced significant weight loss (Group 1, Δ=-3.5±0.7; Group 2, Δ=-3.7±0.8; Group 3, Δ=-4.3±0.9) with weight reduction among Group 3 (CLA+exercise) being clinically significant (Pi-Sunyer, 1996) as subjects lost 5.4% of their weight, on average. However, no significant between-group differences were found. Similarly, all groups experienced significant BMI reduction (Group 1, $p<0.001$; Group 2, $p<0.001$; Group 3, $p<0.001$ – BMI at week 12 compared to baseline BMI) but with no statistically significant differences between the groups. A similar result was shown for WHR compared to the baseline WHR.

Noticeably, there was a significant between-group difference for BFM between Group 2 and Group 3, compared to Group 1 (Group 2 compared to Group 1, $p<0.005$; Group 3 compared to Group 1, $p<0.005$), while the difference between Group 2 and Group 3 is not significant ($p=1.0$). This finding is in addition to significant within-group difference in

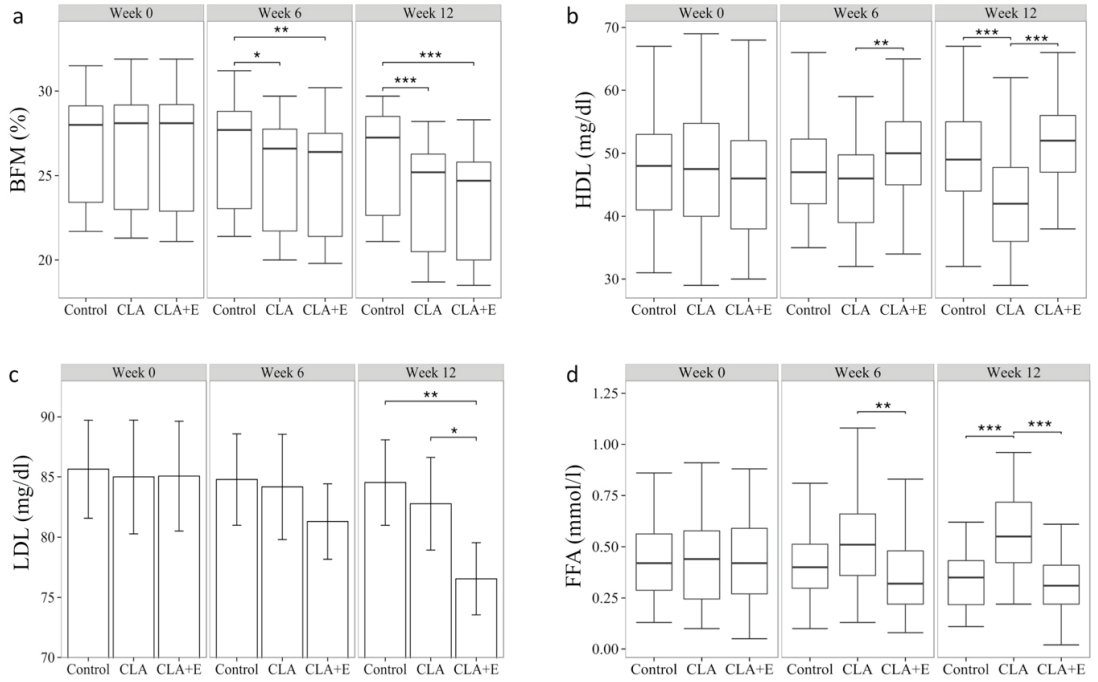

Figure 2. Changes of BFM and select blood profiles across treatment groups during the study (a. BFM changes, b. HDL-chol changes, c. LDL-chol changes, d. FFA changes during the study)

*$p<0.05$

**$p<0.01$

***$p< 0.001$

BFM reduction among all groups. Figure 2a illustrates the differences in BFM among the three study groups.

Effects of CLA on blood profiles

Table 4 summarises the results of analyses on subjects' blood profiles among the study groups. After 12 weeks, there was no significant between-group differences pertaining to FBS, TG, and LDL-chol compared to the baseline values. For FBS, the only significant difference was the within-group reduction among Group 3 (p=0.002). We observed difference pertaining to reduction in TG among Group 2 (Δ=-3.2±8.9) and Group 3 (Δ=-5.0±11.4). LDL-chol reduction was only significant among Group 3 (p<0.0005). Furthermore, participants in Group 3 experienced significant within-group increase of HDL-chol (p<0.0005) while HLD blood levels of those in Group 2 decreased significantly (p=0.006), HDL3 (HDL-chol at week 12) compared to HDL1 (HDL-chol at week 0).

There was a significant decrease in HDL-chol level among Group 2 when comparing HDL-chol at week 12 compared to baseline level. There was also a significant between-group difference when comparing HDL-chol at week 12 between Group 2 and Group 1. Figures 2b and 2c show the alterations in HDL-chol & LDL-chol levels among the three groups, respectively.

There was a significant between-group difference when comparing FFA levels between Group 2 at the 6-week and 12-week point compared to the other groups. Another finding was that FFA level increased in Group 2 (p<0.0005) while Group 1 and Group 3 showed decreases in their FFA levels (p<0.0005 and p=0.003, respectively) (Figure 2d) shows differences in FFA levels during the study among all groups.

DISCUSSION

This study showed that consuming CLA as a soft gel supplement containing two isomers for 12 weeks significantly decreased BFM. The present study found significant changes in BW, BMI, WHR, and BFM within all groups. This could be due to on the weight reduction diet taken by all groups. However, both the intervention groups 2 and 3 experienced almost five times more reduction in BFM compared with the control group. This finding suggests an improvement in body weight reduction obtained through CLA consumption, an effect that was not improved any further by adding aerobic exercising for 160 minutes/week. This might be due to an increase in lean body mass (LBM) that was reported in other studies (Steck *et al.*, 2007; Dilzer & Park, 2012). Unfortunately, this study did not consider LBM for evaluation.

This study revealed statistically significant reduction of FBS in Group 3 after 12 weeks compared to the other groups. This is in accordance to findings of other studies that have shown exercising or physical activity decreases fasting blood sugar levels among both healthy and diabetic population (Ossanloo, Najar & Zafari, 2012). Nevertheless, other studies reported a non-significant difference of FBS with CLA supplementation used among diabetic subjects (Racine *et al.*, 2010; Sluijs *et al.*, 2010).

No significant between-group difference was recorded for blood triglyceride levels. This finding is in accordance with of other investigations (Racine *et al.*, 2010; Sluijs *et al.*, 2010). There is inconsistency in previous studies for the effects of CLA supplementation on LDL-chol concentrations (Dilzer & Park, 2012). We found that exercising appears to exert a significant effect

on LDL-chol, with the participants in Group 3 experiencing significant LDL-chol decrease compared to other groups. Other studies have reported exercising decreases LDL-chol levels among healthy, diabetic, and atherosclerosis patients irrespective of their age (Lira *et al.*, 2010; Kelley & Kelley, 2007). This study confirmed the reduction of HDL-chol level in Group 2 compared to the other groups. This negative effect of CLA has been reported by many studies (Gaullier *et al.*, 2005; Racine *et al.*, 2010; Sluijs *et al.*, 2010).

Perhaps the most important finding of this study was that CLA supplementation elevated FFA concentrations significantly comparing between groups 1 and 2. This unfavourable increase was controlled by aerobic exercise (comparing between groups 2 and 3).

Overall, this study found that CLA supplementation for 12 weeks has a positive impact on body fat mass reduction of overweight individuals with marginal BFM percentages along with the negative effects of decreasing HDL-chol and increasing FFA levels. The combination of CLA with exercise will be beneficial on body composition and would not add adverse effects to health. This could be one of the way to reduce increased adiposity and potentially lower the risk of other diseases associated with obesity.

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Authors' contributions

Hanieh F and Loh SP designed the study. Hanieh F and Abas M helped in data collection. Hanieh F analyzed the data. All authors discussed the results and commented on the manuscript.

Conflict of interest

The authors declared no conflict of interests.

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Factors associated with stunting among *Orang Asli* preschool children in Negeri Sembilan, Malaysia

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ABSTRACT

Introduction: Childhood stunting is recognised as one of the most significant barriers to human development. This cross-sectional study aimed to determine the factors associated with stunting among *Orang Asli* (OA) preschool children in Negeri Sembilan, Malaysia. **Methods:** A total of 264 children (50.9% boys and 49.1% girls) aged 2-6 years (*M*=4.04, *SD*=1.21 years) including their mothers from 14 OA villages in Negeri Sembilan participated in this study. Mothers were interviewed to obtain information regarding socioeconomic status, sanitation facility and personal hygiene. The height of the children and their mothers were measured. Venous blood samples were drawn from the children to estimate haemoglobin level, and stool samples were collected to screen for intestinal parasitic infections. **Results:** Approximately one third of the children (35.6%) and 7.8% of the mothers were stunted. One in five of the children were anaemic (21.6%), while one- third had intestinal parasitic infections (35.0%). Low birth weight (AOR=2.526, 95% CI: 1.310-4.872; *p*=0.006), anaemia (AOR=2.742, 95% CI: 1.265-5.945; *p*=0.011), presence of intestinal parasitic infections (AOR=2.235, 95% CI: 1.310-3.813, *p*=0.003), not wearing shoes (AOR=2.602, 95% CI: 1.453-4.660; *p*=0.001), absence of piped water at home (AOR=2.395, 95% CI: 1.047-5.476; *p*=0.039), dirty nails (AOR=1.956, 95% CI: 1.163-3.289, *p*=0.011), and stunted mothers (AOR=3.443, 95% CI: 1.334-8.890; *p*=0.011) were identified as significant factors for childhood stunting. **Conclusion:** It is suggested that the factors identified associated with childhood stunting be included in future intervention programmes that address stunting among OA children.

Keywords: Haemoglobin level, sanitation and hygiene, maternal stature, parasitic infection, stunting, *Orang Asli* children

INTRODUCTION

Malnutrition, specifically protein-energy malnutrition, exposes children to increased risk of morbidity and mortality. Indeed, it is a serious cause which impedes child growth and development (UNICEF / WHO / World Bank Group, 2017). Basically, stunting, which is one of the various forms of malnutrition, is defined as height-for-age *z*-score (HAZ) of less than -2 standard deviation (SD) below the median of a reference standard (WHO, 2006). It is a well-established indicator of chronic undernutrition which shows long-term,

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cumulative insufficiencies of nutrition and suboptimal health condition that is often due to maternal undernutrition (WHO, 2006).

Globally, the prevalence of childhood stunting has decreased from 32.7% in 2000 to 22.9% in 2016, but it is declining too slowly (UNICEF/WHO/World Bank Group, 2017). Notwithstanding the decline in prevalence of stunting, an estimated 155 million children worldwide below the age of 5 were stunted, with more than half from Asia (UNICEF/WHO/World Bank Group, 2017). With respect to this matter, indigenous people continue to be among the most underprivileged population stricken with deprived health and social outcomes. Moreover, several studies have shown that indigenous children had high prevalence of stunting. For example, the prevalence of stunting was 63.7% in Guatemala (Ramirez-zea *et al.*, 2014), 50.0% in Peru (Anticona & San Sebastian, 2014), and 25.7% in Brazil (Horta *et al.*, 2013).

In 2015, indigenous peoples were approximately 13.8% of the 31,660,700 population in Malaysia. Specifically, in Peninsular Malaysia, indigenous peoples are known as *Orang Asli* (OA), accounting for 210,000 or 0.7% of the 31,005,066 population (Hansen, Jepsen & Jacquelin, 2017). The OA community has distinctive language, cultures and beliefs. Most of these people are hardcore poor and have relatively low socioeconomic status, lack of healthcare awareness, poor sanitation facilities, and unable to provide essential needs such as appropriate clothings and nutritious food for the whole family (Masron *et al.*, 2013). Despite the fact that most Malaysians are categorised under the upper middle-income group, the majority of OA population in Peninsular Malaysia are still struggling with poverty, poor nutritional and health status, especially in young children (Ahmed *et al.*, 2012; Chua *et al.*, 2012; Wong *et al.*, 2015). Overall, the poverty among Malaysians had significantly reduced from 3.8% in 2009 to 0.6% in 2014, but poverty rates among OA population remained high at 34.0% (EPU, 2016). In relation to this finding, OA is ascertained to be among the poorest populations in Malaysia.

Stunting in OA children, especially those below the age of 5, is one of the main concerns of public health in Malaysia. In this context, several studies in Malaysia showed that the prevalence of stunting among the OA children were in the range of 40-76% (Ahmed *et al.*, 2012; Chua *et al.*, 2012; Wong *et al.*, 2018). Clearly, this indicates that these children have a higher tendency to get common infections and diseases such as anaemia later in life, as well as jeopardising their cognitive development. A previous study showed that stunting has a long term adverse effect on adult cognitive ability, reduces school attainment, and limits income levels (Hoddinott *et al.*, 2013).

There are multiple factors known to be associated with stunting such as poor socioeconomic status (Rahman *et al.*, 2016), poor sanitation (Alelign, Degarege & Erko, 2015; Rah *et al.*, 2015), low level of haemoglobin (Al-Qaoud, Al-Shami & Prakash, 2015; Leite *et al.*, 2013), stunted mothers (Walker *et al.*, 2015) and intestinal parasitic infections (Ahmed *et al.*, 2012; Sanchez *et al.*, 2013). Although the prevalence of stunting in OA children were well-documented, its specific determinants toward stunting are inconclusive. Therefore, this study aimed to identify the factors associated with stunting among OA preschool children in Negeri Sembilan.

MATERIALS AND METHODS

Study setting and subjects

A cross-sectional study was conducted among OA children aged 2-6 years in the state of Negeri Sembilan, Malaysia

from April 2015 to January 2016. Out of seven districts in Negeri Sembilan, two districts were purposely selected due to the high number of OA villages, namely Jempol (16 villages) and Kuala Pilah (14 villages). However, only 14 (six from Jempol and eight from Kuala Pilah) out of 30 villages agreed to participate in this study. The other 16 villages did not participate in this study due to several reasons, including no preschool children, no leader (*Tok Batin*) and not allowing outsiders to enter village due to villagers' behaviours and culture of not talking and mixing with outsiders. From these 14 participated villages, a list of 280 children aged 2-6 years old was obtained from the *Tok Batin* from each village. Overall, 264 children completed the questionnaires and measurements of the study with an overall response rate of 94.3%. Ten parents refused to let their children to participate in this study while another six children had moved to other villages.

Procedures

In order to conduct this study, ethics approval was first obtained from the Ethics Committee for Research Involving Human Subjects (JKEUPM) of Universiti Putra Malaysia [Reference No.: FPSK (FR15) P001]. This study was conducted under the permission obtained from the Department of Orang Asli Development (JAKOA) [Reference No.: JAKOA/ PP.30.032Jld31(05)]. Written informed consent forms were acquired from the mothers prior to the data collection.

There were two visits conducted in the OA villages. During the first visit, stool samples of the children were collected while the mothers were interviewed to complete a questionnaire. In the following visit, respondents were gathered in a village hall for blood withdrawal by a paediatrician and anthropometric measurements by the researchers.

Instruments

Face-to-face interview

Socioeconomic background

In this study, a face-to-face interview was conducted using a Malay language questionnaire which required the mothers to provide information on socioeconomic background, including age, sex, sub-tribe, child's birth weight, household size, parents' education level, occupation status, and monthly household income.

Sanitation and hygiene

Personal hygiene and sanitation facilities' questionnaires were adapted from Al-Delaimy et al. (2014) and assessed in this study through two methods, namely observation and interview. During the home visit, observations were focused on the personal hygiene of the children. For example, the practice of cutting fingernails and wearing shoes outside the house were taken into account. On the other hand, both interviews and observations were conducted to inquire about sanitation facilities with regards to the availability of functioning toilets and piped water at home. A binary-choice (yes/no) style was utilised.

Haemoglobin concentration

Haemoglobin (Hb) concentration of children was measured through 3 ml of venous blood sample withdrawn by a paediatrician. Specifically, anaemia in children under age of 5 years is defined when Hb concentration is <11g/ dL, whereas, anaemia for children 5 years and above is defined when Hb concentration is <11.5 g/dL (WHO, 2011). Mild anaemia is defined when Hb concentration is between 10.0-10.9 g/dL for children under 5 years and 11.0-11.4 g/dL for children 5 years and above. Moderate anaemia is defined when Hb concentration is between 7.0-9.9 g/dL for children under 5 years and 8.0-10.9 g/dL for children 5 years and

above. Severe anaemia is defined when Hb concentration less than 7 g/dL for children under 5 years and less than 8 g/dL for children 5 years and above (WHO, 2011). All laboratory analyses were then outsourced to an accredited laboratory.

Parasitic infections

Mothers were asked to scoop a fresh, thumb-sized stool sample from their children without any urination, water or sand, and to store it in a stool container that was provided to them. Mothers informed the person in charge of each village to contact the researcher via phone to collect the stool sample. The stool samples were put in a suitable ice box and transported to an accredited laboratory for further analysis. The transportation time took between 30 minutes to one hour to reach the laboratory. In the laboratory, stool samples were analysed to check for the presence of intestinal parasites, namely *Trichuris trichiuria* and *Ascaris lumbricoides*. Particularly, direct microscopy (iodine) method was employed to analyse the presence of intestinal parasites.

Anthropometric measurement

Anthropometric measurements were conducted on the children and their mothers. Height was measured by using a SECA body meter 206 (SECA, Germany) and rounded to the nearest 0.1 cm whereas weight was measured by using a TANITA Digital Weighing Scale HD-314 (TANITA Corporation, USA) to the nearest 0.1 kg. Duplicate results were obtained and the average of the duplicate results was recorded. Based on the results gathered, the mean *z*-score for height-for-age (HAZ) for children was computed according to the WHO Growth Reference 2007 (≥5 years old) and WHO Child Growth Standards 2006 (<5 years old) (WHO, 2006, 2007) by using the WHO AnthroPlus Version 1.0.4 software (WHO, Geneva, Switzerland). Mothers with height shorter than 145 cm were considered as stunted (Subramanian *et al.,* 2009).

Statistical analysis

The analysis of data was performed using IBM SPSS Statistics 22.0 (IBM Corp., Armonk, NY, USA). The distributions for quantitative variables were checked for normality. Univariate analysis was applied to analyse descriptive data for all variables in this study. Besides, the chi-square test was used to determine the associations between categorical variables and stunting. Additionally, binary logistic regression analysis was performed to determine the risk factors of stunting. All variables that possessed a $p<0.25$ in the simple logistic regression analysis were included in the multiple logistic regression analysis. This criterion of $p<0.25$ was employed based on the evidence proposing that the threshold ($p<0.05$) might exclude variables which are significant (Hosmer & Lemeshow, 1989). The acceptable level of statistical significance for all tests was set at $p<0.05$.

RESULTS

Table 1 shows the characteristics of the study respondents. The prevalence rates of severely stunted and stunted children were 6.4% and 29.2%, respectively. The mean age of the OA children was 4.04±1.21 years, ranging from 2 to 6 years. Majority of the children (59.1%) were of the Temuan sub-tribe. More than half (54.9%) of the respondents had household size of 5-8 family members.

More stunted children had low birth weight and were anaemic (mild, moderate, and severe) as compared to non-stunted children (Table 1). In terms of intestinal parasitic infections, 10 children refused to provide stool samples. Out of 254 stool samples,

Table 1. Sociodemographic characteristics, haemoglobin level, and intestinal parasitic infections of the respondents (*n*=264)

Characteristics	*n (%)*			X^2	*p-value*
	Total (n=264)	*Stunted (n=97)*	*Non-stunted (n=167)*		
Children					
Sex				0.001	0.979
Boy	135 (51.1)	49 (50.5)	86 (51.5)		
Girl	129 (48.9)	48 (49.5)	81 (48.5)		
Age (years)				0.035	0.852
Toddlers (2-3)	92 (34.8)	35 (36.1)	57 (34.1)		
Pre-schoolers (4-6)	172 (65.2)	62 (63.9)	110 (65.9)		
Sub-tribe				6.499	0.039*
Temuan	156 (59.1)	54 (55.7)	102 (61.1)		
Semelai	96 (36.4)	42 (43.3)	54 (32.3)		
Others	12 (4.5)	1 (1.0)	11 (6.6)		
Birth weight (kg)				5.876	0.015*
Low (<2.5)	54 (20.5)	28 (28.9)	26 (15.6)		
Normal (≥2.5)	210 (79.5)	69 (71.1)	141 (84.4)		
Education				0.441	0.507
No schooling	144 (54.5)	56 (57.7)	88 (52.7)		
Pre-school	120 (45.5)	41 (42.3)	79 (47.3)		
Household size (members)				3.056	0.217
1-4	94 (35.6)	35 (36.1)	59 (35.3)		
5-8	145 (54.9)	49 (50.5)	96 (57.5)		
9-19	25 (9.5)	13 (13.4)	12 (7.2)		
Haemoglobin (g/dL)				7.900	0.019*
Normal	207 (78.4)	67 (69.1)	140 (83.8)		
Mild anaemia	34 (12.9)	18 (18.6)	16 (9.6)		
Moderate/severe anaemia	23 (8.7)	12 (12.4)	11 (6.6)		
Parasite infection (*n*=254)				8.876	0.003*
Yes	89 (35.0)	44 (47.3)	45 (28.0)		
No	165 (65.0)	49 (52.7)	116 (72.0)		
Types of parasites				0.135	0.935
Trichuris trichiuria only	47 (52.8)	24 (54.5)	23 (51.1)		
Ascaris lumbricoides only	10 (11.2)	5 (11.4)	5 (11.1)		
Both	32 (36.0)	15 (34.1)	17 (37.8)		
Parents					
Education levels					
Father (*n*=250)				4.640	0.098
No schooling	33 (13.2)	12 (13.0)	21 (13.3)		
Primary school	126 (50.4)	54 (58.7)	72 (45.6)		
Secondary school	91 (36.4)	26 (28.3)	65 (41.1)		
Mother				6.897	0.032*
No schooling	34 (13.0)	12 (12.4)	22 (13.5)		
Primary school	113 (42.5)	52 (53.6)	61 (37.4)		
Secondary school	117 (44.5)	33 (34.0)	80 (49.1)		
Household income (RM)				6.242	0.044*
≤580†	101 (38.3)	44 (45.4)	57 (34.1)		
581–940‡	76 (28.8)	30 (30.9)	46 (27.5)		
>940	87 (33.0)	23 (23.7)	64 (38.3)		
Income per capita (RM)				9.187	0.010*
≤140†	142 (53.8)	62 (63.9)	80 (47.9)		
141-240‡	61 (23.1)	22 (22.7)	39 (23.4)		
>240	61 (23.1)	13 (13.4)	48 (28.7)		
Maternal height (cm)				5.094	0.024*
<145	21 (8.0)	13 (13.4)	8 (4.8)		
≥145	243 (92.0)	84 (86.6)	159 (95.2)		

RM = Ringgit Malaysia

†Hard core poverty income (Economic Planning Unit, 2014)

‡Poverty income (Economic Planning Unit, 2014)

*Chi-square test, significant at $p<0.05$

Table 2. Sanitation and hygiene of the children

Variables	n (%)			X^2	p-value
	Total (n=264)	Stunted (n=97)	Non-stunted (n=167)		
Personal hygiene					
Wash hands using soap				1.149	0.284
Yes	199 (75.4)	69 (71.1)	130 (77.8)		
No	65 (24.6)	28 (28.9)	37 (22.2)		
Wearing shoes outside house				9.613	0.002*
Yes	201 (76.1)	63 (31.3)	138 (82.6)		
No	63 (23.9)	34 (35.1)	29 (17.4)		
Nails cleanliness				6.513	0.011*
Yes	169 (64.0)	52 (53.6)	117 (70.1)		
No	95 (36.0)	45 (46.4)	50 (29.9)		
Sanitation facilities					
Presence of toilet				4.135	0.042*
Yes	82 (31.1)	59 (60.8)	123 (73.7)		
No	182 (68.9)	38 (39.2)	44 (26.3)		
Presence of piped water				4.854	0.028*
Yes	234 (88.6)	80 (82.5)	154 (92.2)		
No	30 (11.4)	17 (56.7)	13 (7.8)		

*Chi-square test, significant at $p<0.05$

35.0% of the children were infected with intestinal parasites. Majority of the children were infected by *Trichuris trichiuria* (52.8%) (47/89), followed by *Ascaris lumbricoides* (11.2%) (10/89) while 36.0% (32/89) were infected by both. More stunted children were infected by intestinal parasites (47.3%) as compared to non-stunted children (28.0%, p=0.003), but there was no significant association between the types of parasite and stunting. Mothers with no schooling (X^2=6.897, p=0.032), low household income (X^2=6.242, p=0.044), low income per capita (X^2=9.187, p=0.010) and short stature (X^2=5.094, p=0.024) were significantly associated with childhood stunting.

There was a higher percentage of stunted children who did not wear their shoes outside the house (35.1%) compared to non-stunted children (17.4%; p=0.002) (Table 2). Among the stunted children, there was a greater percentage of children who had dirty nails (46.4%) compared to non-stunted children (29.9%; p=0.011). Besides, more stunted children did not have toilet facility at home (p=0.042) and functioning water pipes (p=0.028) compared to non-stunted children. However, there was no significant association between hand washing using soap and stunting.

Table 3 outlines the binary logistic regression for unadjusted and adjusted odds ratios (OR). After the data were adjusted for age, sex, mother's education, household size and monthly household income, the logistic regression analysis results showed that child's birth weight, anaemia status, presence of parasitic infections, maternal stature, not wearing shoes outside the house, having dirty nails, and absence of piped water at home were significantly associated with stunting in OA children. In fact, children with stunted mothers were 3.443 times (95% CI: 1.334-8.890;

Table 3. Unadjusted and adjusted odds ratios (OR) and 95% confidence intervals (CIs) among stunted and non-stunted children

Variables	*Unadjusted*			*Adjusted*[†]		
	OR	*95% CI*	*p-value*	*OR*	*95% CI*	*p-value*
Birth weight (g)						
Normal	1.00			1.00		
Low	2.201	1.200-4.036	0.011	2.526	1.310-4.872	0.006[*]
Maternal stature						
Normal	1.00			1.00		
Stunting	3.076	1.226-7.715	0.017	3.443	1.334-8.890	0.011[*]
Haemoglobin level						
Normal	1.00			1.00		
Mild anaemia	2.351	1.129-4.896	0.022	2.742	1.265-5.945	0.011[*]
Moderate & severe anaemia	2.280	0.957-5.432	0.063	2.171	0.893-5.274	0.087
Presence of parasites						
Negative	1.00			1.00		
Positive	2.315	1.358-3.945	0.002	2.235	1.310-3.813	0.003[*]
Wash hands using soap						
Yes	1.00					
No	1.426	0.805-2.524	0.224	-	-	-
Wearing shoes outside house						
Yes	1.00			1.00		
No	2.568	1.441-4.578	0.001	2.602	1.453-4.660	0.001[*]
Nails cleanliness						
Yes	1.00			1.00		
No	2.025	1.206-3.401	0.008	1.956	1.163-3.289	0.011[*]
Presence of toilet						
Yes	1.00					
No	1.800	1.056-3.070	0.031	-	-	-
Presence of piped water						
Yes	1.00			1.00		
No	2.517	1.164-5.442	0.019	2.395	1.047-5.476	0.039[*]

OR = Odds ratio; CI = Confidence Interval

[†]Data were adjusted for age (months), sub-tribe, sex, mother's education, household size, and monthly household income

[*]Significant at $p<0.05$

p=0.011) more vulnerable to stunting, compared to children with non-stunted mothers. Children with low birth weight were 2.526 times (95% CI: 1.310-4.872; p=0.006) more likely to become stunted as compared to children with normal birth weight.

Anaemic children were 2.742 times (95% CI: 1.265-5.945; p=0.011) more likely to become stunted, compared to non-anaemic children. Furthermore, children infected with parasites were 2.235 times (95% CI: 1.310-3.813, p=0.003) more likely to become stunted,

compared to non-infected children. Children who did not wear shoes were 2.602 times (95% CI: 1.453-4.660, p=0.001) more likely to become stunted, compared to those children who wore shoes outside the house. Children with dirty nails were 1.956 times (95% CI: 1.163-3.289, p=0.011) more likely to become stunted as compared to those children with clean nails. Families without piped water inside the house were 2.395 times (95% CI: 1.047-5.476, p=0.039) more likely to have stunted children, compared to those with piped water.

DISCUSSION

This study showed a high prevalence of stunting (35.6%) among the OA children. The prevalence obtained in this study was comparatively higher than a study conducted among OA children in Raub, Pahang which reported 28.0% (Ahmed *et al.*, 2012). In contrast, a few other studies presented a higher prevalence of stunting among OA children, ranging from 41.0% to 64.0% (Chua *et al.*, 2012; Geik, Sedek & Awang, 2016; Wong *et al.*, 2015). The difference might be due to factors including locations and sub-tribes studied. The current study included mainly Temuan, while other studies included Jah-hut and Temiar sub-tribes. The Jah-hut and Temiar usually live close to or in the forest and are involved in gathering and hunting, while the Temuan adopt agriculture practices and manages their own rubber or oil palm (Masron *et al.*, 2013). According to Anuar *et al.* (2012), the Temuan have better housing conditions and provision of basic amenities, compared to the Jah-hut and Temiar. Nevertheless, the high prevalence of stunting in this study reflects the persistence of poor nutrition and the high prevalence of infections among the OA children (Chua *et al.*, 2012; Geik *et al.*, 2016).

Consistent with previous studies (Subramanian *et al.*, 2009; Felisbino-Mendes, Villamor & Velasquez-Melendez, 2014), the present study showed that stunted growth of mothers was closely associated with stunting in children. According to a related finding, mothers of short stature were discovered to produce children with short height (Felisbino-Mendes *et al.*, 2014). The association between low maternal height and childhood stunting may be explained by as the shorter mothers having a smaller uterine size which may lead to inadequate nutrient supply to the foetus (Zhang *et al.*, 2007). Consequently, this may also result in biological changes including membrane stretching and cervical restriction that increase the possibility of preterm delivery, low birth weight, and other health outcomes (Subramanian *et al.*, 2009). This association suggests an intergenerational transfer of poor health from mothers to their children (Subramanian *et al.*, 2009).

This study also showed that children with low birth weight had higher likelihood to become stunted. The tendency of children with low birth weight having poorer height status was reported in other studies (Wong, Moy & Sulochana, 2014; Rahman *et al.*, 2016). For instance, Rahman *et al.* (2016) explained that children with low birth weight had significantly increased risk of malnutrition after controlling for confounders. Similarly, low birth weight was significantly associated with malnutrition among Malaysian children (Wong *et al.*, 2014). As mentioned earlier, biological changes of stunted mothers could be one of the reasons that might increase the risk of low birth weight and the occurrence of malnutrition. One possible link identified between these variables was low birth weight children are more susceptible to various infections, diseases, loss of appetite, and

poor nutrition as compared to normal birth weight children (Rahman *et al.*, 2016).

The association between children's haemoglobin levels and stunting was significantly shown in the present study, even after controlling the confounding variables. A similar association was observed in several other studies. For instance, Leite *et al.* (2013) presented that height-for-age was associated with anaemia among indigenous children in Brazil. Another study found that Kuwaiti preschool children aged 4-5 years who were moderately and severely stunted were 2.3 times more prone to be anaemic (Al-Qaoud *et al.*, 2015). According to Leite *et al.* (2013), stunting and anaemia are usually affected by a set of common causes including socioeconomic status, sanitation and parasitic disease.

The current finding depicted that parasitic infection was significantly related with stunting whereby the majority were infected by *Trichuris trichuria*. However, no significant association between the types of parasites and stunting was found and this is inconsistent with other studies. Sanchez *et al.* (2013) revealed that children with more than one parasites and moderately susceptible to heavy infections were associated with decreasing weight-for-age and height-for-age. Another study among OA school children in Raub, Pahang also depicted that *Ascaris* and *Trichuris* infections were significant predictors for stunting (Ahmed *et al.*, 2012). Parasitic infections were common in the OA children due to their exposure to impoverished living conditions such as poor sanitation and hygiene, poor housing and overcrowding problem. It is also important to note that heavily infected children usually have reduced appetite and poor absorption of micronutrients such as iron and iodine, which eventually leads to stunting (Hall, Hewitt, Tuffrey & De Silva, 2008).

With respect to sanitation facility and personal hygiene, it was found that children who did not wear shoes outside their house, did not have piped water inside the house and had dirty nails were at a significantly higher likelihood of becoming stunted, compared to non-stunted children. Similar to these findings, Wolde, Berhan & Chala (2015) reported that children with poor personal hygiene exhibited higher likelihood to become stunted. Poor sanitation and hygiene also increase the vulnerability of the children to parasitic infections which might cause them to have poor appetite and nutrition-deficient. On the other hand, findings revealed that hand washing using soap and availability of toilet were not significantly associated with stunting. This is in line with the findings of a study among OA pre-schoolers in Gua Musang, Kelantan, where presence of toilet was concluded to be not significantly associated with stunting (Geik *et al.*, 2016). This is mainly due to their habit of not utilising the toilet. Instead, the drain, river or bushes seems more preferable for their daily defecation. However, the children in this study were not affected by this practice as there exists a possibility of their parents cleaning them up after defecation, thus reducing the risk of infection (Geik *et al.*, 2016).

As this was a cross-sectional study, the causality between factors associated with childhood stunting could not be established. As haemoglobin concentration was used as a proxy indicator of anaemia, further studies should include serum ferritin and iron as indicators in order to identify the different types of anaemia. A sufficiently sensitive technique should be used in the future to increase parasite detection rate particularly with light infections. Egg counts should also be included in the future studies to determine the intensity of parasitic infections.

Despite these limitations, the current study has specific identified contributing factors to childhood stunting in indigenous populations, which may be useful for interventions to improve the nutritional status of OA children.

CONCLUSION

Childhood stunting remains a significant public health concern among OA children. This study highlighted early nutrition and environmental factors were crucial aspects in connection with stunting in OA children. Therefore, these identified factors should be addressed in nutrition improvement programmes for OA children.

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Authors' contributions

Siti Fatihah M, carried out data collection, data analysis, data interpretation, and drafted the manuscript; Gan WY, principal investigor, conceptualized and designed the study, collected data, prepared the draft of the manuscript and reviewed the manuscript; Norhasmah S, assisted in drafting of the manuscript and reviewed the manuscript; Zalilah MS, assisted in drafting of the manuscript, reviewed the manuscript.

Conflict of interest

The authors have no conflict of interest.

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Correlations between anthropometric measurements, biochemical indicators, dietary intake and Dialysis Malnutrition Score among haemodialysis patients in Sibu, Sarawak

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ABSTRACT

Introduction: Malnutrition is a common problem associated with increased risk of morbidity and mortality among haemodialysis (HD) patients. **Methods:** This study determined the correlation between anthropometric measurements, biochemical indicators, dietary intake and dialysis malnutrition score among HD patients in Sibu, Sarawak. A total of 55 patients were recruited by purposive sampling and their biochemical parameters were retrieved from dialysis records. Anthropometric measurements and dietary intake were determined using standardised protocols while Dialysis Malnutrition Score (DMS) was computed to determine patients' nutritional status. **Results:** Mean age of the patients was 53.0±12.2 years. Mean DMS was low, indicating low tendency of malnutrition among the patients. Approximately one-third of the patients had high interdialytic weight gain (IDWG), indicating a poor adherence on fluid recommendation. Mean intakes of dietary energy (DEI) and protein (DPI) were low, with only approximately 15% achieving the recommendations according to Kidney Disease Outcomes Quality Initiative (K/DOQI). Increase in age (r=0.337, p=0.012) and dialysis vintage (r=0.403, p=0.002) were associated with poorer nutritional status while higher BMI, MUAC, and serum albumin were associated with better nutritional status. **Conclusion:** This study revealed a high proportion of the HD patients with poor adherence on fluid intake, and the prevalence of inadequate DEI and DPI, indicating the importance of regular dietary counselling for HD patients. In view of their non-invasive nature and close relationship with nutritional status, body mass index, mid-upper arm circumference, and serum albumin should be included as part of the comprehensive periodic nutrition assessment of HD patients.

Keywords: Haemodialysis, Dialysis Malnutrition Score, dietary intakes, anthropometric parameters

INTRODUCTION

Chronic kidney disease (CKD) is defined as the progressive loss of kidney functions and performance of nephrons over a period of at least three months and leading to permanent damage to the kidneys (Kidney Disease: Improving Global Outcomes, 2012). When the glomerular

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filtration pressure and hyperfiltration keep increasing due to fewer functional nephrons, hyperfiltration will accelerate the evolution of CKD to end stage renal disease (ESRD) (McPhee & Ganong, 2006). ESRD is defined as total and permanent loss of kidney function at which renal replacement therapy is required to sustain life.

Haemodialysis (HD) is a long term renal replacement therapy, which replaces the renal functions partially. It requires a fistula created via surgery access the bloodstream by connecting an artery and a vein. During the HD process, waste products and electrolytes will be removed by diffusion, ultrafiltration, and osmosis from the blood to the dialysate (Mahan, Escott-Stump & Raymond, 2012). Globally, by the end of 2014, HD remains as the most common treatment modality among the dialysis population compared to peritoneal dialysis, with around 88% of all incident ESRD cases began the renal replacement therapy with HD (Saran *et al.*, 2017). A similar situation prevails in Malaysia where more than 90% of dialysis patients were on HD treatment between 2005 and 2014 (Goh *et al.*, 2015). Nonetheless, the survival rate of HD patients decreased with prolonged dialysis vintage (Wong & Ong, 2015). Together with lower survival rate, protein energy wasting (PEW) is very common among HD patients. Malnutrition is common among HD patients, varying widely from 29-91%, depending on the population studied (Chan, Kelly, Batterham & Tapsell, 2012; Janardhan *et al.*, 2011; Mohammed, Farhood & AtheemWtwt, 2014). Harvinder *et al.* (2016) reported majority of HD patients were malnourished, regardless of the nutritional status assessment tools used.

Assessment of nutritional status is an integral part of care for CKD patients to provide early nutrition intervention to those who are at risk of malnutrition. The Dialysis Malnutrition Score (DMS), a modified Subjective Global Assessment (SGA) tool to detect the presence of malnutrition, was recommended by European Best Practice Guidelines (EBPG) on Nutrition (Fouque *et al.*, 2007) and Kidney Disease Outcomes Quality Initiative (K/DOQI) (2000) as a predictor tool of malnutrition in HD patients. DMS has also been suggested to be a more practical tool in Malaysia dialysis settings due to its relatively quick, easy, inexpensive to perform, more objective than SGA, and requires no laboratory markers (Harvinder *et al.*, 2016; K/DOQI, 2000). Several studies among Asian dialysis population have reported DMS as a useful and reliable index to detect malnutrition (Harvinder *et al.*, 2016; Janardhan *et al.*, 2011).

While available data indicates a continual increase in the dialysis treatment rate in Sarawak, Malaysia (Goh *et al.*, 2015), studies on nutritional status among HD patients in Sibu, Sarawak are scarce. This study was carried out to determine the nutritional status of HD patients by using the DMS and its correlation with anthropometric measurements, biochemical indicators, and dietary intake.

MATERIALS AND METHODS

This was a cross-sectional study that employed purposive sampling based on pre-determined inclusion criteria for the selection of HD patients. A total of 55 HD patients with informed consent were recruited from SJAM-KPS Haemodialysis Centre 8 (Sibu) Sarawak in Jan-Feb, 2014. All recruited patients met the inclusion criteria of: (1) above 21 years old; (2) undergone HD treatment thrice weekly for at least three months; (3) ability to communicate in Malay, English or Mandarin language. Patients were excluded if they presented with psychological problems such as

dementia and mental illness; hospitalised in the past one month prior to study enrolment; and had hepatitis previously. Ethical approval was obtained from the Ethics Committee for Research Involving Human Subjects, Universiti Putra Malaysia (project identification: UPM/TNCPI/RMC/1.4.18.1 (JKEUPM)/F2). Subject anonymity and confidentially were maintained.

A pre-tested questionnaire was administered to obtain information on socio-demographic background of the patients, clinical history such as dialysis vintage, presence of co-morbidity, and accessibility with dietitians. Patients' body weight and height were measured using Detecto 6868 Bariatric Flip Seat Scale and Stand-alone Stadiometer (SECA 214, Germany), respectively. Body mass index (BMI) of patients was computed using Weight (kg)/(Height x Height) (m^2) formula based on dry weight. Presence of PEW was ascertained when BMI was less than 18.5 kg m^{-2} (Kanazawa *et al.*, 2017). This cut-off is adopted after taken into consideration the recommendation from the International Society of Renal Nutrition and Metabolism (ISRNM), as well as the adjustment made for diagnostic criterion for PEW for Southeast Asian HD patients (Kanazawa *et al.*, 2017).

As an indicator of fluid compliance, interdialytic weight gain (IDWG) was calculated by subtracting post-dialysis weight of the previous dialysis session from the pre-dialysis weight (Bots *et al.*, 2004). This was then compared to the recommendation by EBPG on Nutrition (Fouque *et al.*, 2007), with an IDWG of 4 to 4.5% is considered as acceptable range. Mid-upper arm circumference (MUAC) of the non-fistula arm was measured using a flexible, non-stretchable measuring tape at the midpoint of the upper arm, between acromion and olecranon process, after completion of dialysis session. A MUAC of ≥23 cm is desired as MUAC of <23 cm was strongly associated with BMI <18.5 kg m^{-2} and with increased risk of malnutrition as well as mortality (Tang *et al.*, 2013). Serum albumin and total cholesterol for the last three measurements were obtained retrospectively from medical record as secondary data. The desirable serum albumin levels and total cholesterol were ≥40 g/L and 3.9-5.2 mmol/L, respectively, based on K/DOQI guidelines (2000).

Dietary intake on non-dialysis day of the patients was obtained through 24-hour dietary recall. The quantity of food consumed by the patients was estimated using household measurement tools. Standard calibrated household measuring cups, glasses, spoons and bowls were used during the interview session to help the patients to estimate food portions. Consumed foods and drinks were converted into grams before nutrient analysis using Nutritionist Pro™ Diet Analysis software: Version 2.4.1 (Axxya, USA), with USDA Food Database and Malaysian Food Composition Tables (Tee *et al.*, 1997) as the food databases. Food labels were used whenever possible. Adequacy of dietary intakes (total energy, protein, fluid, sodium, potassium, phosphorus, and calcium) were compared with K/DOQI Recommendations for Nutritional Management (2000) and EBPG (2007).

The nutritional status of the patients was assessed using a fully quantitative scoring system (DMS) developed by Kalantar-Zadeh *et al.* (1999). It comprised five components of medical history (weight change, dietary intake, gastrointestinal symptoms, functional capacity, and co-morbidity) and two components of physical assessments (loss of subcutaneous fat and signs of muscle wasting). The scoring scheme used is described below:

- For 'Weight change' component, the overall change in the post-dialysis

dry weight in the past six months was considered as follow. Score of 1 was given if there was no weight change or if the patient had gained weight. Minor weight loss (<5%), weight loss of 5-10%, weight loss of 10-15%, or any weight loss over 15% during the last six months was given a score of 2 to 5, respectively.

- For 'Dietary intake' component, 1 score was given if it was a regular solid intake with no recent changes in the amount of meals, a score of 2 for sub-optimal solid diet, 3 for full liquid diet or moderate overall decrease, 4 for hypo-caloric liquid and 5 for starvation.
- For 'Gastrointestinal symptoms' component, patients were given a score of 1 if there were no symptoms, 2 for nausea, 3 for vomiting or moderate gastrointestinal symptoms, 4 for diarrhoea and 5 for severe anorexia.
- For 'Functional capacity' component, a score of 1 if patients had normal functional capacity or any improvement in the level of previous functional impairment, 2 for difficulty with ambulation, 3 for difficulty with normal activity, 4 for restricted to solely light activity and 5 for persistent bed/ chair-ridden with no or little activity.
- Patients who had been dialysed for less than one year or healthy otherwise will be given a score of 1 for 'Co-morbidity' component. This was followed by a score of 2 if the subject had been dialysed for 1 to 2 years or if there was any mild co-morbidity, 3 if the subject had been dialysed for 2 to 4 years or if there was moderate co-morbidity or if the patient aged more than 75 years old, 4 if the subject had been dialysed for more than 4 years or if there was severe co-morbidity, and 5 if there were very severe and multiple co-morbidities.

The second part of the DMS comprised physical examination. 'Body fat stores or subcutaneous fat' was determined by assessing the fat deposition below the eyes, triceps, biceps and in the chest area. Sign of muscle wasting was determined by examining temple, clavicle, scapula, ribs, quadriceps, knee and interosseous muscles. Both components under physical examination were given a score of 1-5 to represent normal to very severe changes. Each component had a score of 1 (normal) to 5 (severe). Summation of the scores of the seven components provides a continuous score with possible range of 7 (normal) to 35 (severely malnourished), with higher score indicates higher risk of PEW (Kalantar-Zadeh *et al.*, 1999), and reflects a more severe degree of PEW. The approval of DMS use in this study was obtained from the author.

Statistical analysis

The results were analysed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., USA). Pearson product-moment correlation coefficient analysis was carried out for parametric data while Spearman's rank correlation coefficient was performed for non-parametric data to determine correlations between DMS of patients and anthropometric measurements, biochemical indicators, and dietary intake. Independent-samples *t*-test was performed to compare the mean of two groups. Statistical significant was set at p-value<0.05. Based on calculation according to Cole (1997), with a total of 55 patients, this study achieved study power of 80% and sensitivity and specificity of 90% for the correlation between DMS and related variables.

RESULTS

A total of 55 patients comprising 74.5% men was included in the study (Table

1). Their mean age was 53.0±12.2 years, ranging from 28 to 78 years. Majority were Chinese (58.2%), followed by *Bumiputera* (34.5%) and Malay (7.3%). More than 80% of them were married. Approximately 62% had moderate to high educational levels while 12.7% had no formal schooling.

Close to 90% of the patients were unemployed, while the rest were employed or working in family business. The working patients had monthly income of less than Ringgit Malaysia (RM) 2,300. Approximately 70% of the patients had household monthly income less than RM2,300 while the rest earned between RM2,300 to RM5,599. These income levels are respectively classified as low income and middle-income households based on the Tenth Malaysia Plan. Many of the patients were economically dependent on government subsidies, charity organisations, Social Security Organisation (SOCSO), and support from family members and relatives.

Majority of the patients (81.8%) suffered from co-morbidities along with kidney failure with 40.0% of them presented with more than one co-morbidities. Hypertension was most prevalent (76.4%), followed by diabetes mellitus (40.0%). Mean dialysis vintage was 2.67±0.64 years with a range of 3 months to 14 years. Approximately three quarters of them had at least a previous encounter with dietitians, indicating relatively high accessibility of the patients to dietitians.

Low mean DMS (11.85±2.26) indicated that the patients had a low risk of malnutrition. The DMS of women (12.64±2.59) was approximately 1.1 units higher than men (11.59±2.10), suggesting that women had a higher risk of malnutrition. Similarly, DMS in older patients (12.79±2.58) was higher than their younger counterparts (11.54±2.08), suggesting a poorer nutritional status among the older patients. However, the mean differences of DMS were not significant between sex and age groups.

Mean BMI and MUAC of the patients were 23.8±4.1 kg m^{-2} and 27.2±3.4 cm, respectively (Table 2). One-third of them exceeded the recommended IDWG of 4.5%, whereby more men and younger patients with IDWG greater than 4.5%. There was no sex-specific difference after adjustment for dry weight. In general, mean serum albumin and cholesterol levels of the patients were within the desirable range, with no significant differences between sex and the age groups. Nonetheless, approximately 10% and 30% patients had hypoalbuminemia and hypocholesterolemia, respectively.

Mean DEI and DPI of the patients were inadequate (Table 3), with only 14.5% and 16.4% achieved the recommendations, respectively (Table 4). Mean fluid intake was 922±384 ml/day, ranged widely from 600 to 3250 ml/day. Approximately 40% of the patients had excessive sodium intake according to K/DOQI (2000) and EBPG (2007) recommendations, with significantly higher sodium intake in men. Primary food sources of sodium included hawker foods, processed foods, and frequent use of condiments. Mean potassium intake was below the recommended level of 1950 to 2730 mg/day. Mean phosphate intake was low (543.5±210.5 mg/day) with significant higher intake among men (t=3.383, p=0.002). While mean dietary calcium intake (187.9±76.5 mg/day) was well below the recommended level of 500 mg/day, 61.8% of the patients reported excess calcium intake, mainly from supplements.

Pearson product-moment correlation coefficient analysis (Table 5) showed medium but significant positive correlation between age and DMS (r=0.337, p=0.012), independent of the presence of co-morbidities, suggesting

Table 1. Distribution of patients by socio-demographic and clinical backgrounds (*n*=55)

Variables	*Number, n (%)*	*Mean±SD*	*Range*
Sex			
Male	41 (74.5)		
Female	14 (25.5)		
Ethnicity			
Chinese	32 (58.2)		
Bumiputera[†]	19 (34.5)		
Malay	4 (7.3)		
Age group (years)		53.0±12.2	28-78
<60	41 (74.5)		
≥60	14 (25.5)		
Marital status			
Single	8 (14.6)		
Married	46 (83.6)		
Widow/ Widower	1 (1.8)		
Educational level			
No formal education	7 (12.7)		
Primary school	14 (25.5)		
Secondary school	29 (52.7)		
Tertiary	5 (9.1)		
Working status			
Unemployed	49 (89.0)		
Part time	3 (5.5)		
Full time	3 (5.5)		
Personal monthly income[‡]			
<RM 2300	55 (100.0)		
RM 2300-RM 5599	0 (0.0)		
≥RM 5600	0 (0.0)		
Household monthly income[‡]			
<RM 2300	39 (70.9)		
RM 2300-RM 5599	16 (29.1)		
≥RM 5600	0 (0.0)		
Presence of co-morbidity			
Yes	45 (81.8)		
No	10 (18.2)		
Number of co-morbidity			
0	10 (18.2)		
1	23 (41.8)		
≥2	22 (40.0)		
Co-morbidities[§]			
Hypertension	42 (76.4)		
Diabetes mellitus	22 (40.0)		
Dyslipidaemia	9 (16.4)		
Cardiovascular disease	2 (3.6)		
Anaemia	1 (1.8)		
Gout	1 (1.8)		
Dialysis vintage		2.67±0.64 years	3 months-14 years
Encounters with dietitians			
Yes	40 (72.7)		
No	15 (27.3)		

[†]Natives of Sarawak
[‡]Classified according to 10th Malaysia Plan, US$1.00=RM3.90
[§]Multiple responses

Table 2. Anthropometric measurements, biochemical indicators and Dialysis Malnutrition Score of patients according to sex and age (years) (n=55)

Measurements	*Age<60 (n=41)*	*Age≥60 (n=14)*	*Male (n=41)*	*Female (n=14)*	*Total*	*Range*
Height (cm)	161.8±0.1	157.2±0.1	162.1±0.1	156.2±0.1	160.6±1.0	149-179
Dry weight (kg)	63.6±14.9	56.4±10.7	64.5±15.0	53.9±7.6	61.8±14.3	37.0-100.5
Body Mass Index (BMI) (kg m^{-2})	24.1±4.3	22.7±3.5	24.4±4.5	22.0±2.1*	23.8±4.1	15.0-32.4
<18.5	4 (9.8)	2 (14.3)	5 (12.2)	1 (7.1)	6 (10.9)	
≥18.5	37 (90.2)	12 (85.7)	36 (87.8)	13 (92.9)	49 (89.1)	
Interdialytic Weight Gain (IDWG) (kg)	2.8±1.1	2.2±0.6*	2.8±1.0	2.1±1.0*	2.6±1.0	0.4-6.3
Interdialytic Weight Gain (IDWG) (%)	4.4±1.6	3.9±0.9	4.4±1.4	3.9±1.6	4.3±1.4	0.7-7.9
<4	16 (39.0)	6 (42.9)	16 (39.0)	6 (42.8)	22 (40.0)	
4-4.5	8 (19.5)	6 (42.9)	10 (24.4)	4 (28.6)	14 (25.5)	
>4.5	17 (41.5)	2 (14.2)	15 (36.6)	4 (28.6)	19 (34.5)	
Mid-Upper Arm Circumference (MUAC) (cm)	27.6±3.7	26.2±2.3	27.5±3.6	26.4±2.8	27.2±3.4	19.6-35.4
<23	6 (14.6)	1 (7.1)	5 (12.2)	2 (14.3)	7 (12.7)	
≥23	35 (85.4)	13 (92.9)	36 (87.8)	12 (85.7)	48 (87.3)	
Serum albumin (g/L)	43.1±2.6	42.2±2.8	42.9±2.9	42.5±1.9	42.8±2.7	35.0-48.0
<40	5 (12.2)	2 (14.3)	7 (17.1)	0 (0.0)	7 (12.7)	
≥40	36 (87.8)	12 (85.7)	34 (82.9)	14 (100.0)	48 (87.3)	
Total cholesterol (mmol/L)	4.3±1.0	4.1±0.6	4.1±0.8	4.6±1.0	4.2±0.9	2.4-6.5
<3.9	14 (34.1)	5 (35.7)	14 (34.1)	5 (35.7)	19 (34.5)	
3.9-5.2	20 (48.8)	8 (57.2)	23 (56.1)	5 (35.7)	28 (50.9)	
≥5.2	7 (17.1)	1 (7.1)	4 (9.8)	4 (28.6)	8 (14.6)	
Dialysis Malnutrition Score (DMS)	11.54±2.08	12.79±2.58	11.59±2.10	12.64±2.59	11.85±2.26	9-18

Data were presented as mean±SD or n (%).
*Independent t-test is significant at p<0.05.

Table 3. Mean daily dietary intake among patients according to sex and age (years) (*n*=55)

Nutrients	*Mean±SD*				*Mean±SD (Range)*
	Male (n=41)	*Female (n=14)*	*Age<60 (n=41)*	*Age≥60 (n=14)*	*Total*
Energy (kcal/kg/day)	21±9	19±5	21±8	20±8	21±8 (6-44)
Protein (g/kg/day)	0.82±0.43	0.64±0.23	0.82±0.43	0.65±0.27	0.77±0.39 (0.13-2.08)
Fluid (ml/day)	979±428	756±85	891±224	1014±667	922±384 (600-3250)
Sodium (mg/day)	2398.7±942.3	1715.1±656.4*	2234.4±882.5	2196.3±1067.8	2224.7±922.9 (1054-4533)
Potassium (mg/day)	1118.3±502.9	926.1±428.2	1129.6±461.4	893.2±539.7	1069.4±488.5 (245-2529)
Phosphate (mg/day)	583.1±222.0	427.4±113.3*	564.0±204.6	483.4±223.7	543.5±210.5 (128-951)
Calcium (mg/day)	1937.8±726.1	1820.4±603.7	2085.8±584.1	1387.1±746*	1907.9±693.5 (64-3408)
Diet	201.2±77.6	148.9±60.0*	198.0±76.5	158.6±71.3	187.9±76.5 (53-371)
Supplement	1763.6±716.2	1671.4±585.0	1887.8±583.2	1228.6±727*	1720.0±680.5 (0-3200)

*Independent-samples *t*-test is significant at $p<0.05$.

Table 4. Adequacy of dietary intake among patients according to sex and age (years) (*n*=55)

Nutrients	*Recommendations*	*Number of patients achieved recommendations, n (%)*				
		Age<60 (n=41)	*Age≥60 (n=14)*	*Male (n=41)*	*Female (n=14)*	*Total (n=55)*
Energy (kcal/kg/day)	35 for age<60† 30-35 for age≥60†	3 (7.3)	1 (7.1)	7 (17.1)	1 (7.1)	8 (14.5)
Protein (g/kg/day)	≥1.1‡	9 (22.0)	0 (0.0)	8 (19.5)	1 (7.1)	9 (16.4)
Fluid (ml/day)	500-750‡	16 (39.0)	5 (35.7)	12 (29.3)	9 (64.3)	21 (38.2)
Sodium (mg/day)	2000-2300‡	4 (9.8)	0 (0.0)	3 (7.3)	1 (7.1)	4 (7.3)
Potassium (mg/day)	1950-2730‡	1 (2.4)	1 (7.1)	2 (4.9)	0 (0.0)	2 (3.6)
Phosphate (mg/day)	800-1000†,‡	3 (7.3)	3 (21.4)	6 (14.6)	0 (0.0)	6 (10.9)
Elemental calcium (mg/day)	<2000†,‡	20 (48.8)	11 (78.6)	22 (53.7)	9 (64.3)	31 (56.4)
Diet	<500	41 (100.0)	14 (100.0)	41 (100.0)	14 (100.0)	55 (100.0)
Supplement	<1500	12 (29.3)	9 (64.3)	17 (41.5)	4 (28.6)	21 (38.2)

†K/DOQI (2000)
‡EBPG (2007)

Table 5. Correlation of Dialysis Malnutrition Score with socio-demographic and clinical backgrounds, anthropometric measurements, biochemical indicators, and dietary intake

Variables	*DMS*	
	r	*P*
Socio-demographic and clinical backgrounds		
Age	0.337	0.012*
Sex	0.189	0.167
Ethnic group	-0.039	0.779
Marital status	0.003	0.980
Educational level	-0.223	0.102
Occupation	-0.007	0.958
Household income	-0.166	0.232
Presence of co-morbidity	0.039	0.777
Number of co-morbidity	-0.041	0.764
Dialysis vintage	0.403	0.002**
Encounter with dietitian	-0.100	0.466
Anthropometric measurements		
Mean BMI	-0.459	0.000**
Mean IDWG (%)	0.037	0.788
Mean MUAC	-0.520	0.000**
Biochemical indicators		
Mean serum albumin	-0.284	0.036*
Mean total cholesterol	-0.127	0.356
Dietary intake		
Dietary energy intake	-0.095	0.491
Dietary protein intake	-0.082	0.552
Fluid intake	-0.103	0.455
Sodium intake	-0.100	0.469
Potassium intake	-0.093	0.499
Calcium intake	-0.187	0.172
Phosphate intake	-0.091	0.509

*Correlation is significant at $p<0.05$.
**Correlation is significant at $p<0.01$.

older age was associated with poorer nutritional status. Longer dialysis vintage had a significant positive moderate impact on DMS (r=0.403, p=0.002). BMI (r=-0.459, p<0.01), MUAC (r=-0.520, p<0.01) and serum albumin (r=-0.284, p=0.036) were negatively correlated with DMS. There were no significant correlations between DMS and other variables including education level, household family income or dietary intakes.

DISCUSSION

Malnutrition is often associated with mortality risk (Mohammed *et al.*, 2014). Mean DMS of the current study was relatively lower than that reported by other studies (Janardhan *et al.*, 2011; Mohammed *et al.*, 2014), but was comparable with studies in Malaysia (Harvinder *et al.*, 2016; Sahathevan *et al.*, 2015). Mean BMI of the patients was comparable to the national data among dialysis patients as reported in the 22nd National Renal Registry of Malaysia (Abdul Halim *et al.*, 2015). While morbid obesity should be avoided, higher BMI should be maintained among dialysis population, attributed to the "obesity paradox" or "reverse epidemiology", whereby higher BMI is paradoxically

associated with better survival in patients with ESRD. This survival advantage of large BMI has been consistently reported for HD patients across regional differences (Cabezas-Rodriguez *et al.*, 2013; Wong & Ong, 2015), including Malaysia (Abdul Halim *et al.*, 2015). The current finding of approximately one in ten of the patients were underweight is similar to that in the national data among dialysis patients (Abdul Halim *et al.*, 2015). Despite BMI being not a sensitive marker, a low BMI is associated with higher mortality risk (Abdul Halim *et al.*, 2015) and PEW (Kanazawa *et al.*, 2017), thus patients with BMI below the desirable range should receive close monitoring. Significant mean BMI differences between sex was expected due to the differences in the body composition (Tang *et al.*, 2013). The findings of this study suggested the needs of nutritional intervention such as comprehensive dietary counselling and renal nutritional supplement to improve the body weight status and muscle mass of the HD patients.

Interdialytic weight gain is a common used index in assessing fluid and dietary compliance among HD population, with increased of IDWG often associated with hypertension, acute pulmonary edema, and congestive heart failure (Bots *et al.*, 2004). This study showed comparable proportions of patients with excessive IDWG, fluid and dietary sodium intakes. These findings are not unexpected as excessive dietary sodium intake will increase thirst, leads to higher fluid intake and excessive IDWG eventually (Fouque *et al.*, 2007). Men and younger patients had poorer fluid compliance, which may be attributed to lower health awareness (Chan, Zalilah & Hii, 2012; Park *et al.*, 2008).

Despite its limitations, serum albumin is commonly used as an objective data to identify malnutrition due to low cost and widely available (Friedman & Fadem, 2010). Mean serum albumin of the patients in this study was higher than the national mean reading (Abdul Halim *et al.*, 2015) indicating lower risk of malnutrition among the patients.

Mean total cholesterol of the patients was relatively low, with approximately one in three patients with hypocholesterolemia, compared to the national data among dialysis patients (Abdul Halim *et al.*, 2015). The lower mean cholesterol level and the high proportion of hypocholesterolemia in this study warrant further investigation.

Mean DPI of the studied patients was far lower when compared to other studies and recommended intake of 1.1 to 1.2 g/kg/day (Cupisti *et al.*, 2010; Fouque *et al.*, 2007; K/DOQI, 2000). Inadequate protein intake was associated with increased mortality (Jadeja & Kher, 2012). Reduced food intake could be affected by loss of appetite (Sahathevan *et al.*, 2015) or nausea when toxins removal by dialysis was inadequate (Cupisti *et al.*, 2010) and dietary restrictions (Fouque *et al.*, 2007).

Lower dietary phosphorus intake may be attributed by the low protein intake as food sources high in protein are generally good sources of phosphorus (Fouque *et al.*, 2007). Low dietary calcium intake among the patients may be due to omission of milk or dairy products, with the intention to control serum phosphorus levels (Cupisti *et al.*, 2010). On the other hand, more than half of the patients had excessive calcium intake from supplements. Various dietary restrictions amongst HD patients aimed at keeping IDWG, serum phosphorus and potassium levels within desirable range may have resulted in limited food choices.

Our findings are in concordance with Chan *et al.* (2012) who found that DMS was positively correlated with age and dialysis vintage. Longer HD treatment was associated with poorer nutritional

status as dialysis treatment is a catabolic process. On the other hand, BMI, MUAC, and serum albumin were negatively correlated with DMS, which were in congruence with Kalantar-Zadeh *et al.* (1999), Janardhan *et al.* (2011) and Harvinder *et al.* (2016). However, as BMI does not discriminate between muscle mass and fat mass, we are not able to delineate whether muscle mass or body fat confers the nutritional status advantage in our study.

Studies should investigate further the association between energy or protein intake with DMS. Despite the strong biological plausibility of nutritional interventions to improve health, empirical evidence on their effectiveness or significant correlations with nutrition status in cross sectional studies is lacking (Chen *et al.*, 2013). Possible attributions include day-to-day variations in dietary intake and lack of objective measures of food intakes. The use of one-day food recall in the current study may have also contributed to the lack of significant correlation between DMS and dietary intake in this study.

CONCLUSION

This study revealed overall unsatisfactory dietary intake among the haemodialysis patients, indicating the need for regular individual dietetic counselling and assessment of anthropometric and biochemical status.

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Authors' contributions
Lina Ho LL conceptualized and designed the study, conducted the data collection, analysis and interpretation, and prepared the draft of the manuscript; Chan YM advised on study conceptualization, data analysis and interpretation, and reviewed the manuscript.

Conflict of interest
The authors declare that they have no competing interest.

Glossary of abbreviations
HD – Haemodialysis
SJAM-KPS – St. John Ambulance of Malaysia Kawasan Pantai Selangor
DMS – Dialysis Malnutrition Score
BMI – Body mass index
MUAC – Mid-upper arm circumference
IDWG – Interdialytic weight gain
CKD – Chronic kidney disease
ESRD – End-stage renal disease
PEW – Protein energy wasting
SGA – Subjective Global Assessment
K/DOQI – Kidney Disease Outcomes Quality Initiative
EBPG – European Best Practice Guidelines
ISRNM – International Society of Renal Nutrition and Metabolism
DEI – Dietary energy intake
DPI – Dietary protein intake

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Comparison of dietary intake, energy adequacy and anthropometric parameters between Indian junior male and female hockey players

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ABSTRACT

Introduction: Athletes' performance is highly depended on their nutritional status for optimising performance. This study is aimed at assessing and comparing adequacy intake of nutrients and energy between male and female Indian hockey players. **Methods:** A total of 40 Indian junior national hockey players with an equal number of males and females were selected randomly by the Sports Authority of India, Kolkata. Mean age of males was 18.2±2.3 years while that of females was 17.1±2.2 years. Dietary intake was assessed based on a 3-consecutive-day, 24-hour dietary recall and frequency intake questionnaire. Dietary intake adequacy was determined according to the Recommended Dietary Allowance for India (2010). Energy requirement was estimated by the basal metabolic rate based on the Harris-Benedict formula and multiplied by an index of physical activity. Various anthropometric parameters were assessed using standard procedures. **Results:** Total energy intake was significantly lower in both male (2622±450 kcal) and female (1848±236 kcal) when compared with their total energy expenditure (male: 3621±127 kcal, female: 3049±115 kcal; $p<0.00$). Dietary intake consisted of low fat (male: 53.5±14.01g; female: 34.0±8.33 g) and high carbohydrate (male: 431.7±85.90 g; female: 317.5±45.69 g). Insufficient intake of iron, folic acid, zinc, B-vitamins and vitamin-C were found among female participants, but not in the males. Significant differences were observed in muscle mass and haemoglobin level between the sexes. **Conclusion:** The study revealed inadequate dietary intake among hockey players, especially among the females. Individualised nutritional orientation, nutrition education and dietary interventions are recommended for Indian hockey players towards improving their performance.

Keywords: Hockey players, India, total energy expenditure, vitamins, minerals, nutrition

INTRODUCTION

The importance of nutrition in endurance sports is well established. Athletes' performance is highly depended on their nutritional status; hence, adequate nutrition is necessary to optimise their performance. Hockey is characterised by high intensity passages of play, mixed with low intensity activities, including standing, walking, jogging. Players must perform continuously for 70 minutes with just one 5-10 mins interval. Good aerobic endurance is required to support repetitive bouts of high intensity exercise

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(Bishop *et al.,* 2015). The intermittent high intensity pattern of activity during matches requires a high function of both aerobic and anaerobic energy delivery pathways (Manna *et al., 2010*). Performance during intermittent sports is dependent upon a combination of anaerobic and aerobic energy systems, both of which rely on muscle glycogen and/or blood glucose as an important substrate for energy production (Baker *et al.,* 2015).

The role of a balanced diet is well recognised for helping to maximise the physical efficiency of bodily function and hence improve the effectiveness of training. All the macro, micro nutrients, fluids and electrolytes play a decisive role in body composition of athletes (Thomas *et al.*, 2013). High intensity training demands a higher nutritional need. Moreover, because of the heightened requirement for micro- and macro-nutrients, during training, athletes are often much more vulnerable to any deficiencies, compared to the general population (Nowacka *et al.,* 2010). Recent studies describe that an athlete's diet is not fulfilling the energy (Coutinho *et al.,* 2016; Sangeetha *et al.,* 2014), carbohydrates requirements (Wardenaar *et al.* 2017) and also deficit in intake of vitamins and minerals (Wardenaar *et al.*, 2017, Raizel *et al.*, 2017).

In India, there are limited published data on the nutritional assessment and dietary intake of elite Indian hockey players. Hence, the aim of the present study was to compare dietary intake, energy intake versus energy expenditure, and anthropometric parameters between Indian male and female junior players, in Kolkata.

MATRERIALS AND METHODS

Forty young hockey players (20 male and 20 female) with an age range of 13-22 years representing India were selected randomly by the Sports Authority of India (SAI) in Kolkata. These players were at least state level performers with minimum of 3-4 years of formal training history. They belonged to almost the same socio-economic status and have similar dietary intake during training. Participants signed an informed consent form before the verbal interview and testing. Inclusion criteria included being medically fit with no history of hereditary and cardio respiratory diseases. All participants were clinically examined by the SAI physicians, who specialised in Sports Medicine (Debnath *et al.*, 2016). Various anthropometric parameters were assessed in the Human Performance Laboratory at Sports Authority of India, Kolkata.

Training regimen

The training programme applied to the present subjects consisted of aerobic and anaerobic training, scrimmaging, and different resistance training along with flexibility exercises. By and large, all the players underwent training on an average duration of 4-5 hours a day. One-hour training session both in the morning and afternoon was fixed for all the players to improve the physical fitness component while the rest of the sessions were fixed for skill/technical and tactical training. Total training period was about 30 hours in a week excluding Sunday. Players had also undergone mental training sessions in addition to the physical and skill/technical training programmes.

Dietary assessment

The dietary assessment was based on three consecutive 24-hours dietary recall method. This is the most commonly used dietary assessment method to estimate dietary intake (Shim *et al.,* 2014; Wierniuk & Włodarek, 2013). The serving sizes of the meals consumed by athletes were recorded according to home-based

measurements followed by conversion into grams and milligrams. The 'Diet soft' Software package (Invincible Ideas, Delhi) was used to determine calorie and the nutritive values of foods consumed based on Indian standards (Narasinga & Sivakumar, 2010).

Energy expenditure

Basal metabolic rate (BMR) was calculated using modified Haris-Benedict equation (Wierniuk & Włodarek, 2013) followed by multiplying an index of physical activity, assumed here as 2.3, for individuals performing heavy physical activity (Narasinga & Sivakumar, 2010).

Anthropometric measurements

Height (cm) and body weight (kg) were measured by anthropometric rod and digital weighing instruments respectively, using standard procedures (Debnath *et al.*, 2016). Body Mass Index (BMI) was calculated from body height and weight measurements.

Body composition including lean body mass (LBM), fat free mass (FFM), fat mass (%) were measured by using a multi-frequency bioelectrical impedance analyser (Maltron Bioscan 920- 2, Made in UK) (Bolanowski & Nilsson, 2001). Total body electrical impedance to an alternate current (0.2 mA) with four different frequencies (5, 50, 100 and 200 KHz) was measured. Measurements were taken using the standard testing manual of Maltron International. The laboratory tests were performed at a room temperature varying from 23-25°C with the relative humidity varying between 50-60%.

Statistical analysis

Data were analysed using the SPSS software version 16.0 for Windows (IBM Corp., USA). All values expressed as means±standard deviation (SD). A confidence level at 95% (p<0.05) was considered as significant. Parametric test one-way ANOVA was done for normally distributed data. As the nutrient consumption data was not normally distributed, non-parametric Mann–Whitney test was used to study the differences between male and female hockey players.

RESULTS

Table 1 represents descriptive statistics of all the anthropometric and nutritional parameters of both the male and female Indian hockey players respectively. There is no significant difference in the mean age between the sexes, while a significant difference was shown in the height and weight, but not in their mean body mass index (BMI) status (Table 2). Similarly, fat free mass was also found to be higher in male players (87.6±5.82%) than the female (83.0±7.77%), but the difference was statistically insignificant. Haemoglobin (Hb) level was significantly higher in male players as compared to their female counterparts.

Table 3 represents the intake of macronutrients of male and female hockey players. The total energy consumption was significantly higher in the males (2622±450 kcal) than female athletes (1848±236 kcal). The protein intake of the male players was significantly higher (110.9±15.20 g and 77.4±9.74 g, respectively). The average protein intake was adequate among both groups (>1.5 g/kg body weight). A significant difference was also observed in total fat and carbohydrate consumption between the sexes. Carbohydrates provide 65% of total calorie intake, while fat and protein provide 18% and 17% respectively among the male players. On the other hand, carbohydrates, fat and protein contributed 67%, 16% and 17% of total calories, respectively for the female players.

Table 1. Anthropometric and nutritional parameters of Indian junior male and female hockey players

Parameters	*Male*					*Female*				
	Range	*Minimum*	*Maximum*	*Mean±SD*	*Std. Error*	*Range*	*Minimum*	*Maximum*	*Mean±SD*	*Std. Error*
Age (yrs)	7.4	12.8	20.2	17.1±2.21	0.50	7.9	14.0	21.9	18.2±2.27	0.51
Height (cm)	21.0	156.0	177.0	168.4±1.24	5.54	10.0	153.0	163.0	158.3±3.48	0.78
Weight (kg)	17.0	53.0	70.0	58.4±0.91	4.07	17.0	37.0	54.0	48.6±4.86	1.09
BMI	4.1	18.7	22.8	20.6±0.28	1.26	6.2	15.8	22.0	19.5±1.77	0.40
Muscle mass (kg)	23.1	70.0	93.1	87.6±1.30	5.82	32.5	64.0	96.5	83.0±7.77	1.74
Fat mass (%)	23.0	6.9	30.0	12.4±1.29	5.78	35.5	3.5	39.0	17.3±8.50	1.90
Haemoglobin (g/dl)	2.0	11.4	13.4	12.3±0.13	0.57	2.6	9.4	12.0	10.6±0.69	0.15
Energy (kcal)	1285	2013	3298	2622±450	101	908	1428	2336	1848±236	53
Protein (g)	50.5	83.7	134.2	110.9±15.20	3.40	36.0	56.9	92.9	77.4±9.74	2.18
Fat (g)	47.1	37.4	84.6	53.5±14.01	3.13	31.2	18.6	49.8	34.0±8.33	1.86
Carbohydrate (g)	283.2	293.0	576.2	431.7±85.90	19.21	163.2	235.9	399.1	317.5±45.69	10.22
Dietary fibre (DF) (g)	8.6	9.5	18.0	13.6±2.31	0.52	5.0	7.6	12.6	11.1±1.97	0.44
Insoluble DF (g)	6.6	6.9	13.5	10.8±1.99	0.44	4.3	6.1	10.5	9.0±1.83	0.41
Soluble DF (g)	2.4	2.2	4.6	2.8±0.67	0.15	0.6	1.5	2.1	1.9±0.22	0.05
Calcium (mg)	900.2	500.7	1400.8	845.5±358.86	80.24	386.3	490.6	876.9	678.0±81.32	18.18
Phosphorous (mg)	1188.3	1720.1	2908.3	2364.0±384.97	86.08	779.9	1221.7	2001.6	1594.6±237.91	53.20
Iron (mg)	18.4	10.8	29.2	20.0±4.92	1.10	16.2	1.2	17.4	11.2±3.78	0.85
Zinc (mg)	7.2	6.5	13.7	10.1±2.49	0.56	3.7	5.2	9.0	6.9±1.19	0.27
Beta carotene (µg)	2756.0	652.6	3408.6	1674.6±779.02	174.19	718.5	391.7	1110.3	683.5±210.32	47.03
Retinol (µg)	457.0	426.0	883.0	620.0±165.68	37.05	84.0	378.0	462.0	415.2±27.23	6.09
Thiamine (mg)	2.0	0.5	2.6	1.7±0.53	0.12	1.0	0.4	1.4	0.8±0.36	0.08
Riboflavin (mg)	1.3	0.5	1.7	1.2±0.27	0.06	1.0	0.0	1.0	0.7±0.26	0.06
Niacin (mg)	17.4	3.4	20.8	12.6±4.49	1.00	7.9	4.1	12.0	8.5±1.74	0.39
Pyridoxine (mg)	0.7	0.9	1.6	1.1±0.20	0.04	0.5	0.4	0.9	0.6±0.11	0.03
Folic acid (µg)	190.1	207.8	398.0	281.2±51.75	11.57	83.3	139.6	222.8	180.0±27.88	6.24
Vitamin C (mg)	108.5	26.4	134.9	60.1±32.79	7.33	22.1	18.2	40.3	27.2±7.70	1.72
Vitamin B_{12} (mg)	1.9	1.4	3.2	2.3±0.74	0.17	0.0	1.6	1.6	1.6±0.00	0.00

Table 2. Mean, SD and level of significance of various anthropometric parameters and haemoglobin level of Indian junior male and female hockey players

Parameters	*Male (n=20)*	*Female (n=20)*	*F-value*
Age (yrs)	17.1±2.21	18.2±2.27	0.10
Height (cm)	168.4±1.24	158.3±3.48	47.7**
Weight (kg)	58.4±0.91	48.6±4.86	47.8**
BMI	20.6±0.28	19.5±1.77	5.1
Muscle Mass (kg)	87.6±1.30	83.0±7.77	148.4**
Fat free Mass (%)	87.6±5.82	83.0±7.77	4.6
Fat mass (%)	12.4±1.29	17.3±8.50	4.5
Haemoglobin(g/dl)	12.3±0.13	10.6±0.69	96.0**

**$p<0.01$

Table 3. Mean, SD and level of significance of macronutrients and dietary fibres consumption of elite Indian male and female hockey players

Nutrient	*Male (n=20)*	*Female (n=20)*	*U-value*
Total Energy Intake (kcal)	2621.9±450.31	1847.5±235.53	16.0**
Total Energy Expenditure (kcal)	3621.4±126.49	3048.5±114.81	0.0**
Protein(g)	110.9±15.20	77.4±9.74	11.0**
Fat(g)	53.5±14.01	34.0±8.33	34.0**
Carbohydrates(g)	431.7±85.90	317.5±45.69	48.0**
Total Dietary Fibre (g)	13.6±2.31	11.1±1.97	70.0**
Insoluble Dietary Fibre(g)	10.8±1.99	9.0±1.83	82.0*
Soluble Dietary Fibre(g)	2.8±0.67	1.9±0.22	78.0*

*$p<0.05$; **$p<0.01$

Table 4. Comparison of Mean, SD and level of significance of minerals and vitamins intake of elite Indian male and female hockey players

Minerals	*Male (n=20)*	*Female (n=20)*	*U value and level of significance*	*% below of RDA†*	
				Male	*Female*
Zinc(mg)	10.1±2.49	6.9±1.19	50.0**	65	100
Iron(mg)	20.0±4.92	11.2±3.78	34.0**	30	100
Phosphorous(mg)	2364.0±384.97	1594.6±237.91	18.0**	-	-
Calcium(mg)	845.6±358.86	678.0±81.32	186.0	35	10
B-carotene (µg)	1674.6±779.02	683.5±210.32	5.0**	100	100
Retinol (µg)	620.0±165.68	415.2±27.23	26.0**	11	100
Pyridoxine (mg)	1.1±0.20	0.6±0.11	22.0**	100	100
Thiamine (mg)	1.7±0.53	0.8±0.36	40.0**	40	90
Folic Acid (µg)	281.2±51.75	180.0±27.88	8.0**	-	65
Vitamin-C (mg)	60.1±32.79	27.2±7.70	36.0**	50	100
Vitamin-B12 (µg)	2.3±0.74	1.6±0.00	30.5**	-	-
Vitamin-B2 (mg)	1.2±0.27	0.7±0.26	10.0**	100	100
Niacin (mg)	12.6±4.49	8.5±1.74	102.0*	100	100

*$p<0.05$; **$p<0.01$

†RDA source: Narasinga & Sivakumar (2010). Nutrients Requirements & Recommended Dietary Allowances for Indians. (1990, Reprinted 2008) 2nd Edition – 2010.

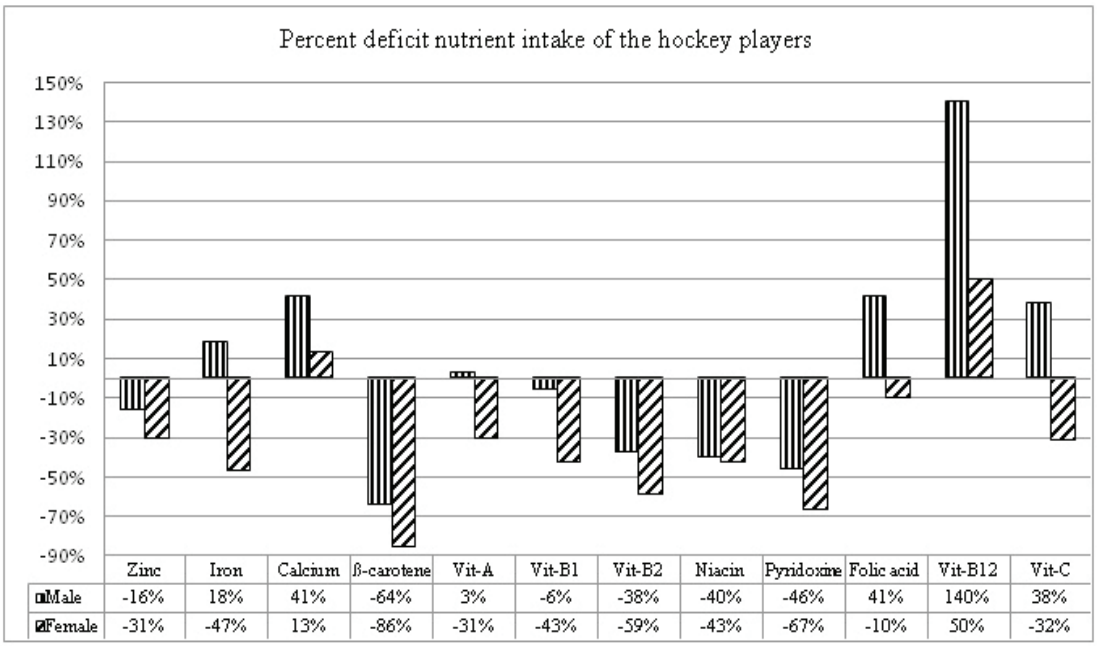
Total energy intake and total energy expenditure

Total energy intake (TEI) and total energy expenditure (TEE) among male and female athletes are was also described in Table 3. In male and female athletes average daily calorie intake was found to be 2622±450 kcal and 1848±236 kcal respectively whereas their total energy expenditure was 3621±126 kcal and 3049±115 kcal.

Micronutrient intake

Average intake of minerals and vitamins of the present subjects and adequacy of micronutrient intakes, that were computed based on Indian RDA, 2010 (Narasinga & Sivakumar, 2010) reference values, are presented in Table 4. Adequate intake of calcium and phosphorous was found in both groups whereas intake of iron and zinc was inadequate among male (65%) and female (100%) players. A higher proportion of female players showed deficit in intake of retinol (100%), vitamin C (100%) and folic acid (65%) when compared to their male counterparts. Table 4 revealed that male players met the RDA for these minerals. Intake of B-vitamins (thiamine, niacin, pyridoxine & riboflavin) was inadequate in males and females, except for vitamin B_{12}. Intakes of vitamins and minerals were significantly higher among male players, except for calcium.

Comparison of the average percent deficit intake of vitamins and minerals as compared to the RDA for both male and female hockey players. Zinc and iron intake were deficient in 31% and 47% respectively among the female players, whereas in male players, zinc deficiency wasat16% while iron intake exceeded the RDA by 18%. Calcium intake exceeded the RDA (male: 41%; female: 13%). Female players showed deficient intake



	Zinc	Iron	Calcium	ß-carotene	Vit-A	Vit-B1	Vit-B2	Niacin	Pyridoxine	Folic acid	Vit-B12	Vit-C
Male	-16%	18%	41%	-64%	3%	-6%	-38%	-40%	-46%	41%	140%	38%
Female	-31%	-47%	13%	-86%	-31%	-43%	-59%	-43%	-67%	-10%	50%	-32%

Figure 1. Percent deficit of various nutrient intakes of the hockey players as compared to RDA†.

†RDA source: Narasinga & Sivakumar (2010). Nutrients Requirements & Recommended Dietary Allowances for Indians. (1990, Reprinted 2008) 2nd Edition – 2010.

in all B-vitamins i.e., B_1 (43%), B_2 (59%), niacin (43%), pyridoxine (67%), folic acid (10%) along with vitamin C (32%) and vitamin A (31%). In male players, vitamin B_1, vitamin B_2, niacin and pyridoxine intake were deficient by 6%, 38%, 40%, 46% respectively. Vitamin B_{12} intake was found to exceed RDA in males (140%) and females (50%).

DISCUSSION

For optimal performance in sports, adequate nutrition and physical training are essential. Athletes have poor understanding of nutrition which may directly affect their nutritional status (Coutinho *et al.,* 2016). In India there are very few studies available on individual athletes' needs for energy and nutrients.

The present study showed that both males and females had normal BMI. The average muscle mass of male and female players were 25.4 kg and 16.4 kg respectively. It is known that the lower the fat mass proportion, the greater the musculature, and more active mass is required in most sports disciplines (Coutinho *et al.,* 2016).

The study revealed that female players had greater fat mass than male players though was not statistically different. According to the American College of Sports Medicine, the average body fat percentage for male soccer should be within 7-12%. This study recorded the body fat of the players in the "good" category (Thompson *et al.,* 2010). Singh *et al.* (2010) found that the average BMI of Indian hockey players was 22.3±1.75 and body fat percentage was 7.8±3.86. Another study (Sharma & Kailashiya, 2017) revealed that body fat percent of male hockey players was 18.7±5.16 and female hockey players was 24.9±3.91. These findings are closely related with current findings.

Hb level indicates the iron status of the human body (Damodar *et al.,* 2013). Hb_{mass} is often regarded as a key limiting factor to maximum O_2 uptake (VO_2max), which in turn is a strong predictor of endurance performance. Endurance training likely impacts other haematological variables: blood volume (BV) changes generally outpace Hb_{mass} increase, mainly due to an exercise-induced plasma volume (PV) expansion, resulting in lower haemoglobin concentration ([Hb]) and haematocrit levels (Hct) in endurance athletes (Brocherie *et al.*, 2015).

The present study observed low haemoglobin level in male and female hockey players. In contrast, Manna *et al.* (2011) found normal haemoglobin level among male field hockey players. Previous studies revealed that, in female athletes, intense physical exercise leads to early stages of depletion of Hb and other blood cell parameters (Alam *et al.,* 2014; Martínez *et al.,* 2011). It is estimated that 75% of anaemia cases are related to iron deficiency followed by folic acid and vitamin B_{12} deficiency (Haidar, 2010). The present study also revealed that 30% male and 100% female hockey players were deficient in dietary iron intake.

Various studies (Coutinho *et al.,* 2016; Sangeetha *et al.,* 2014) have found energy inadequacy among male and female players. The present study revealed that calorific value of the diet was inadequate for both males and females. Male and female players were consuming 73% and 61% respectively of their total energy expenditure. Almost similar observations were reported by Sangeetha and her colleagues (2014) and Wierniuk & Włdarek (2013) on volleyball players in Poland. Female athletes were reported to have a tendency to follow a restrictive eating habit or chronic dieting to achieve and maintain a low body weight (Hoogenboom *et al.*, 2009; Sundgot-Borgen *et al.*, 2007).

Protein is essential to maintain athletes' body composition and muscle strength. The Indian Council of Medical Research recommends a daily protein need for Indian sedentary individual of 0.8 g/kg body weight (Narasinga & Sivakumar, 2010) and for endurance athletes and bodybuilders it can be go up to 1.0 to 1.5 grams per kilogram of bodyweight (Nutrition and Hydration Guidelines, 2007). According to the American College of Sports Medicine (ACSM) (Potgieter *et al.,* 2013) recommendation, strength and endurance athletes need 1.2-1.7 g/kg body weight/day and guidelines advice that these requirements should be achieve through diet alone. Additional supplementation is not necessary, especially when the energy intake is optimal (Potgieter *et al.,* 2013, Rodriquez *et al.* 2009). We have found the average intake of protein was >1.5 g/kg body weight/day which is considered as adequate according to the recommendations mentioned above.

Fat requirements of athletes are similar, the amount depends largely on the training status and goals of the athletes (Potgieter *et al.*, 2013). A moderate quantity of dietary fat with a balance between saturated and unsaturated fatty acids are desirable for athletes. Dietary intake of fat should not be more than 30% of total daily caloric intake (Nutrition and Hydration Guidelines, 2007). Subjects of this study consumed inadequate amount of fat which may negatively affect training, nutrient density of the diet and the ability to consistently improve their athletic performance (Nutrition and Hydration Guidelines, 2007; Zapolska *et al.,* 2014). The tendency of restrictive eating and chronic dieting for weight loss may be associated with low fat intake in these players.

Carbohydrates are the primary source of energy, and stored muscle glycogen supply fuel for muscle contraction. Sufficient amount of carbohydrate intake reduces post exercise recovery time and helps to restore carbohydrate store for the next practice/training session. The daily requirement for carbohydrate is highly individualised, depending on gender, type of sports, intensity of training, length of practice, condition of environment etc (Potgieter *et al.*, 2013). According to Nutrition and Hydration Guidelines for Excellence in Sports Performance recommendation, carbohydrate should contribute 55% of total energy intake (Nutrition and Hydration Guidelines, 2007). ACSM recommend 6-10 g of carbohydrate per kg body weight per day for athletes. In this study we have also observed an adequate intake of carbohydrate (male: 65%; female: 67%) in both the groups (Potgieter *et al.*, 2013). But low scores were noted in female footballers in Greece (Papadopoulou *et al.,* 2010), India (Jain *et al.,* 2008) and the USA (Papandreou *et al.,* 2006).

Vitamins and minerals are essential for metabolic functions as they are act as cofactors for various enzymes involved in metabolism. Additional supplementation of micronutrients is not recommended for athletes if they are consuming adequate amounts of energy and on a healthy and balance diet. Athletes who restrict their energy intake or restrict certain types of food, especially for a long period to meet weight loss goals, may need supplementation (IOC, 2011, Potgieter *et al.*, 2013, Rodriquez *et al.,* 2009). Previous research reported that calcium, vitamin D, iron, and some antioxidants deficiency are common among athletes (Wardenaar *et al.*, 2017, Raizel*et al.*, 2017).

Intake of calcium and phosphorous was adequate in this study, whereas intake of iron and zinc was inadequate among male (65%) and female (100%) players. Martínez *et al.* (2011) observed

that carotenes, vitamin A, vitamin E, vitamin D, and folic acid deficiency in both boys and girls; girls also had inadequate intake of iron and calcium. In the present study, we have also observed that female athletes were found to be deficit in retinol (100%), vitamin C (100%) and folic acid (65%).

It is generally assumed that athletes with a poor thiamin and riboflavin status have a reduced ability to perform physical activity, especially performing maximal work (Wardenaar *et al.*, 2017).This study found intakes of B-vitamins except vitamin B_{12} in the daily diet were below the RDA levels. Energy intake inadequacy negatively reflects intake of vitamins and minerals (Wardenaar *et al.*, 2017). Athletes should be encouraged to select foods rich in B-vitamins like fruits, vegetables, legumes and milk to meet the dietary requirements for specific B vitamins.

CONCLUSION

The hockey players of the present study were shown to consume inadequate energy, fat, vitamins and minerals. The female players were found to be deficient in intake of several B-vitamins and iron.

The results of the present study may assist sports nutritionists, coaches and trainers to prepare individualised nutrition education programmes, and dietary interventions for Indian hockey players to improve their performance.

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Authors' contributions

Roy M, manuscript preparation, statistical process, data collection and analysis; Chatterjee S, review of literature, Data collection and analysis; Dey SK, study design, manuscript preparation and correction.

Conflict of interest

There is no conflict of interests among the authors.

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Decreased weight gain and enhanced serum biochemical parameters in rats after vitamin D and Ca supplementation

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ABSTRACT

Introduction: Obese individuals tend to have lower plasma concentrations of calcidiol and higher levels of plasma parathyroid hormone (PTH). Objective of this study was to evaluate the influence of vitamin D and Ca supplementation on weight gain and biochemical parameters in rats fed a high-fat high-calorie diet. **Methods:** Fifty-six male Sprague-Dawley rats were assigned randomly into 4 groups of 14 rats each, and receiving diets as follows: (1) high fat (HF) 40% total energy from fat; (2) high fat & vitamin D (HF-D) 2000 IU vit D/kg diet; (3) high fat & Ca (HF-Ca) 7 g Ca/kg of diet; and (4) high fat & vitamin D & Ca (HF-D & Ca) (2000 IU of vit D+7 g Ca/kg of diet). Measured variables included body weight gains, food intake, serum triglycerides, cholesterol, insulin, glucose, ALT, and AST at 5 weeks and 10 weeks of the trial. **Results:** Lowest amount of weight gain and feeding efficiency ratio were recorded for the (HF-D & Ca) group. Rats in the HF-D group had the lowest circulating cholesterol. No significant differences in food intake, blood glucose, insulin, triglycerides, ALT and AST were found among the treatment groups. **Conclusion:** This study showed that diet supplemented with vitamin D and Ca combined appeared to mitigate weight gain in weight-induced rats, while vitamin D supplementation alone lowered serum cholesterol concentrations. Further studies are recommended to confirm these results.

Keywords: Obesity, calcium, rats, vitamin D

INTRODUCTION

Obesity is a major global health challenge with on the rise prevalence regardless of gender, age, socioeconomic status, or geographic location that is increasing considerably in both genders and across all ages. Vitamin D deficiency is a common problem that may lead to the development of several health problems (Lamendola *et al.*, 2012). Many studies showed that obesity or overweight status is in close association with compromised vitamin D status (Tolassa *et al.*, 2016; Vanlint, 2013). The National Health and Nutrition Examination Survey (NHANES III) data reported that white women with normal BMI (18.5 to 25 kg/m^2) had higher 25(OH) D serum levels

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compared to those with BMI ≥30 kg/m^2. Increasingly more studies have shown the close association between vitamin D and obesity-induced physiological complications such as diabetes and cardiovascular disease (Anderson *et al.,* 2010).

Total body fat is associated positively with parathyroid hormone (PTH) and inversely with 25(OH) D levels, and also for its essential role in calcium homeostasis (Pacifico *et al.*, 2011). Evidence showed that supplementing Ca and/or vitamin D may contribute to an effective management of weight (Soars *et al.*, 2012). Other studies have showed that vitamin D in fat tissue lowers vitamin D bioavailability by slowing the release of vitamin D when levels are deprived (Roth *et al.*, 2011). Studies have demonstrated that increase in serum vitamin D_3 after sun exposure was 57% less in obese compared with non-obese subjects (Vanlint, 2013). This has led to the hypothesis that the decreased release of endogenously produced vitamin D into circulation is due to increased storage of the synthesised vitamin D in adipocytes of obese subjects (Vilarrasa *et al.*, 2007).

Increasing dietary Ca from 400 to 1000 mg/d for 1 year resulted in a 4.9 kg reduction in body fat (Zemel *et al.*, 2000). In contrast, feeding high Ca diet resulted in less weight gain among rats, compared to the control group fed less Ca (Thomas *et al.,* 2012). However, there is scarce information regarding the role of calcium and/or vitamin D supplements on the etiology of obesity and weight gain upon consumption of high fat. Our hypothesis suggests that adequate levels of Ca and vitamin D_3 supplements may reduce weight gain in rats consuming an extra 7 gm Ca/kg of diet and 2000 IU cholecalciferol/kg of diet daily. The objective of this experiment was to evaluate the influence of vitamin D, Ca and their combination on weight gain and selected biochemical parameters (fasting blood glucose, alanine transferase, aspartate transferase, cholesterol, triglycerides and insulin) among rats fed a high-fat high-calorie diet.

MATERIALS AND METHODS

Design

Fifty-six rats were equally randomised into four treatment groups of 14 rats each group provided with high-fat high-calorie diet and supplemented with vitamin D and Ca as follows; (1) high fat (HF) group (40% of total energy from fat) without any supplements which is more like a control group; (2) high fat vitamin D (HF-D) group (40% of total energy from fat and 2000 IU vitamin D/kg diet); (3) high fat Ca (HF-Ca) group (40% of total energy from fat and 7g Ca/kg diet); and (4) high fat vitamin D and Ca (HF-D & Ca) group (40% of total energy from fat, 2000 IU vitamin D/kg and 7g Ca/kg of diet). The doses of Ca and vitamin D_3 were obtained in this study after an investigation of the literature and scientific resources about tolerable upper intake levels of vitamins and minerals in rats.

Calcium carbonate and vitamin D_3 in powder form were used and they were obtained from Jovet Company (Amman, Jordan).

Animals

Fifty-six male Sprague-Dawley (SD) rats (weighing 215±16.2 g and aged 120±1.5 days) were purchased from the Animal House at Jordan University of Science and Technology (JUST) after receiving the approval of ACUC (Animal Care and Use Committee) at JUST. Rats were individually housed in shoebox cages to properly measure individual feed intake and weight gains throughout the trial period (10 weeks). Identification numbers (ID) were assigned to each rat and the researcher chose even numbers of IDs to be given HF and HF-D while odd numbers of IDs were assigned to HF-Ca and HF-D/Ca treatments for randomisation purposes. Surrounding

climatic conditions were stabilised at thermoneutrality (23°C air temperature, 50-60% relative humidity), while light was cycled every 12 hours, with darkness period from 1900 to 0700 hrs.

Diet composition and preparation

Accentuated BW gain-inducing diet was adopted from Dyets Inc.® (Pennsylvania, USA). Diet components were individually weighed and then mixed for 20 minutes until homogenised. Diets were freshly prepared in batches of 12 kg each and stored in sterile bags and refrigerated until offered to animals (within a week period). The caloric content of the diet was 4120 calories/kg of the diet. Diet composition was as follows: 35.5, 17.8, 1.8, 3.6, 17.8, 0, 8.9, 3.6, 1.8, 0.4, 7.1, and 1.8% for casein, sucrose, coconut oil, DL/methionine, cellulose, corn oil, mineral mix, vitamin mix, choline bitartrate, cholic acid, corn starch and cholesterol, respectively.

Measurements

Weight of the rats were measured and recorded weekly. Daily feed intake was assessed by the difference between food offered *ad libitum*- and feed refusals. Blood triglycerides, cholesterol, glucose, insulin and liver enzymes (ALT and AST) were assessed on week 5 and 10 of the study period.

On the 5th week of the experiment, six rats from each treatment group were sacrificed in order to collect blood samples (via cardiopuncture, upon deep ketamine/xylazine anaesthesia), into vacutainer tubes. The same procedure was done with the remaining rats at week 10 of the trial.

Blood samples were analysed using Beckman Coulter (Access 2) with commercially available reagents (Roche Diagnostic). One blood sample was drawn from each rat via cardiopuncture into 10 vacutainer tubes, and stored for 30 minutes at room temperature before centrifugation (4000-RPM for 5 minutes). Sera were then separated and transferred into endocrine automated analysers (Modular-E170-Roche-Germany) and used for measurements of glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, triglycerides and insulin using electro chemiluminescence immunoassay "ECLIA".

Statistical analyses

All data were analysed using SPSS software version 19.0 for Windows (IBM Corp., USA). One-way ANOVA was used for normally-distributed variables, and *Post-hoc* ANOVA (Least Square Difference) was conducted to determine the difference between variables. *P*-value of ≤0.05 was considered the cut-off level for statistical significance.

RESULTS

At week 5 (Table 1)

No significant differences were observed in the initial and final body weights at week 5 among the treatment groups (Table 1). However, rats in HF-D & Ca groups had lower (p<0.05) body weight gain when compared to rats in the other treatment groups. The lowest weight gain was observed in rats consuming diets supplemented with Ca and vitamin D (HF-D & Ca).

No differences were shown among the different treatment groups with regard to serum concentrations of glucose, ALT, AST, triglycerides or insulin.

At week 10 (Table 2)

Amounts of food consumed by all the studied groups did not differ significantly. However, rats fed high vitamin D and high Ca (HF-D & Ca) gained significantly lowest among of body weight. No differences were detected among different dietary treatments with regard to serum concentrations of glucose, ALT, AST, triglycerides or insulin.

DISCUSSION

Sergeev & Song (2014) reported that

Table 1. Initial and final body weights (BW), food intakes, and feed efficiency ratio (FER) and biochemical tests of rats at 5 weeks

Parameter	*Diets**			
	HF	*HF-D*	*HF-Ca*	*HF-D & Ca*
Initial BW	208.5±20.5	216.2±21.5	220.4±20.4	216.92±2.5
Final BW	306.5±32.0	318.5±21.4	311.2±17.2	297.7±19.2
Weight gain	98.1±25.1[a]	102.3±16.0[a]	90.8±18.6[ab]	80.8±24.1[b]
Food intake (g)	564.0±43.8[a]	597.3±45.6[a]	593.5±42.2[a]	578.3±48.5[a]
FER**	0.2±0.04[a]	0.2 ±0.1[a]	0.2 ±0.03[ab]	0.14 ±0.04[b]
Glucose (mmol/L)	16.6±1.3[a]	17.5 ±1.6[a]	16.5±0.9[a]	16.5±1.3[a]
ALT (U/L)	71.9±4[a]	68.1±4.2[a]	65.5±3.2[a]	66.0±3.8[a]
AST (U/L)	197.0±23.5[a]	171.7±16.4[a]	180.9±26.2[a]	171.4±18.1[a]
Cholesterol (mmol/L)	2.1±0.1[a]	1.8±0.1[b]	2.0±0.1[ab]	2.0±0.1[ab]
Triglyceride (mmol/L)	1.3±0.13[a]	1.2±0.1[a]	1.3±0.1[a]	1.3±0.1[a]
Insulin (u IU/ ML)	0.1±0.02[a]	0.158±0.05[a]	0.14±0.06[a]	0.14±0.06[a]

*Diets; HF: high fat diet group, HF-D: high fat diet plus vitamin D, HF-Ca: high fat diet plus Ca, HF-D/Ca: high fat diet plus vitamin D and Ca
**FER = body weight gain for experimental period/food intake for the experimental period.
Values represent means±SD
Values with different letters ([a] and [b]) within a row are significantly different by LSD test ($p \leq 0.05$)

Table 2. Initial and final body weights (BW), weight gain, food intakes, and feed efficiency ratio (FER) and biochemical tests of rats at 10 weeks

Parameter	*Diet**			
	*HF**	*HF-D*	*HF-Ca*	*HF-D & Ca*
Initial BW (g)	208.5±20.5	216.2±21.5	220.4±20.4	216.9±19.5
Final BW (g)	364.29±47.38	359.3±36.7	362.14± 30.8	347.50±26.6
Weight gain (g)	152.9±34.7[a]	149.3±20.3[a]	143±18.7[a]	128±23.4[b]
Total food intake (g)	980±94.8[b]	1023±71.3[b]	1055±58.9[b]	1005±46.1[b]
FER**	0.15±0.02[a]	0.15±0.01[a]	0.14±0.01[ab]	0.13±0.02[b]
Glucose (mmol)	16.06±1.48[b]	16.43±2.04[b]	17.00±1.51[b]	16.67±1.78[b]
ALT (U/L)	63.82±2.78[a]	64.2±5.71[a]	65.74±3.91[a]	76.18±3.36[a]
AST (U/L)	198.54±43.2	176.33±28.3	151.53±12.8	156.50±20.2
Cholesterol (mmol)	2.02± 0.06[ab]	1.78±0.09[b]	2.01±0.15[ab]	2.04±0.17[ab]
Triglyceride (mmol)	1.54±0.26	1.23±0.17	1.19±0.17	1.20 ±0.17
Insulin	0.08±0.03	0.17±0.04	0.19±0.10	0.21±0.12

*Diets; HF: high fat diet group (n=7), HF-D: high fat diet plus vitamin D (n=7), HF-Ca: high fat diet plus Ca (n=7), HF-D/Ca: high fat diet plus vitamin D and Ca (n=6).
**FER = body weight gain for experimental period/food intake for the experimental period.
Values represent means±SD (n=7).
Values with different letters ([a] and [b]) within a raw are significantly different by LSD test ($p \leq 0.05$).

mice fed fatty diets supplemented with vitamin D (1000 IU/kg of the diet) and Ca (1.2%/kg of the diet) had the lowest fat weight gain and showed improvement in adiposity markers, compared to vitamin D or Ca. Findings of human studies support the possible role of a combined supplementation of calcium and vitamin D on obesity prevention (Vilarrasa *et al.*, 2007; Roth *et al.*, 2011).

Ca and 1,25(OH) D work in a way to control metabolism of lipids in adipose cells by stimulating the oxidation of fatty acid and suppressing the lipogenic process (Mahdieh *et al.*, 2018). Furthermore, Ca has a role in decreasing the absorption of fatty acids through the formation of insoluble Ca and fatty acid soap in the intestine that could increase faecal fat excretion, leading to a decrease in the digestibility of fat (Zhu *et al.,* 2013). Another finding of our study was the cholesterol-lowering effect of vitamin D supplementations to high fat diets. Low vitamin D_3 levels may impair insulin action as well as glucose metabolism and various metabolic processes in adipose and lean tissue (Roth *et al.*, 2011). Asemi *et al.* (2013) noticed a significant reduction in serum total cholesterol concentrations with daily 4000 IU vitamin D given to obese patients for 12 weeks. In cross-sectional studies, serum vitamin D levels were positively correlated with HDL cholesterol (Jorde & Grimnes, 2011). Moreover, the indirect vitamin D immune-modulatory and cytokine suppressive effects can decrease cholesterol synthesis and absorption (Hart *et al.*, 2011). The vitamin D effect in decreasing cholesterol absorption might also be linked with lipid lowering therapy as dietary absorption which can lead to less circulating cholesterol levels rather than reducing endogenous production. It is also believable that vitamin D effects on lipids might increase with underlying abnormal lipid metabolism or metabolic disorders such as hypercholesterolemia or diabetes (Al-Daghri *et al.,* 2012).

In contrast, some investigations found that vitamin D supplementation had no significant effects on serum lipid profile (Wang *et al.,* 2012). Furthermore, in a cross-sectional study, it was found that 25(OH) D levels of >30 ng/ml compared to <20 ng/ml were markedly associated with a healthier lipid profile. On the other hand, and in the same population, it showed no effect on lipid when raising 25(OH) D levels on the short run (Ponda *et al.,* 2012).

CONCLUSION

This study showed that rats fed diet with 2000 IU vitamin D/kg and 7 g Ca/kg of diet appears to mitigate weight gain despite being provided high fat (40% of total energy) for 10 weeks. Further studies are needed to investigate the potential effects of vitamin D in obesity prevention.

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Authors' contributions

Dr Hadil S Subih prepared the manuscript and the study design; Hiba Hamdan did the research in the animal house; Dr Hosam Al-Tamimi assisted in the study design and diet components; Dr Hiba Bawadi revised the manuscript and run the statistical analysis; Dr Sana Janakat revised the manuscript and assisted in study design

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Bioactive and nutritional compounds in virgin coconut oils

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ABSTRACT

Introduction: Virgin coconut oil (VCO) is very much in demand among health-conscious consumers. VCO is produced from fresh coconut milk by using centrifugation (CVCO) or fermentation (FVCO). Since the conditions used for these processes are quite different, this study aimed to investigate their effects on the contents of selected bioactive compounds that have potential health benefits. **Methods**: CVCO and FVCO were produced from the same batch of fresh coconut (*Cocos nucifera L.*) milk. CVCO was obtained by centrifuging coconut milk in three steps with vacuum evaporation, while FVCO was obtained by anaerobically fermenting coconut milk at 35°C for 16 h. The products were analysed for macronutrients, fatty acid profiles, phytosterols and phenolic compounds. Potential health benefits were determined by calculating the chance of fatty acid bioavailability and analysing antioxidant activities. **Results**: Both VCO production processes removed all hydrophilic compounds, with the remaining fat and moisture contents not significantly different at 99.90% and 0.10%, respectively. Their fatty acid profiles were 90% saturated and 60% medium chain (mainly lauric acid). The phenolic compound (originally found high in coconut milk) was present in trace amounts in the VCOs. However, phytosterols became more concentrated. Chances of medium chain fatty acid becoming more available for health benefit were at 54% and 58%, and were insignificant among both VCOs. Fermentation caused more rancidity to the product. **Conclusion**: Both centrifugation and fermentation production processes did not qualitatively and quantitatively affect the bioactive compounds of virgin coconut oil.

Keywords: Centrifugation, fermentation, medium chain fatty acid, phytosterols, virgin coconut oil

INTRODUCTION

Virgin coconut oil (VCO) that is produced in certain countries of Southeast Asia is used as a dietary supplement with the aim of reducing the risk of certain non-communicable diseases (NCDs). The demand for VCO is increasing among consumers globally. A result of market analysis indicated that the compound annual growth rate (CAGR) of global

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VCO market would be around 10% during 2017-2021 (Technavio, 2017).

The key factors of such growth are due to the increased use of natural health products among health-conscious consumers and aging population. However, the quality of VCOs available in the market varies depending on the quality of production facilities, which also affects its market price. As compared to traditional VCO produced by mechanical extraction from copra (sun-dried coconut meat), the new generation of VCO is produced by oil separation from fresh coconut milk. The oil separation process is performed under less severe or mild conditions, which results in products of better quality.

Natural or pure culture lactic acid fermentation, chilling and thawing, centrifugation, or mixed enzyme (cellulose, amylase, polygalacturonase and protease) digestion is the condition that can be used for oil separation. Due to the freshness of raw material and mild production process, these VCOs are believed to maintain the nutrient profile and potential health benefits of coconut.

Commercial VCOs are produced by centrifugation (CVCO) or fermentation (FVCO). CVCO is normally produced industrially in less than 10 minutes by separating VCO from fresh coconut milk using a high speed centrifugal machine. FVCO is produced by small scale or cottage industries using natural fermentation process which takes approximately two weeks.

The oil separation process in CVCO depends on mechanical force, while FVCO relies on the denaturation of a natural emulsifying agent. Both kinds of VCOs are widely available in the market with claims on various health benefits, such as weight and cholesterol reduction, immune system improvement, lower risk of Alzheimer, and antimicrobial growth (DebMandal & Mandal, 2011). These health benefits might be due to available bioactive compounds in the VCOs, which are also found in coconut meat and milk. Coconut meat is known to be a good source of medium chain fatty acid, especially lauric acid, which is directly metabolised into energy with no effects on blood cholesterol.

Phenolic compounds and phytosterols are antioxidants related to risk reduction of non-communicable diseases (NCDs), are also found in both coconut meat and coconut milk. The different production processes of these VCOs could have different effects on the contents and availability of these bioactive compounds. This study was aimed at investigating the effects of VCO production process, i.e. centrifugation and fermentation, on the contents of potential bioactive compounds including medium chain fatty acids, triacylglycerol composition, total phenolic content, phenolic acids and flavonoids and phytosterols.

MATERIALS AND METHODS

Coconut milk

Coconut milk was prepared in batches of three at the Theppadungporn Coconut Co., Ltd., Nakhonpathom, Thailand. The peeled coconut meat was cleaned in chlorinated water, shredded, and expressed to extract coconut milk. The coconut milk was then stored in tightly closed glass bottle at -20°C until analysis.

Virgin coconut oil (VCO)

VCOs were produced from freshly extracted coconut milk of the same batch at the Theppadungporn Coconut Co., Ltd. by using centrifugation and fermentation methods. Centrifuged virgin coconut oil (CVCO) was produced by centrifuging coconut milk in a series of three centrifugal machines (GEA, GEA Westfalia Separator Group GmbH, Oelde, Germany) then residual water

in the oil was finally removed in a vacuum evaporator (Behle Apparate & Behälterbau, H. Behle GmbH, Bielefeld, Germany). Fermented virgin coconut oil (FVCO) was produced by naturally fermenting coconut milk in closed glass jars under anaerobic condition at 35°C for 16 h. The FVCO was harvested and filtered through cheesecloth. The produced VCOs were sampled and stored in closed amber glass bottles at -20°C until analysis.

Proximate analysis

Moisture content was determined by measuring the constant weight after drying in a hot air oven (AOAC INTERNATIONAL, 2012). Total fat content was determined by extracting VCOs or hydrolysed coconut milk with petroleum ether in Soxtec system (Model HT 1043, Tecator Co., Ltd., Hoganas, Sweden) (AOAC INTERNATIONAL, 2012).

Protein content was analysed according to Kjeldahl method (AOAC INTERNATIONAL, 2012), with 6.25 as the multiplication factor for converting total nitrogen into protein content. Ash content was determined after the sample had been burnt in a muffle furnace at 550°C for 2.5 h (AOAC INTERNATIONAL, 2012). Carbohydrate was calculated by subtracting moisture, fat, protein and ash contents from 100 (FAO, 1998).

Fatty acid profile

The extracted oil from coconut milk or VCO was saponified with 0.5M KOH in methanol at 95°C, methylated into fatty acid methyl esters (FAMEs) by adding 14% Boron trifluoride in methanol (Petrović Kezić & Bolanča, 2010). FAMEs were analysed on the DB-23 capillary GC column (60 m x 0.25 mm I.D., 0.25 µm) installed in an Agilent 9860 gas chromatograph system equipped with a flame ionisation detector and a split/splitless injector (Agilent Technologies, Santa Clara, CA, USA). Methyl heptadecanoate, C17:0 was used as the internal standard and helium was the carrier gas. The Supelco™ 37 Component FAME Mix (10 mg/ml) was used as the standard (Sigma-Aldrich, MO, USA.).

Triacylglycerol (TAG) composition

Five milligrams of oil extracted from coconut milk or VCO was mixed with 2 ml of the solvent mixture of methylene chloride: isopropanol: methanol (25:10:65 v/v/v) added with 50 µg butylated hydroxytoluene (BHT)/ml. The mixture (3 µl) was analysed in the High Strength Silica (HSS) T3 column (1.8µm particle 100 x 2.1 mm id, Waters, Milford, Massachusetts, USA) equipped on the quadrupole time-of-flight (TOF) mass spectrometer (MS) (AB SCIEX, TripleTOF 5600) that was operated under the information-dependent MS/MS acquisition mode.

The gradient mobile phase consisted of acetonitrile: water containing 10 mM ammonium formate (60:40 v/v) and isopropanol: acetonitrile: water containing 10 mM ammoniumformate (90:10:5 v/v/v). The scan range of TOF/MS was m/z 70-1,200 and MS/MS was m/z 50-1,200 (Choi *et al.*, 2015). The PeakView™ software (SCIEX, MA, USA.) was used to identify triacylglycerol species and content of each triacylglycerol species was calculated as relative quantification. Moreover, the chance of medium chain fatty acid, MCFA for being on sn-1 and sn-3 in sample was calculated as:

$$\frac{\sum\left(\frac{\text{No. of MCFA on each TAG}}{3}\right)}{\text{No. of TAG in the species}} \times \text{Relative content of each TAG species}$$

Where:

No. of MCFA each TAG = number of MCFA (fatty acids that contain 8-12 carbon atoms) in each TAG species

No. of TAG in the species = total number of TAG possibly in the species

Total phenolic content, phenolic acid and flavonoids

The extract of coconut milk or VCO in 80% methanol was the sample used for analyses. Total phenolic was analysed by using Folin-Ciocalteu assay (Martin *et al.*, 2009), which was measured the absorbance at 755 nm on spectrophotometer (UV1601, Shimadzu, Kyoto, Japan). The total phenolic content was determined regarding the standard curve of gallic acid (0.04-0.20 mg/ml).

The phenolic acid and flavonoids were determined by High Performance Liquid Chromatography (HPLC) with Synergi Hydro-RP column (4 μm particle, 250 x 4.60 mm id, Phenomenex, Torrance, CA, USA) and Allsphere ODS-2 as a guard column (10 x 4.6 mm id, Alltech, Deerfield, IL, USA) using Perkin-Elmer Series 400, equipped with a Hewlett-Packard 1040A photodiode array detector.

The gradient mobile phases system consisting of acetonitrile (mobile phase A) and glacial acetic acid in deionisation water (mobile phase B) with the initial ratio 5% mobile phase A for 3 min then increased to 25% in 27 min and increased to 75% in 5 min were applied. The injection volume was 10 μl and a flow rate of 1 ml/min was used (Lee, Durst & Wrolstad, 2002).

Phenolic acid and flavonoids were detected at 260, 280 and 320 nm. The standards used included chlorogenic acid, orcinol, caffeic acid, epicatechin, caffeine, p-coumeric acid, ferulic acid, rutin, Q-3-rhamnoside, hesperetin, phloridzin, resveratrol and kaempferol.

Antioxidant activity

The antioxidant activities in the 80% methanol extract of coconut milk or VCO were determined as Oxygen Radical Absorbance Capacity (ORAC) (Huang *et al.*, 2002) and Ferric Reducing Antioxidant Power (FRAP) assays (Benzie & Strain, 1996). The ORAC was analysed on 96-well microplate reader (Synergy HT, Bio-Tek Instruments, Winooski, VT, USA.).

The ORAC value was determined regarding the Trolox standard curve of 3.125-100 μM in 75 mM phosphate buffer pH 7.4. The FRAP assay was performed on microplate reader (Synergy HT, Bio-Tek Instruments, Winooski, VT, USA). The FRAP value was determined regarding the Trolox standard curve of 7.8125-250 μM in deionised water.

Phytosterols

After being saponified with potassium hydroxide (KOH) in ethanol, coconut milk or VCO was added with 50 μl of 0.2 mg/ml Δ^7-cholesterol as internal standard. The mixture was then extracted with n-hexane. The extract was dried under nitrogen gas and dissolved in chloroform: methanol solvent (1:3 v/v).

The analysis was performed on Liquid Chromatography–Atmospheric Pressure Chemical Ionization Mass Spectrometry (LC-APCI-MS/MS) with 3 μm particle, 100 x 2 mm id Luna™ C18 (2) column (Phenomenex, Torrance, CA, USA.) operations in positive ion and selective reaction monitoring (SRM) modes. An isocratic mobile phase of acetonitrile: methanol (99:1 v/v) at flow rate 0.6 ml/min was use (Mo *et al.*, 2013). The optimum sensitivity and selectivity for quantitative analysis were established for campesterol, β-sitosterol, stigmasterol, Δ^5-avenasterol, brassicasterol, cycloartenol, β-sitostenol and campestenol.

Quality parameters of VCO

The VCO was determined for acid value (AV), free fatty acid content (calculated as lauric acid), peroxide value (PV), iodine value (IV) according to AOCS Method Cd 3d-63, AOCS Method Cd 8-53,

AOCS Method Ca 5a-40, AOCS Method Cd 1d-92, respectively (AOCS, 1998). Colour was measured in L*a*b* unit by a colorimeter (Color Flex EZ, Color global Co., Ltd., Bangkok, Thailand).

Statistical analysis

The IBM SPSS Statistics 19.0™ software (IBM Corp., Armonk, New York, USA) was used for statistical analysis and determine the significant difference at $p<0.05$. All analyses were performed in triplicate and the results were expressed as mean and standard deviation (SD). One-way ANOVA and Duncan's multiple range tests were conducted to assess difference among mean values from analyses of coconut milk and the VCOs. Student's *t*-test was used to evaluate difference between mean values from chemical analyses of the VCOs.

RESULTS AND DISCUSSION

Macronutrients analysis

Coconut milk (with no water added during the extraction process) contained approximately 60% water, 30% fat and small contents of carbohydrate, protein and ash. For the VCO production, at least 30% fat in the coconut milk was required. Protein as a natural emulsifier could stabilise the fat emulsion in coconut milk (Gonzalez, 1990), which could trouble the oil separation process in the VCO productions.

Oil was separated due to breakage of the fat emulsion in coconut milk, either caused by mechanical force (centrifugation) or protein denaturation (fermentation). Under the commercial VCO production processes, the role of protein as emulsifier in coconut milk could be overcome, which resulted in the

Table 1. Chemical compositions and fatty profiles of coconut milk, centrifuged virgin coconut oil (CVCO) and fermented virgin coconut oil (FVCO)[†]

Parameters	*Content (%)*		
	Coconut milk	*CVCO*	*FVCO*
Moisture[‡]	59.98±0.97[b]	0.11±0.01[a]	0.13±0.02[a]
Protein	3.41±0.02[b]	0.00[a]	0.00[a]
Fat	31.88±1.49[a]	99.90±0.01[b]	99.87±0.01[b]
Ash	0.97±0.08[b]	0.00[a]	0.00[a]
Carbohydrate	4.59±0.29[b]	0.00[a]	0.00[a]
Fatty acids			
C 8:0	3.59±1.24[a]	2.51±1.28[a]	2.26±1.40[a]
C 10:0	5.26±0.61[a]	4.86±0.48[a]	4.71±0.70[a]
C 12:0	47.81±1.84[a]	49.80±1.12[a]	49.48±1.12[a]
C 14:0	19.20±1.29[a]	21.27±0.99[a]	21.41±1.28[a]
C 16:0	11.16±1.70[a]	10.21±0.80[a]	10.43±0.92[a]
C 18:0	4.06±0.39[a]	3.92±0.36[a]	4.00±0.45[a]
C 18:1	7.76±0.83[b]	6.40±0.42[a]	6.66±0.54[a,b]
C 18:2	1.17±0.13[a]	1.02±0.03[a]	1.06±0.06[a]
n-6	1.17±0.13[a]	1.02±0.03[a]	1.06±0.06[a]
n-9	7.76±0.83[b]	6.40±0.41[a]	6.66±0.54[a,b]
S:M:P[§]	1:0.09:0.01	1:0.07:0.01	1:0.07:0.01

[†]Mean±SD (n=3)

[a,b]Different alphabets within the same row denote significant difference at $p<0.05$

[‡]APCC recommendations in 2009 for moisture contents of VCO was 0.1%

[§]S: Saturated fatty acid; M: Monounsaturated fatty acid; P: Polyunsaturated fatty acid

VCOs of the fat and moisture contents regarding the Asian and Pacific Coconut Community (APCC) recommendation, amended in August 2009 (Asian and Pacific Coconut Community, 2009).

There were no significant differences (p>0.05) in fat and moisture contents of the VCOs from both production processes (Table 1). Since ancient times, traditional-pressed copra-coconut oil used in Asian cuisines was the primary source of fat for the population. In contrast, VCO is presently produced from less severe processes, and is marketed as a functional food of high economic value and with claims of potential health benefits.

Fatty acid profile

The fatty acid profile of coconut milk, CVCO and FVCO were mostly not significantly different (p>0.05), except for C18:1 and n-9 fatty acids (Table 1). The VCO production processes did not affect the original fatty acid profile of the coconut milk.

Among the saturated fatty acids, medium chain fatty acid, MCFA (C8-C12) contributed the most to VCOs, of which lauric acid (C12:0) was the major fatty acid (50% of total fatty acids). The contents of MCFA were not significantly different in the VCOs derived from both processes. Compared to fatty acids >C12, MCFA (C8-C12) are metabolised more efficiently and showed less accumulation in the body (Marten, Pfeuffer & Schrezenmeir, 2006). Lauric acid, as compared to myristic acid (C14:0) and palmitic acid (C16:0), increases blood LDL-cholesterol as well as HDL-cholesterol. However lauric acid shows its ability to reduce the ratio of TC/HDL-cholesterol (Mensink, 2016).

Cohort studies reported that lauric acid consumption at 0.63% of energy intake (1.4 g/day) could reduce the risk of type 2 diabetes (Liu *et al.*, 2018). By having lauric acid at 0.7g/day, it could not reduce the risk of coronary heart disease (Zong *et al.*, 2016) and had no effect on BMI (Raatz *et al.*, 2017). Nonetheless, one should be mindful of the saturated fatty acids content in VCO. One serving of VCO as a dietary supplement (e.g. 1 tablespoon, 15 ml or 14.5 g) provides saturated fatty acids amounting to 67% of the recommendation of the World Health Organization (WHO) of <20 g/day (WHO, 2003). Positive correlation between saturated fatty acids and risk of cardiovascular disease has been shown in several studies (Mensink, 2016).

Triacylglycerol composition

The triacylglycerol (TAG) composition is used to determine the proportions of individual TAG molecular species. Based on this data, the potential TAG molecular species are shown in Table 2, Column 2. By using the value of relative content of the molecular species, the proportions (%) of MCFA (C8-12) in sn-1 and sn-3 positions was evaluated. This information is of nutritional relevance because fatty acids at the stereospecific sn-1 and sn-3 positions in the TAG have been shown to have a better chance of being hydrolysed by lipase and hence improving their bioavailability.

TAG 30:0 species means a triglyceride with 30 carbon atoms with no double bond, can be 3 ways of arrangement. The relative content of this species in the coconut milk is 7.07%, of which 6.28% of MFCA are in the sn-1 and sn-3. Table 2 indicates that up to 54-58% of MCFA in coconut milk, CVCO or FVCO were in the sn-1 and/or sn-3 positions. In comparing the products from the 2 processes, the FVCO showed higher proportions of MCFA at these positions.

MCT has been promoted for problems related to fat digestion, metabolism and utilisation (Hamosh *et al.*, 1991; Li *et al.*, 2015). This study showed that the chance of having triacylglycerol molecules as MCT in our studied coconut products was 21-24%.

Table 2. Quantity (%) of MCFA in coconut milk, centrifuged virgin coconut oil (CVCO) and fermented virgin coconut oil (FVCO) at sn-1 and/or sn-3 positions of TAGs[†]

TAG Species	*TAG composition in the species*	*MCFA (%) at sn-1 and sn-3 [Relative content of the TAG species (%)]*		
		Coconut milk	*CVCO*	*FVCO*
TAG 30:0	10:0/10:0/10:0, 8:0/10:0/12:0, 8:0/8:0/14:0	6.28±1.7^{a} [7.07±1.92^{A}]	4.34±0.30^{a} [4.51±0.70^{A}]	4.38±1.30^{a} [4.93±1.46^{A}]
TAG 32:0	10:0/10:0/12:0, 8:0/10:0/14:0, 8:0/12:0/12:0	12.12±1.29^{b} [13.63±1.45^{A}]	10.80±0.15^{a,b} [12.15±0.17^{A}]	8.94±0.60^{a} [10.54±1.35^{A}]
TAG 34:0	10:0/10:0/14:0, 10:0/12:0/12:0	9.81±0.83^{a,b} [11.77±0.99^{A,B}]	12.91±1.47^{b} [15.49±1.76^{B}]	9.31±0.56^{a} [10.70±1.34^{A}]
TAG 36:0	10:0/10:0/16:0, 10:0/12:0/14:0, 12:0/12:0/12:0	9.69±0.69^{a} [12.45±0.89^{A}]	8.22±0.40^{a*} [10.57±0.52^{A*}]	12.32±0.08^{b} [15.78±0.20^{B}]
TAG 38:0	10:0/10:0/18:0, 10:0/12:0/16:0, 10:0/14:0/14:0, 12:0/12:0/14:0	7.76±0.95^{b} [13.30±1.62^{B}]	5.36±0.42^{a*} [9.19±0.71^{A*}]	9.00±0.05^{b} [15.37±0.18^{B}]
TAG 38:1	10:0/10:0/18:1, 8:0/12:0/18:1	1.61±0.52^{a} [2.41±0.79^{A}]	1.51±0.36^{a} [2.27±0.53^{A}]	1.76±0.25^{a} [2.37±0.76^{A}]
TAG 40:0	10:0/10:0/18:0, 12:0/14:0/14:0, 10:0/14:0/16:0, 12:0/12:0/16:0	2.86±0.53^{a} [5.71±1.06^{A}]	3.88±0.36^{a*} [7.76±0.34^{A}]	5.27±0.10^{b} [10.69±0.42^{B}]
TAG 40:1	10:0/12:0/18:1, 8:0/14:0/18:1	0.70±0.24^{a} [1.41±0.47^{A}]	1.05±0.12^{a} [2.00±0.25^{A}]	0.89±0.32^{a} [1.83±0.14^{A}]
TAG 42:0	10:0/14:0/18:0, 10:0/16:0/16:0, 12:0/12:0/18:0, 12:0/14:0/16:0, 14:0/14:0/14:0	1.85±0.37^{a} [5.55±1.10^{A}]	1.91±0.01^{a*} [5.73±0.03^{A*}]	1.44±0.06^{a} [4.20±0.31^{A}]
TAG 42:1	12:0/12:0/18:1, 10:0/14:0/18:1, 8:0/16:0/18:1	1.71±0.16^{b} [3.86±0.37^{B}]	1.04±0.10^{a*} [2.34±0.23^{A*}]	1.62±0.06^{b} [3.74±0.27^{B}]
TAG 44:0	12:0/14:0/18:0, 12:0/16:0/16:0, 10:0/14:0/20:0, 14:0/14:0/16:0, 10:0/16:0/18:0	0.68±0.01^{a} [2.73±0.02^{A}]	1.05±0.51^{a} [4.20±2.05^{A}]	0.65±0.02^{a} [2.56±0.12^{A}]
TAG 44:1	12:0/12:0/20:1, 12:0/14:0/18:1, 10:0/14:0/20:1, 10:0/16:0/18:1	0.92±0.43^{a} [2.75±1.28^{A}]	0.65±0.03^{a} [1.95±0.09^{A}]	0.94±0.18^{a} [2.45±1.06^{A}]
TAG 44:2	12:0/14:0/18:2, 10:0/16:0/18:2	0.43±0.02^{a} [1.28±0.06^{A}]	0.31±0.03^{a} [0.93±0.10^{A}]	0.43±0.05^{a} [1.39±0.32^{A}]
TAG 46:0	12:0/16:0/18:0, 14:0/14:0/18:0, 14:0/16:0/16:0, 10:0/16:0/20:0, 10:0/18:0/18:0	0.18±0.00^{a} [1.10±0.02^{A}]	0.23 ± 0.02^{a} [1.37±0.10^{A}]	0.23±0.03^{a} [1.46±0.30^{A}]
TAG 46:1	12:0/16:0/18:1, 14:0/14:0/18:1, 10:0/18:0/18:1	0.37±0.04^{a} [1.68±0.19^{A}]	0.41±0.11^{a} [1.84±0.51^{A}]	0.39±0.01^{a} [1.81±0.09^{A}]
TAG 46:2	12:0/16:0/18:2, 14:0/14:0/18:2, 10:0/18:0/18:2, 10:0/18:1/18:1	0.30±0.04^{a} [1.81±0.09^{A}]	0.33±0.05^{a} [1.31±0.19^{A}]	0.40±0.06^{a} (1.41±0.50^{A}]
TAG 48:1	12:0/18:0/18:1, 14:0/16:0/18:1	0.23±0.00^{a} [1.33±0.04^{A}]	0.30±0.05^{a} [1.79±0.32^{A}]	0.25±0.01^{a} [1.57±0.14^{A}]
TAG 48:2	12:0/18:0/18:2, 12:0/18:1/18:1, 14:0/16:0/18:2	0.22±0.00^{a} [0.98±0.00 ^{A}]	0.61±0.04^{b} [2.75±0.18^{B*}]	0.24±0.02^{a} [1.07±0.16^{A}]
Total		57.72±3.34^{a} [90.26±4.70^{A}]	54.91±0.39^{a*} [88.63±2.49^{A}]	58.48±0.93^{a} [95.67±0.54^{A}]

[†]Mean±SD (n=3)

^{a,b}Different alphabets denote significant difference (p<0.05) between means within the same species (same row) for coconut milk, CVCO and FVCO

^{A,B}Different alphabets denote significant difference (p<0.05) between means within the same species (same row) for coconut milk, CVCO and FVCO

*Significant mean difference (p<0.05) within the same species (same row) between CVCO and FVCO

Table 3. Total phenolic content, phytosterols and antioxidant activity in coconut milk, centrifuged virgin coconut oil (CVCO) and fermented virgin coconut oil (FVCO)†

Parameter	*Coconut milk*	*CVCO*	*FVCO*
Total phenolic content (mg GAE/100 g)	2911.24±399.02[b]	Not detected[*]	59.44±13.40[a]
Antioxidant activity (µmole Trolox/100 g)			
FRAP	33.24±2.21[b]	0.11±0.01[a*]	0.83±0.12[a]
ORAC	362.37±42.33[b]	0.77±0.26[a*]	5.22±0.42[a]
Phytosterols (mg/100 g)			
Campesterol	2.22±0.17[a]	5.91±0.10[b*]	6.21±0.11[c]
b-sitosterol	20.30±1.72[a]	49.42±0.56[b]	51.57±3.38[b]
Stigmasterol	3.85±0.30[a]	8.50±0.28[b*]	9.20±0.43[c]
D^5-Avenasterol	6.59±0.04[a]	16.53±1.40[b*]	18.26±0.04[c]
Brassesterol	tr[a]	tr[a]	tr[a]
Cycloartenol	1.42±0.11[a]	4.97±0.63[b]	5.30±0.82[b]
b-sitostenol	1.63±0.14[a]	4.14±0.09[b]	4.18±0.32[b]
Campestenol	1.03±0.00[b]	0.65±0.09[a]	0.97±0.32[a]
Total phytosterol	36.01±2.26[a]	89.89±2.08[b*]	95.12±3.37[b]

†Mean±SD (n=3), tr = trace
[a,b,c]Different alphabets within the same row denote significant difference at $p<0.05$
[*]Significant mean difference ($p<0.05$) within the same row between CVCO and FVCO

Table 4. Physicochemical properties of centrifuged virgin coconut oil (CVCO) and fermented virgin coconut oil (FVCO)†

Parameter	*CVCO*	*FVCO*
Moisture content (%)	0.11±0.01[a]	0.13±0.02[a]
Iodine Value (g of iodine/100 g oil)	3.41±0.22[a]	4.21±0.45[a]
Peroxide value (mEq/kg oil)	0.00±0.00[a]	2.39±0.62[b]
Acid value (mg KOH/g oil)	0.08±0.00[a]	0.21±0.02[b]
Free fatty acids (mg lauric acid/g oil)	0.29±0.00[a]	0.76±0.08[b]
Colour‡:		
- L	4.83±0.30[a]	5.07±0.19[a]
- a	-0.26±0.03[a]	-0.31±0.03[a]
- b	0.26±0.04[a]	0.33±0.04[a]

†Mean±SD (n=3)
[a,b]Different alphabets within the same row denote significant difference at $p<0.05$
‡"L" represents the lightness, "a" represents green (-a) to red (+a) colour, and "b" represents blue (-b) to yellow (+b) colour

The MCFA at the sn-1 and sn-3 are partly hydrolysed in the stomach by gastric lipase, but mostly in the intestines by pancreatic and intestinal lipases. The MCFA are eventually metabolised to acetyl CoA for utilisation as energy (Marten, 2006). The position of MCFA on a triglyceride indicates potential bioavailability.

Total phenolic content, phenolic acid, flavonoids and antioxidant activity

The total phenolic content was found not as high in VCOs as in coconut milk (Table

3). Since most phenolic compounds were hydrophilic, they were unfortunately removed during the oil separation processes and are therefore not found in VCOs. FVCO tends to contain slightly higher phenolic content than CVCO. During fermentation, microorganisms digest the TAG into free fatty acids, which tend to lower the hydrophobicity of the coconut oil. The higher free fatty acid content produced in the FVCO leads to more phenolic compounds in the final product (Table 4). As a bioactive compound, polyphenol intake was associated with a 46% reduction in risk for cardiovascular disease when comparing between the highest (1,235 mg/d) and lowest (483 mg/d) quintile of intake (Tresserra-Rimbau *et al.*, 2014).

Phytosterols

Phytosterols are lipophilic, therefore found being more concentrated in the VCOs than coconut milk after being oil-separated. The contents were not significantly different between the VCOs from both production processes (Table 3).

Phytosterols are reported to reduce cholesterol absorption due to the similarity of their chemical structures (Ostlund, 2004). Phytosterols compete with cholesterol in mixing with micelles, leading to reduced cholesterol absorption in the small intestine (Mel'nikov, Seijen ten Hoorn & Eijkelenboom, 2004).

A serving (80 g) of coconut milk containing 30 mg phytosterols is close to the amount in nuts such as almond (37.78 mg / serving) and walnut (37.99 mg / serving) (Kornsteiner-Krenn, Wagner & Elmadfa, 2013). However, in the case of VCOs which are the extracts from coconut milk, one serving provides only 13.5 mg phytosterols, this amount is too low for considering its phytosterols as a bioactive compound. It has been suggested that phytosterol intake must be up to 800-1000 and 2000 mg/d for reducing 5% (Berger, Jones & Abumweis, 2004), and 10% of blood LDL-cholesterol (Normén, Frohlich & Trautwein, 2004), respectively.

Quality parameters of VCO

Differences in the production processes resulted in different contents of the quality parameters in the VCOs. Factors that related to oil quality deterioration were found to be significantly higher (p<0.05) in the FVCO (Table 4). Both VCOs had a similar iodine value which indicates no changes in the fatty acid profile during the production processes. Due to uncontrollable factors and longer production period of fermentation, the FVCO had higher acid and peroxide values due to increased hydrolytic and oxidative rancidity than CVCO (Raghavendra & Raghavarao, 2011). Differences in VCO production processes did not affect the colour of the products. The final products from both production processes passed the Asian and Pacific Coconut Community (APCC) standards for VCO (Asian and Pacific Coconut Community, 2009).

CONCLUSION

The quality of most bioactive compounds found in fresh coconut milk remains unaltered by the production processes of VCOs, except for phenolic compounds. Lacking of hydrophilic phenolic compounds in the VCOs could negatively affect their antioxidant activities. The most promising bioactive compound in VCOs was MCFA, of which lauric acid was the main contributor. The low levels of phytosterols in both VCOs were not deemed to have potential health benefits.

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Authors' contributions

Chavasit V, designed the experiment and wrote the manuscript; Ngampeerapong C, performed the experiments, analysed the data and drafted the manuscript; Durst RW, designed the methodology and analysed the data.

Conflicts of interest

None.

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Effects of ripening stage and cooking methods on available glucose, resistant starch and estimated glycemic index of bananas (*Musa sapientum;* Nam-wa variety)

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ABSTRACT

Introduction: Resistant starch (RS) has been associated with health benefits including reduced cholesterol absorption, and also been considered as a prebiotic. Little is known of the RS contents of bananas from Thailand. **Methods:** This study determined the digestibility of starch in bananas (Nam-wa variety) at different ripeness stages, based on roasting and boiling. *In vitro* glycemic response of the bananas was also investigated. Based on peel colour, banana maturity stages were classified into 8 stages namely, unripe (stages 3–5) and ripe (stages 6–8). Analysis methods used were *in-vitro* enzymatic digestion and HPLC analysis. **Results:** Unripened bananas contained less total sugar compared to ripened bananas. Rapidly Available Glucose (RAG) and Slowly Available Glucose (SAG) contents increased in tandem with progression of the ripening stage. However, there was no significant difference in the RS content with ripening stage (p>0.05). The RS content also did not show significant difference between the cooking methods. Boiling of banana at the same ripening stage considerably reduced the estimated glycemic index (eGI) (34-56), whilst roasting did not produce any marked changes in pGI (53-56). **Conclusion:** The RAG and SAG amounts in the bananas studied were found to be directly related to ripening stage. Boiling was shown to be a better cooking method for lowering the pGI of bananas compared to roasting.

Keyword: Banana, cooking, glycemic index, processing, resistant starch

INTRODUCTION

Bananas are a leading food crop worldwide, secondary to rice, wheat, corn and potatoes. They are a rich source of carbohydrates, vitamins and minerals. The high carbohydrate content of bananas makes them a staple calorie resource for over 500 million inhabitants of tropical countries (Aurore, Parfait & Fahrasmane, 2009). Banana is a rich source of vitamin B6, vitamin C and potassium.

Harvested bananas pass through several physiological development stages, namely the pre-climacteric (green) stage, the climacteric stage, the ripening stage and the ripe and senescence stage (Robinson & Sauco, 2011). Unripe

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bananas are a rich source of starch, accounting for 70–80% of the overall composition, whereas in ripe bananas, the amount of sugar is increased with the reduction on the starch content.

Different sources of carbohydrates affect the blood glucose response differently after consumption. Blood glucose response is normally estimated by the glycemic index, which compares the response of a test food to that of a reference food, usually glucose or white bread. The primary factor that affects the glycemic index is the rate or absorption of carbohydrates in the presence of food. The rapidly available glucose (RAG) and slowly available glucose (SAG), which are nutritionally classified according to the glucose availability, have been shown to influence the glycemic index (GI) (Englyst *et al.*, 1999). Foster-Powell, Holt & Brand-Miller (2002) reported that the addition of high fibre to food products reduced the food's GI value.

Many studies have reported that the GI of bananas ranges from 53 to 70, leading to bananas being considered to be a medium-GI food (Foster-Powell *et al.*, 2002; Yusof, Talib & Karim, 2005; Hettiaratchi, Ekanayake & Welihinda, 2011). In general, the different GI values of bananas could be due to differences in ripening stages and cultivars. Unripe (green) bananas can be cooked e.g. by roasting or boiling, like it is practiced for plantain and cooking bananas, depending on cultivar and preferences (Dufour *et al.*, 2011). The cooking method has been found to have an impact on the starch content, resistant starch (RS) and GI (Moongngarm *et al.*, 2014).

The starch digestion rate in releasing glucose into the bloodstream at a slower rate results in reduced glycemic and insulinemic postprandial responses from food products containing high amounts of dietary fibre (Yamada *et al.*, 2005). In addition, the RS acts as a fermentation substrate in the colon, similar to non-starch carbohydrates, with positive implications for the prevention of food-borne diseases, such as colon cancer and hypolipidemia. Odenigbo *et al.* (2013) showed that cooked bananas had a lower RS content than uncooked bananas. The RS content of bananas decreased during postharvest treatments (Wang *et al.*, 2014).

The RS of bananas has been studied primarily in the form of banana flour. Thus, the results from these studies do not represent the actual RS in bananas. There are limited studies that investigated the effects of processing, roasting and boiling on the RS content, SAG, RAG and GI of bananas available in Thailand. The objective of this study was to determine the digestibility of starch in bananas (the Nam-wa variety) at different ripeness stages using two cooking methods, roasting and boiling, and investigate the *in vitro* glycemic response. The studies were conducted for 2–3 different stages but not the completely ripe stage. The processing of bananas could influence their RS content and food microstructure. However, the slowly digested starch (SDS), rapidly digested starch (RDS), RS and GI of bananas at different ripening stages, as well as the effect of cooking on these compounds under the same experimental conditions, have not been previously investigated.

MATERIALS AND METHODS

Sample preparation

Freshly harvested bunches of green (unripe) bananas (*Musa sapientum*) were obtained from the Nonthaburi province, Thailand, in 2014. After species identification (as *Musa sapientum*, triploid hybrid banana), the fruits were classified as non-plantain cooking banana according to the dry matter content ranging between 30-33% (Dufour *et al.*, 2009), the ripening process

was conducted in house by leaving the bananas at room temperature. The ripening of the bananas was assessed by the peel colour and divided into 8 stages,: green (stage 1), green with a trace of yellow (stage 2), more green than yellow (stage 3), more yellow than green (stage 4), yellow with a green tip (stage 5), all yellow (stage 6), yellow with a few brown spots (stage 7) and yellow with many brown spots (stage 8). Fresh bananas at ripening stages 3-8 were used to study the RAG, SAG, RS and eGI, whereas bananas at ripening stages 3-5 were used to study the effect of cooking on the RAG, SAG, RS and eGI. Stage 1 and 2 were used for nutritional composition analysis and sugar but not for cooking effect.

Colour measurement

The peel colour was measured using a colorimeter (Minolta, CM 600d, US). The bananas' outer peels were cut into small pieces and placed in a glass dish, covering the entire base of the dish. Measurements were performed in triplicate.

Cooking methods

Roasting

Banana roasting was conducted as follows. A whole unpeeled banana was placed on a wire mesh (width 11.5 x 11.5 cm) over a preheated charcoal stove. The banana sample was frequently turned to prevent charring and burning. The banana was cooked for 40 min until a golden-brown colour was obtained. Roasting temperature was in between 100-110°C The sample was then divided into 2 lots for immediate analysis and left at room temperature prior to analysis.

Boiling

An unpeeled whole banana was boiled in water (gentle boil at 100°C) for 30 min. The ratio of water to banana was 1:3 (v/w). The banana sample had a soft texture and became purple.

Both roasted and boiled bananas were removed from the heat source and left at room temperature (25-30°C) for 0 min and 120 min to study the effect of cooling on RS formation. Sample was then divided into 2 lots for immediately analysis and left at room temperature prior analysis.

Proximate analysis

All of the nutritional components were analyzed according to the AOAC (2012) method, including the carbohydrate, protein (991.20), fat (932.06), dietary fibre (991.43), sugar (980.13), ash (930.30) and moisture content (926.12).

Sugar analysis

The method used for sugar analysis was adopted from the AOAC method (977.20). Banana samples of approximately 5 g were ground. Then, 85% EtOH was added and placed in a water bath at 60°C for 1 h. The extraction was performed in triplicate. The solution was evaporated using a rotary evaporator (Buchi, Switzerland) until completely dry. The residue was redissolved with 3 ml of distilled water, filtered through a 0.22 µm PTFE prior HPLC analysis.

RAG, SAG and estimated glycemic index analysis

The *in vitro* starch digestibility was assessed according to the protocol developed by Goni *et al.* (1996). In brief, approximately 100 mg of a sample were weighed in a 50 ml tube. Potassium chloride buffer was added to the sample, and 0.2 ml of a pepsin solution was then added. The sample was then incubated in a water bath at 40°C for 60 min with constant shaking. Then, the pH was adjusted to 6.9 using a 0.1 M Tris-maleate buffer. After 1 ml of the α-amylase solution was added,

the mixture was incubated at 37°C for 2 hours with constant shaking. The samples that were removed at 30 and 120 min were considered the RAG and SAG, respectively. (Goni, Garcia-Alonso & Saura-Calixto, 1997).

Measurement of RS

The RS measurement was performed according to Goni, Garcia-Alonso & Saura-Calixto (1997). In brief, a digested sample was incubated at 37°C for 16 h. The sample was then centrifuged the pellets were washed with l0 ml of distilled water. Before the addition of the enzyme, the pellets were washed with distilled water and this was followed by the addition of 3 ml of a 0.4 M sodium acetate buffer and 80 µL of amyloglucosidase. The mixture was incubated in a water bath at 60°C for 45 min with constant shaking. The glucose was converted into starch by applying a factor of 0.9, which included the conversion of RAG and SAG into RDS and SDS, respectively.

Tannin screening method

The screening of tannin was performed according to Geetha & Geetha (2014). In brief, banana samples (peel or pulp) were ground with a blender and diluted with DI water. The aliquot was then made to react with lead acetate anhydrous (1% solution). The formation of a red colour solution indicated the presence of tannin. Qualitative test was performed by comparing with a tanning standard as a positive control.

Statistical analysis

All of the values shown are the mean averages of triplicate determinations. The glycemic index and starch fraction after hydrolysis were analyzed by one-way analysis of variance using SPSS version 19, Mahidol University at a 95% confidence interval. All of the data are reported as the means and standard errors of the mean (mean±SEM). The area under the curve associated with a change in the glucose level was calculated using GraphPad Prism version 5.01 (GraphPad software, CA, USA).

RESULTS AND DISCUSSION

Proximate analysis and ripening stages

The strong correlation between fruit colour and ripening makes it feasible to evaluate the ripening level based on colour (Zhang *et al.*, 2014). In this experiment, bananas at 8 different ripening stages were classified into 2 main groups, unripe (stages 3-5) and ripe (stages 6-8). This classification was different in comparison with other studies that divided ripening stage into either 5 stages (Khawas *et al.*, 2014) or 7 stages (Chiun *et al.*, 2015). The banana pulp was composed of carbohydrates, proteins, lipids, ash, and dietary fibre (Table 1). With regards to energy (kcal), no significant difference was noted between fresh bananas and processed bananas (roasted and boiled) at different ripening stages. The moisture content of bananas at different ripening stages varied from 66-69% DW in fresh bananas. It must be noted that the water percentage increases in the pulp during ripening due to the respiratory breakdown of starch and the osmotic movement of water from peel to pulp. In roasted bananas, water constituted approximately 56-59% DW, compared with 67-70% DW in boiled. During boiling, excessive water was used, whereas during roasting, most of the moisture content originated from intracellular water.

Changes in the carbohydrate content in banana pulp during ripening were due to conversion of starch to sugars. However, the total carbohydrate content was not significantly different among the fresh, roasted and boiled bananas. The lipid content remained constant during the ripening process. Lipids

Table 1. Proximate analysis of fresh, roasted and boiled bananas at different maturity stages (g/100 g DW)

Sample	*Energy*^{ns}	*Mo*	*P*^{ns}	*TF*^{ns}	*CHO*^{ns}	*TDF*	*IDF*^{ns}	*SDF*	*Ash*^{ns}
Raw banana									
Stage 3	388.64±0.13	66.54±0.40^{a}	3.08±0.04	nd	94.08±0.07	6.47±0.03^{a}	2.94±0.03	3.53±0.00^{a}	2.84±0.03
Stage 4	387.02±0.06	67.49±0.08^{a}	3.04±0.08	nd	93.71±0.09	7.47±0.15^{a}	2.83±0.14	4.64±0.01^{a}	3.24±0.01
Stage 5	386.79±0.06	66.08±0.27^{a}	3.07±0.06	nd	93.63±0.07	6.38±0.07^{a}	1.98±0.03	4.41±0.10^{a}	3.30±0.01
Stage 6	388.46±0.63	69.03±0.15^{a}	3.37±0.13	0.27±0.11	93.12±0.03	7.75±0.04^{a}	2.66±0.17	5.08±0.14^{b}	3.23±0.02
Stage 7	387.92±0.49	69.24±0.15^{a}	3.27±0.05	0.15±0.07	93.39±0.08	7.83±0.05^{a}	2.52±0.10	5.31±0.05^{b}	3.20±0.04
Stage 8	388.80±0.19	69.21±0.26^{a}	3.28±0.12	0.219±0.00	93.26±0.17	8.80±0.12^{b}	2.14±0.16	6.66±0.04^{c}	3.17±0.05
Roasted banana									
Stage 3	388.75±0.17	56.55±0.36^{b}	3.24±0.04	0.30±0.07	93.27±0.07	12.53±0.41^{c}	3.77±0.13	8.76±0.54^{d}	3.19±0.04
Stage 4	388.44±0.04	58.18±0.20^{b}	3.11±0.05	0.30±0.02	93.33±0.10	9.60±0.17^{b}	3.13±0.08	6.47±0.26^{c}	3.26±0.03
Stage 5	389.31±0.38	58.64±0.11^{b}	3.16±0.01	0.34±0.14	93.41±0.21	8.44±0.02^{b}	2.77±0.06	5.67±0.04^{b}	3.09±0.08
Boiled banana									
Stage 3	388.86±0.37	67.52±1.27^{a}	3.85±0.28	0.38±0.01	92.50±0.36	7.28±0.09^{a}	1.22±0.03	6.07±0.06^{c}	3.26±0.08
Stage 4	385.91±0.24	71.11±0.11^{c}	3.98±0.16	0.38±0.00	91.64±0.11	9.66±0.23^{b}	3.10±0.06	6.56±0.29^{c}	3.99±0.06
Stage 5	386.93±0.08	70.33±0.25^{c}	3.52±0.15	nd	93.21±0.13	8.76±0.03^{b}	3.05±0.19	5.71±0.17^{b}	3.27±0.02

Values expressed are mean±standard deviation of triplicates analysis

$^{a, b, c}$ Different alphabets within the same column indicate a significant difference at $p<0.05$

Mo = Moisture, P = Protein, TF = Total fat, CHO = Carbohydrate, TDF = Total Dietary Fibre, SDF = Soluble Dietary Fibre, IDF = Insoluble Dietary Fibre, and nd = Not detected. ns = not significantly different at $p<0.05$

were not detected in fresh bananas at stages 3-5 but appeared subsequently in stages 6-8. Only 3% protein content was detected, and this value did not markedly change during ripening, (Robinson & Sauco, 2011). As ripening progresses, the water-insoluble fibre decreases, and the soluble fibre increases significantly (3-6%) except at stage 5. This agreed with the softer in texture as they ripen. The cell wall of the less-mature fruit is generally more compact due to the pectin molecules being tightly bound in the cell wall, which could contribute to the firmness of the fruits Table 1shows that banana contains high soluble fibre which is likely to be pectin.

Effect of ripening stage on glucose availability and RS

The RAG, SAG and RS contents are shown in Figure 1, in terms of glucose units formed by starch hydrolysis. An increase in the RAG content was observed with advancing ripening stage. The RAG contents of stages 3 (12.5 g/100 g DW) and 4 (12 g/100 g DW) were not very different compared with that obtained in stage 5 (19 g/100 g DW). A marked change was observed during the transition from stage 5 to stage 6 (32 g/100g DW). In stage 6, the entire banana turned yellow (as indicated in the methods section). By comparison, bananas contain a lower RAG (40%) compared to cornflakes (70%) and biscuits (50%) (Bhavya & Prakash, 2012).

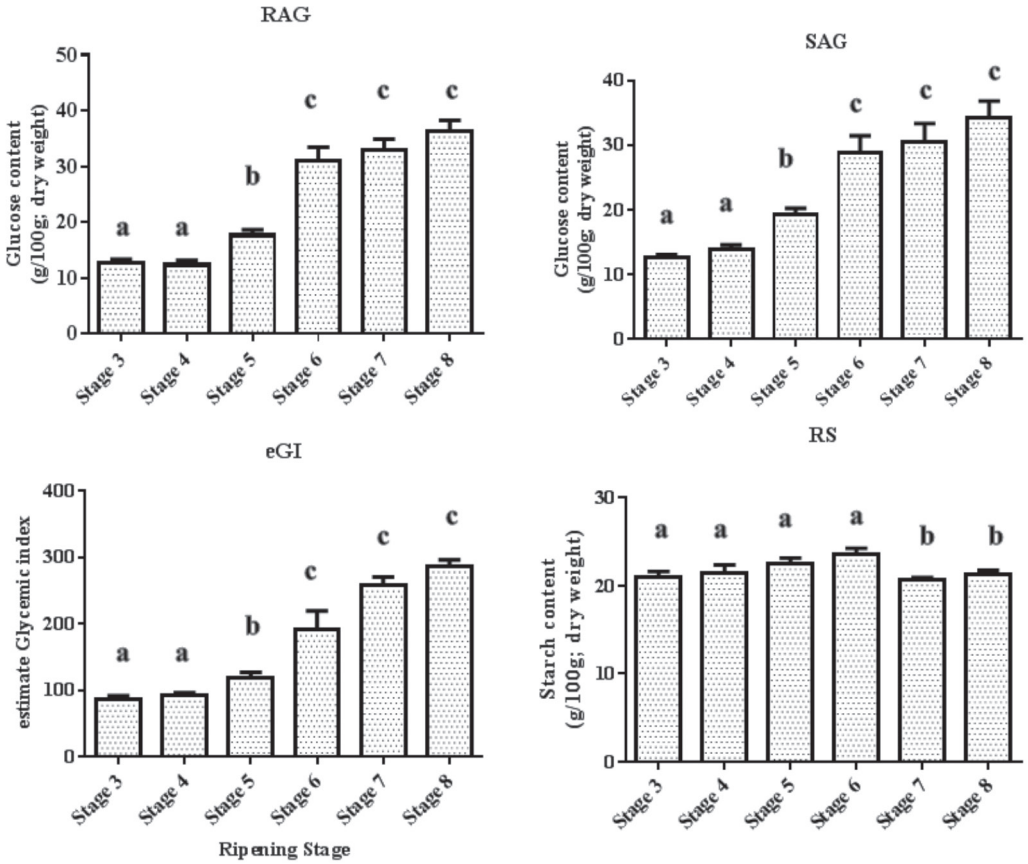

Figure 1. *In vitro* starch hydrolysis of banana at different ripening stages (g/100 g DW).
[a,b, c] Different alphabets indicate significant difference at $p<0.05$

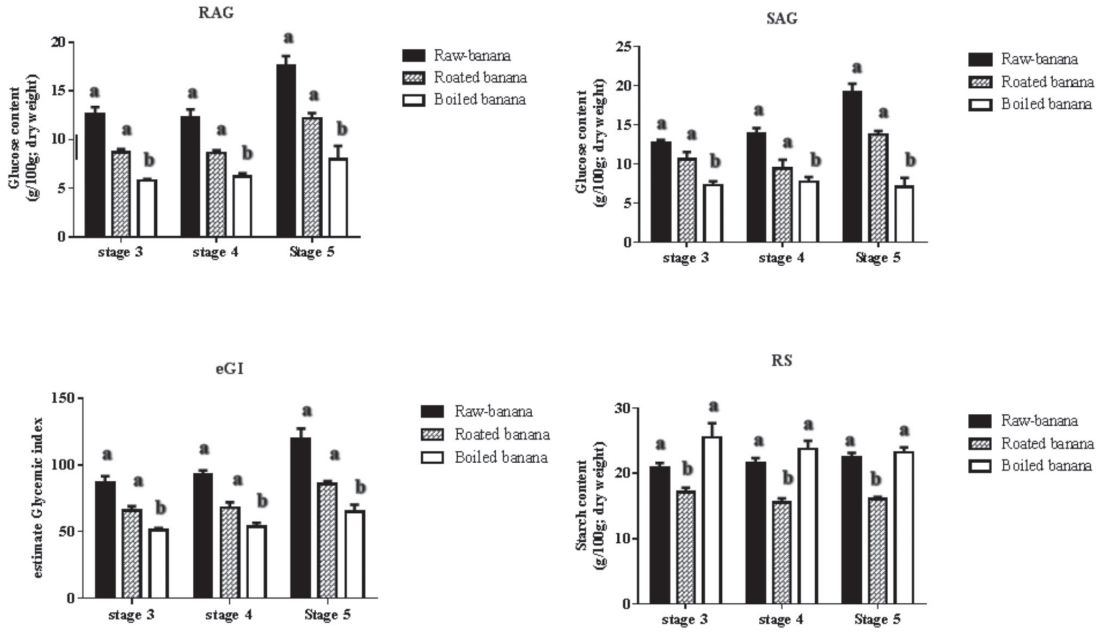

Figure 2. RAG, SAG, RS content and pGI of banana stage 4, 5, 6 cooked by different methods. [a, b, c] Different alphabets indicate significant difference at $p<0.05$

Figure 2 shows that ripe bananas have a high sugar content. This finding indicates a direct correlation between the RAG and SAG and eGI, which is in agreement with the results of several studies (Bhavya & Prakash, 2012; Englyst & Englyst, 2005). The estimated GI was quite high when was compared with sucrose of the same weight. RS was detected in all stage of bananas. This finding implies that most raw banana starch is resistant to enzymes found in nature. This type of starch known as RS2 represents starch that is in a certain granular form and resistant to enzyme digestion according to the Englyst classification, RS type 1-5.

In raw starch granules; starch is tightly packed in a radial pattern and is relatively dehydrated. This compact structure limits the accessibility of digestive enzymes, various amylases, and accounts for the resistant nature of RS2. The RS2 has slightly increased at the beginning from stage 3 to stage 6 but then declined from stage 7 to stage 8. This shows that RS2 formed progressively as banana was ripening and declined after the peel colour turned entirely into yellow. The decreased of RS3 at stage 7 and 8 (Figure 1) can be explained by the increase in moisture content (Table 1) hence RS2 was rehydrated and therefore became less resistant. Moreover, as ripening progresses, pectin esterase, α- and α-amylase activities increase which loosens starch that is tightly packed, results in available starch (Soares *et al.*, 2011).

Three types of sugars were detected in fresh bananas, namely sucrose, fructose and glucose (Table 2). However, the concentration varied with the ripening stage. Ripe bananas had a higher sucrose content compared with unripe bananas. The glucose and fructose concentrations exhibited a similar trend, increasing from stage 3 to stage 8 with a slightly lower concentration at stage 8.

Table 2. Fructose, glucose and sucrose of fresh and cooked banana of different maturity stages (g/100g DW)

Maturity stages	*Fructose*	*Glucose*	*Sucrose*
Fresh banana			
Stage 3	4.68±0.40[b]	3.73±0.03[b]	16.71±0.50[a]
Stage 4	6.49±0.50[b]	5.68±0.72[b]	17.65±0.29[a]
Stage 5	5.49±0.28[b]	4.63±0.40[b]	27.32±0.46[b]
Stage 6	8.68±0.30[c]	7.81±0.25[c]	36.75±0.15[c]
Stage 7	10.14±0.50[c]	9.12±0.40[c]	40.91±0.23[c]
Stage 8	9.77±0.42[c]	8.76±0.30[c]	44.07±0.33[c]
Roasted banana			
Stage 3	2.00±0.47[a]	0.63±0.75[a]	20.58±0.49[a]
Stage 4	2.39±0.70[a]	0.67±0.44[a]	26.45±0.19[b]
Stage 5	2.31±0.12[a]	0.89±0.39[a]	30.72±0.18[b]
Boiled banana			
Stage 3	1.25±0.26[a]	0.24±0.16[a]	17.78±0.34[a]
Stage 4	1.40±0.70[a]	0.44±0.22[a]	26.25±0.04[b]
Stage 5	3.10±0.21[b]	1.17±0.31[a]	32.55±0.40[b]

Values are mean±SD
[a, b, c] Different alphabets indicate significant difference at $p<0.05$

Effect of cooking on glucose availability, RS content and eGI

Table 2 shows higher content of sucrose in roasted and boiled bananas. This may be explained by cell wall degradation by high heat and starch degradation. Higher content of sucrose is also due partly to lower moisture content in cooked banana. The reduction of glucose and fructose was caused by their participation in the Maillard reaction, as evidenced by the brown colour of the roasted and boiled bananas.

Reduction of glucose and fructose varied at each stage, indicating that cooking method influences the release of glucose molecules differently, as shown in Figure 2. Roasting and boiling significantly reduced the RAG of bananas at stages 3, 4 and 5. These stages were chosen for their cooking applications. Bananas at stages 1-2 were very green and hard, whereas those are stages 7-8 were overripe and very soft; thus, none of these four stages are suitable for cooking. Roasting relatively reduced the RAG via the Maillard reaction by producing a Maillard reaction byproduct (MRP), which is the chemical reaction of reducing sugar and protein present in banana. In addition, the MRP was found to exhibit α-amylase activity (Chung, Lee & Rhee, 2011).

Boiling yielded the lowest RAG and SAG contents detected, which may be due to the leaching of sugar into the boiling water, compared to starch, which is less mobilized. The RS content level was ranked highest in boiling, followed by roasting. Increase in the RS content by boiling whole bananas was more likely due to the migration of starch from the banana peel into the pulp, while roasting led to significant reduction of RS (Figure 2). Boiling provided moist heat enabling starch to be gelatinized prior to recrystallization to form RS (specifically RS3). Therefore, conditions that could fully gelatinize banana starch favor the formation of RS3.

The eGI of boiled bananas was lower than that of the roasted sample due to

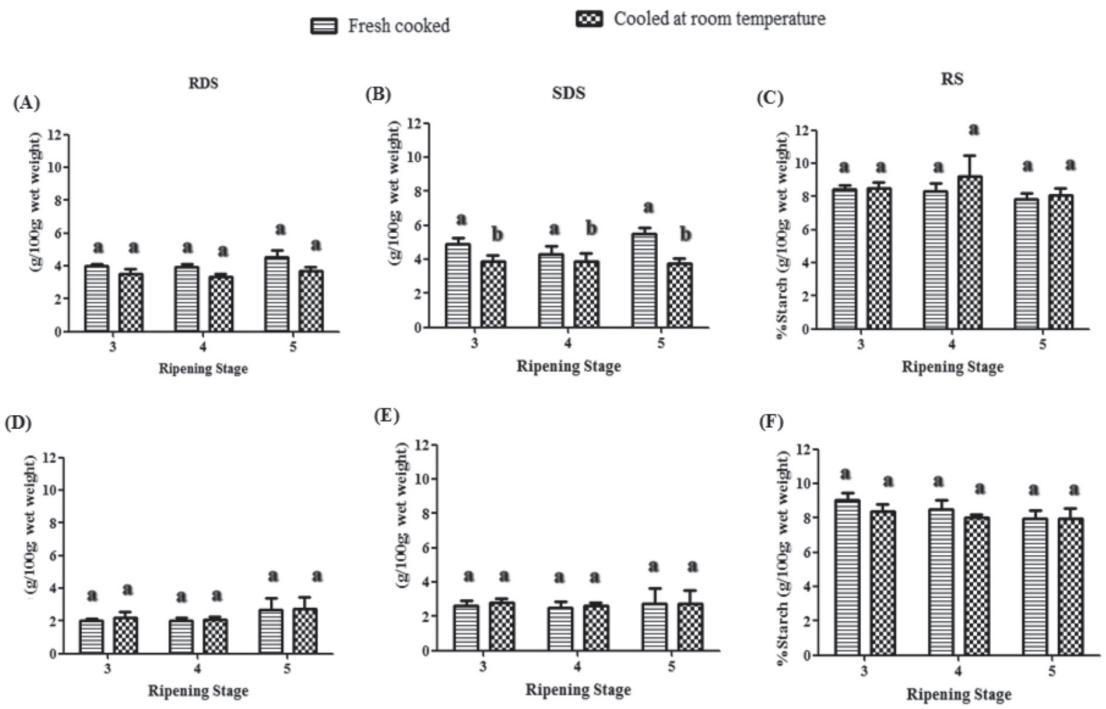

Figure 2. RAG, SAG, RS content and pGI of banana stage 4, 5, 6 cooked by different methods. [a, b, c] Different alphabets indicate significant difference at $p<0.05$

the whole, unpeeled banana being boiled and the tannins in the banana exhibiting α-amylase inhibition; therefore, the reduction of the GI of boiled bananas maybe due not only to a low sugar concentration but also other factors that inhibit α-amylase activity. Moreover, the consumption of RS proved an improvement in the glucose metabolism (Tabibloghmany & Ehsandoost, 2014). Thus, the presence of RS could provide health benefits, including an increase in the total dietary fibre. Boiling is a better method compared with roasting because it results in lower RAG and SAG contents, which determines the GI, as well as a high RS content.

Effect of storage conditions on RAG, SAG, RS and eGI

Several studies have shown that retrogradation results in RS formation. Thus, cooking and cooling have been found to increase the RS content (Arcila, Weier & Rose, 2015).

As shown in Figure 3, the RS increased after the roasted bananas were maintained at room temperature for 120 mins, compared with 0 min. This finding may be explained by the recrystallization of complex carbohydrates during cooling. This result is in agreement with the hardening of the outer banana pulp, whereas in boiled bananas, the trend was not apparent. In addition, stages 3 and 4 showed the reduction of RS after the bananas were left to cool at room temperature. After being cooled at room temperature, the final temperature of the roasted and boiled bananas was 30°C and 29°C, respectively. The heat transfer rate of the roasted and boiled bananas was 0.6°C/min and 0.5°C/min, respectively. These heating rates

were not different; therefore, it was postulated that the differences in the RS formation were due to the differences in the moisture content, type of heat and microstructure. Consequently, a different rearrangement of amylose and amylopectin might have occurred.

Roasting resulted in starch hydrolysis due to the decrease in the RDS and DSD content (Figure 3) because heat was involved in the process and bananas contain both reducing sugars and proteins (Tables 1 and 2). It is assumed that the Maillard reaction would occur in both boiled and roasted bananas. In terms of the Maillard reaction and its properties, eating cooked bananas could result in increased blood sugar via α-amylase inhibition. Furthermore, a MRP has been associated with impairments in glucose metabolism. However, the active dose of the MRP has not yet been defined. From the experiment, the reduction in temperature was not an effective procedure for improving RS formation in both roasted and boiled bananas. The formation of RS was limited by the hydrolysis of starch during heating, leading to a low amylose and amylopectin content and thereby reducing retrogradation. Thus, no significant difference was observed. Therefore, it is hypothesized that a rapid reduction of temperature could assist in RS formation through the retrogradation process. In addition, RS is not only chemically distinct, but its physiological properties, including fermentation characteristics and crystal formation, need to be studied to fully understand the function of RS2 which is naturally found in banana and RS3 that is formed in bananas (*Musa sapientum*) after cooking.

CONCLUSION

This study showed that the RS content range of 20-25 g/100 g DW in the bananas studied (Nam-wa variety) did not vary with maturity. The RAG and SAG amounts increased with progression of maturity. The RS content of the bananas increased with boiling compared to roasting. The beneficial properties of RS in bananas should be further investigated.

Authors' contributions

SC performed the experiment, collected data and performed data evaluation. WS designed experiment, performed experiment, interpreted the result, prepare manuscript, corrected manuscript. WK designed experiment.

Conflict of interest

The authors declared no conflict of interest.

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SHORT COMMUNICATION

Proximate composition, short and medium-chain fatty acids of selected powdered goats milk

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ABSTRACT

Introduction: Goats milk provides health benefits due to its unique fatty acid composition that comprises relatively high amounts of short- and medium-chain fatty acids, which make goats milk easy to digest. **Methods:** A total of 20 powdered goats milk samples were selected based on ease of availability in shops in Kubang Kerian, Kelantan. Proximate composition and fatty acids, specifically C6:0, C8:0 and C10:0 were determined using AOAC methods (2000), and gas-chromatography, respectively. Results were compared with commercial pure goats milk (CBM®). **Results:** Wide variations in the proximate composition and fatty acid contents were found among the samples when compared with the CBM® sample. The mean range values for energy were 368 to 498 kcal/100 g, moisture: 2.46 to 4.28 g/100 g, ash: 2.04 to 6.61 g/100 g, protein: 2.80 to 26.24 g/100 g, fat: 1.68 to 25.90 g/100 g and carbohydrates: 44.81 to 87.64 g/100 g. The total short and medium-chain fatty acids contents ranged from 3.22% to 12.97%. **Conclusion:** There is a need for standardisation of the proximate composition and fatty acids contents of goats milk available in Malaysia.

Keywords: Goats milk, proximate composition, medium-chain fatty acids (MCFA)

INTRODUCTION

Although there is no official statistical record of the current production of goats milk in Malaysia (Shanmugavelu & Quaza Nizamuddin, 2013), goats milk consumption is perceived to have become popular among Muslim consumers, because of the claim that it is a kind of prophetic food with health benefits (Rani *et al.,* 2016).

While goats milk is mainly sold in fresh liquid form in the market, it is also available in powdered form. The nutritional quality and taste of goats milk is usually compared to cows milk. Owing to its relatively high amount of short- and medium- chain fatty acids content, goats milk facilitates nutrient absorption, especially in improving fat absorption (Zenebe *et al.,* 2014; Alferez *et al.*, 2001).

The most reported health benefits of goat's milk are its advantages in improving weight and undernutrition.

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This was reported by a research conducted in Madagascar amongst thirty hospitalised malnourished children (1 to 5 years old) which succeeded in increasing their body weight by 9% (Razafindrakoto *et al.,* 1994). Meanwhile, a study in New Zealand with seventy-two new born infants showed that infants who were fed with goat's milk gained an average of 309 gram more weight than before over the 168-day of study period (Grant *et al.,* 2005).

There are limited studies on the proximate and fatty acids analyses of powdered goats milk available in Malaysia. This study aimed to determine the proximate and fatty acids contents of selected powdered goats milk, with focus on short- and medium- chain fatty acids contents.

MATERIALS AND METHODS

Sampling

A total of twenty powdered goats milk samples of different brands were bought from various supermarkets, small sundry shops and through online shopping websites. The supermarkets and small sundry shops were located in Kubang Kerian, Kelantan. The samples' prices ranged from RM7 to RM11 per 100 g. The inclusion criteria include goats milk that are suitable for four years old and above, while flavoured goat' milk was excluded. As a reference, a pure full cream goats milk sample (powdered form) was obtained from The Netherlands (CBM®). Most goats milk in the Malaysian market generally use CBM® as their base ingredient.

Proximate analysis

The samples were analysed individually in triplicate. Proximate analysis was undertaken based on AOAC (2000). Moisture content was obtained using air-oven dried method (105°C). Ash was determined by incineration in a muffle furnace at 550°C. Protein content was determined by micro-Kjeldahl analyser, i.e. Kjeltec Auto 2300 Analyzer, Denmark. The composition of fat in powdered goats milk was determined by Modified Mojonnier method. Carbohydrate was determined by subtracting from 100, the sum of moisture, ash, protein and fat percentage. Total energy was calculated as: Energy = (protein x 4) + (fat x 9) + (carbohydrate x 4).

Short and medium-chain fatty acids analysis

The fatty acids profile in powdered goat's milk was determined by Gas Chromatography (GC) method (Christie, 1989). Data obtained from chromatogram was analysed. Peak identification was based on retention time of reference standards based on peak area percentages (Supelco® 37, Bellefonte, PA).

Statistical analysis

The results were analysed by applying descriptive statistical analysis using mean value, standard deviation (SD), maximum and minimum values (IBM SPSS Statistics Version 22). ANOVA analysis was carried out to determine the differences and to compare using the Duncan test with 5% of significance.

RESULTS AND DISCUSSION

Out of twenty analysed samples, only nine contain pure goat's milk (based on the ingredient's label). This indicates that most of the samples analysed in this study were not pure goat's milk, but contained a mixture of other ingredients, such as non-dairy creamer and extracts of raisin, honey, pomegranate, and other miscellaneous ingredients.

Proximate analysis

The proximate analysis of the powdered goats milk compared with the reference

Table 1. Proximate analysis of 20 samples of powdered goat's milk and reference value (g/100 g)

Sample	*Moisture*	*Ash*	*Protein*	*Fat*	*Carbohydrate*	*Energy (kcal/ 100 g)*
	Mean±SD					
G1	4.11^{h}±0.02	2.45^{d}±0.06	2.92^{a,b}±0.01	16.38^{j}±0.24	74.14^{i}±0.28	456^{k}±1
G2	4.13^{h}±0.06	2.85^{g}±0.01	9.33^{h}±0.04	9.33^{c}±1.12	74.36^{i}±1.10	419^{c}±6
G3†	3.96^{g}±0.06	2.44^{c,d}±0.01	3.07^{b}±0.02	14.50^{h,i}±0.18	76.04^{j}±0.26	447^{i}±1
G4†	3.91^{g}±0.01	2.45^{d}±0.01	3.06^{b}±0.01	11.66^{f,g}±0.13	78.92^{l,m}±0.14	433^{f,g}±1
G5†	4.28^{i}±0.01	2.50^{e}±0.01	3.02^{a,b}±0.02	11.65^{f,g}±0.02	78.55^{k,l}±0.03	431^{f,g}±0
G6	3.33^{e}±0.01	2.04^{a}±0.00	2.80^{a}±0.03	4.19^{b}±0.40	87.64^{q}±0.44	399^{b}±2
G7	3.12^{d}±0.08	3.69^{i}±0.00	8.34^{f}±0.00	13.84^{h}±0.31	71.01^{h}±0.23	442^{h}±2
G8†	3.31^{e}±0.06	3.69^{i}±0.01	15.66^{j}±0.02	16.73^{j,k}±0.11	60.62^{e}±0.08	456^{k}±1
G9	2.68^{b}±0.01	3.28^{h}±0.02	5.78^{e}±0.07	10.07^{d}±0.11	78.19^{k}±0.14	427^{d,e}±1
G10	2.46^{a}±0.00	5.92^{k}±0.00	21.37^{k}±0.10	14.90^{i}±0.63	55.36^{d}±0.52	441^{h}±3
G11	3.51^{f}±0.05	6.61^{n}±0.07	26.24^{n}±0.38	1.68^{a}±0.13	61.96^{f}±0.23	368^{a}±1
G12†	3.31^{e}±0.06	2.41^{c,d}±0.02	3.16^{b}±0.00	10.46^{d,e}±0.03	80.66^{n}±0.05	429^{e,f}±1
G13†	3.49^{f}±0.01	2.43^{c,d}±0.00	3.16^{b}±0.01	11.45^{f}±0.13	79.46^{m}±0.11	434^{g}±1
G14†	3.01^{c}±0.00	2.35^{b}±0.02	3.10^{b}±0.02	10.22^{d}±0.46	81.32^{o}±0.40	430^{e,f}±3
G15	2.75^{b}±0.15	2.40^{b,c,d}±0.01	3.51^{c}±0.27	12.31^{g}±0.03	79.04^{l,m}±0.14	441^{h}±1
G16†	3.13^{d}±0.04	2.39^{b,c}±0.07	3.13^{b}±0.02	9.18^{c}±0.03	82.17^{p}±0.02	424^{d}±0
G17†	2.77^{b}±0.02	6.19^{l,m}±0.00	24.59^{l}±0.12	21.65^{m}±0.65	44.81^{a}±0.51	472^{l}±4
G18	3.39^{e}±0.01	4.69^{j}±0.01	12.57^{i}±0.15	25.90^{n}±0.33	53.46^{c}±0.20	498^{m}±2
G19	3.92^{g}±0.03	2.74^{f}±0.06	4.74^{d}±0.06	19.35^{l}±0.55	69.25^{g}±0.52	470^{l}±3
G20	3.06^{c,d}±0.01	6.16^{l}±0.04	9.06^{g}±0.11	10.97^{e,f}±0.17	70.74^{h}±0.30	418^{c}±1
Reference	2.43^{a}±0.03	6.23^{m}±0.00	25.18^{m}±0.27	17.21^{k}±0.46	48.96^{b}±0.16	451^{i}±3

†Samples that based on ingredient list were pure goat's milk and not added with other ingredients.
^{a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q}Different alphabets in the same column denote significant difference according to Duncan's test (5%).

are shown in Table 1. The reference sample composition was found to be consistent with the findings of Reddy *et al.,* (2014), Batiston *et al.,* (2012), and Park (2000) for energy, ash and protein contents. The proximate analysis of the commercial samples of powdered goats milk showed that Sample G5 provided the highest moisture content. (4.28 g/100 g), while Sample G10 had the lowest (2.46 g/100 g), compared to the reference value (2.43 g/100 g).

The ash content of powdered goat's milk samples ranged between 2.04 g/100 g to 6.61 g/100 g and only two samples of powdered goat's milk; Sample G17 and Sample G20 were comparable to the value of the reference sample (6.23 g/100 g).

Overall, wide variations were found for energy, protein and fat contents among the studied samples. The energy value of the powdered goat's milk varied from 368 kcal/100 g to 498 kcal/100 g. Most of the samples showed statistically significant difference when compared against the reference sample (451 kcal/100 g). In terms of the protein content, Sample G11 had the highest value (26.24 g/100 g), while Sample G6 had the lowest (2.80 g/100 g), compared with the reference protein value of 25.18 g/100 g. Meanwhile, the highest and the lowest fat contents were 25.90 g/100 g (Sample G18) and 1.68 g/100 g (Sample G11), respectively. According to the label of the sample which contained the lowest fat content, its first ingredient was listed as skim milk, and this may explain the low amount of its total fat content. The carbohydrate content of powdered goats milk was wide, ranging between 44.81 g/100 g to 87.64 g/100 g. Only Sample G17 was quite close to that of the reference sample. The high content of carbohydrate in the analysed samples may be attributed to the added ingredients, such as dates, raisin, and honey, such as shown for Sample G6 which had the highest carbohydrate content (87.64%) and it had added honey and dates.

Fatty acids analysis

Table 2 shows the short and medium-chain fatty acids contents in commercial powdered goat's milk samples and the reference sample. Haenlein (2004) reported that goats milk was higher in C6 to C10 than cows milk. This study showed that the total medium-chain fatty acids (MCFA) ranged between 3.22% (Sample G3) to 12.97% (Sample G17), compared to that in the reference sample (11.66%). The result also showed that 12 samples contained C6:0 to C10, while eight samples had only C8:0 and C10:0. Based on ANOVA, there was a significant difference in the total fatty acids and C10:0 for all samples compared to the reference sample. This may be due to the added ingredients in the studied goats milk, especially those added with palm oil or non-dairy creamer.

The richness of short and medium-chain fatty acids in goats milk helps to facilitate improvement of nutrient absorption and energy production in the human body (Zenebe *et al.,* 2014). Apart from that, these fatty acids in goats milk have been recognised as unique lipid with health benefits claimed for treating malabsorption syndromes, chyluria, steatorrhea, hyperlipoproteinnemia, and for premature infant feeding (Vaquil & Rathee, 2017).

According to Salari *et al.,* (2016), quality of fatty acid profile can be affected by season. Some fatty acids were found to be significantly reduced during summer. Norris *et al.,* (2011) also found that the fat content was lower in Saanen breed as compared to the other breeds, such as Toggenburg and British Alpine even though it produces more milk. In this study, most

Table 2. C6:0, C8:0, C10:0 and total MCFA of present study and reference value (%)

Sample	*C6:0*	*C8:0*	*C10:0*	*Total MCFA (%)*
	Mean±SD			
G1	0.76^{d}±0.06	2.18^{c}±0.05	2.83^{c}±0.08	5.78^{c}±0.20
G2	1.37^{f}±1.57	$2.69^{d,e}$±0.25	5.66^{g}±0.46	9.72^{h}±0.86
G3	$0.07^{a,b}$±0.00	1.47^{a}±0.02	1.68^{a}±0.02	3.22^{a}±0.04
G4	1.52^{g}±0.09	2.87^{e}±0.06	5.76^{g}±0.10	10.14^{h}±0.25
G5	0.15^{b}±0.00	1.28^{a}±0.02	1.96^{a}±0.00	3.39^{a}±0.01
G6	0.51^{c}±0.02	$2.10^{b,c}$±0.09	3.79^{d}±0.08	6.40^{d}±0.18
G7	1.01^{e}±0.24	$2.04^{b,c}$±0.17	3.59^{d}±0.16	6.64^{d}±0.56
G8	-	3.39^{f}±0.04	4.53^{e}±0.06	$7.92^{e,f}$±0.10
G9	0.01^{a}±0.01	2.75^{e}±0.34	2.29^{b}±0.14	5.05^{b}±0.48
G10	1.71^{h}±1.63	1.44^{a}±0.11	5.56^{g}±0.24	8.71^{g}±0.51
G11	$0.02^{a,b}$±0.00	1.91^{b}±0.02	6.52^{h}±0.09	$8.45^{f,g}$±0.11
G12	-	3.89^{h}±0.13	3.56^{d}±0.07	7.45^{e}±0.21
G13	-	3.87^{h}±0.01	3.62^{d}±0.03	7.49^{e}±0.04
G14	-	3.86^{h}±0.00	3.62^{d}±0.04	7.47^{e}±0.04
G15	-	3.83^{h}±0.01	3.55^{d}±0.01	7.38^{e}±0.02
G16	-	4.18^{i}±0.01	3.71^{d}±0.06	$7.89^{e,f}$±0.08
G17	$0.02^{a,b}$±0.00	2.88^{e}±0.13	10.07^{k}±0.45	12.97^{j}±0.58
G18	-	$3.44^{f,g}$±0.09	5.22^{f}±0.10	8.67^{g}±0.02
G19	-	5.27^{j} ±0.03	4.63^{e}±0.08	9.90^{h}±0.06
G20	1.64^{h}±0.06	3.61^{g}±0.08	7.20^{i}±0.14	12.45^{j}±0.28
Reference	$0.01^{a,b}$±0.00	2.54^{d}±0.03	9.10^{j}±0.08	11.66^{i}±0.11

Medium-Chain Fatty Acids (MCFA) [C6:0 to C10:0].

^{a,b,c,d,e,f,g,h,i,j,k}Different alphabets in the same column denote significant difference according to Duncan's test (5%).

of the commercial powdered goats milk samples was used CBM® as a base with different percentages and the breed used by CBM® was known as Saanen breed. Thus, variation in the fatty acid does contribute to the difference in short and medium-chain fatty acids content. The low short and medium-chain fatty acids content in the present study could also be due to the low proportion of goat's milk incorporated in the commercial goats milk powder samples. As stated earlier, some of the goat's milk samples in this study were not purely goats milk but consisted of other ingredients. Furthermore, the fatty acid profile of this study do not originated from goats milk only, but also from other added ingredients. Thus, a large difference in fatty acid contents as compared to other studies would be expected.

CONCLUSION

Considering the increasing importance of goat's milk to human nutrition especially for its fatty acids believed to aid in digestion, these findings indicate the need to standardise the proximate and fatty acids contents of goats milk in Malaysia.

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Authors' contributions

Juliana S carried out the experiment, analysed the data and wrote the manuscript with the support from all authors, Marina AM help with the data analysis, Shariza AR & Sakinah H help supervise the project and writing process.

Conflict of interest

The authors declare that they have no conflicting interests either financial or non-financial.

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SHORT COMMUNICATION

Cadmium and lead contents and potential health risk of brown rice (NSIC Rc222 *Tubigan 18*) cultivated in selected provinces in the Philippines

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ABSTRACT

Introduction: Brown rice is promoted for a healthier rice-consuming population as it renders numerous nutritional benefits due to its fiber and germ, yet may contain high concentrations of metal elements from environmental effluents. The purpose of this study is to identify the potential health risk of brown rice cultivated in different major islands in the Philippines. **Methods:** Concentrations of heavy metals cadmium (Cd) and lead (Pb) were investigated on brown rice of a popular modern rice variety (NSIC Rc222) cultivated from top rice-producing provinces in Luzon, Visayas and Mindanao, namely Nueva Ecija, Iloilo and Bukidnon, respectively, through non-probability sampling. Total Hazard Quotient (THQ) and Combined Total Hazard Quotient (CTHQ), as developed by US EPA, were used to calculate the potential hazard. **Results:** Cd levels of brown rice from different sites were found to be below the maximum level of 0.1 mg/kg. However, Pb content from all sites exceeds the 0.2 mg/kg allowable level as recommended by the Joint FAO/WHO Food Standards Programme. Brown rice from Ilo-ilo had the highest Pb content while Nueva Ecija the lowest. THQ values were all below 1.0 but contribution of Pb to CTHQ was higher than that for Cd. **Conclusion:** The findings suggest consuming brown rice from the studied sites has low probability of inducing carcinogenic effects in the long run, but Pb has a greater contribution in the hazard risk as compared to Cd. Further studies on heavy metals especially Pb in brown rice consumed in the Philippines are suggested.

Keywords: Brown rice, cadmium, lead, hazard identification, total hazard quotient

INTRODUCTION

Rice, aside from being the staple food in the Philippines, is also one of the country's major agricultural products. Rice is consumed by 94.8% of the population at 290 g per capita; the richest household has relatively lower consumption at 264 g, as compared to poor households at 309 g (FNRI, 2013). It is the major source of energy due to its high carbohydrate content.

There are two common types of available rice in the market, namely, white rice and brown rice. The difference between the two is in the degree of polishing. In brown rice, only the outer covering is removed, leaving the bran intact whereas white rice is milled and

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polished leading to the removal of husk bran and germ. Due to polishing, white rice loses most of its nutritional content and health promoting activities from fiber, antioxidants, minerals, vitamins and phenolic compounds (Yang *et al.*, 2016). Thus, brown rice is being promoted for a healthier rice-consuming population.

However, rice is also prone to different environmental hazards from water, soil and air. Solidum (2014) showed that all rice varieties sold in Metro Manila market contained lead, and the regular *Malagkit* and NFA rice exceeded the permitted limit for lead. Concern for this matter was raised by the Department of Agriculture – Philippine Rice Research Institute (DA-PhilRice) due to the alarming increase of levels of heavy metals in rice. Further, different rice samples from Asia and Europe were reported to be contaminated with such heavy metals too (Oplas, 2013).

Human exposures to heavy metals have increased dramatically (Cherfi *et al.*, 2016) and it has been reported that the main route of exposure to heavy metal of most people is through the diet. It is important to identify hazards in rice, as it constitutes a major part of the diet among Filipinos.

The general objective of this study was to identify the potential health risk of brown rice cultivated in the Philippines. Specifically, the study aimed to determine concentrations of cadmium (Cd) and lead (Pb) of brown rice of NSIC Rc222 (*Tubigan 18*) cultivated in three major rice producing islands in the country, namely Nueva Ecija in Luzon, Ilo-ilo in Visayas, and Bukidnon in Mindanao.

MATERIALS AND METHODS

Raw materials and sample preparation

Inbred NSIC Rc222 raw rice paddies grown during the 2016 wet season were collected from Munoz, Nueva Ecija, Pototan, Ilo-ilo and Musuan, Bukidnon. Rice growing procedure followed the protocols of National Cooperative Test (NCT) for Rice (BPI, 2014). Briefly, seedlings aged 18 to 21 days old were transplanted at 1-2 seedlings per hill in each plot. Fertilizer (N, P2O5, K2O) were applied at 7 days, 21 days and 28 days after transplanting at a rate of 120-60-60. Crop management practices followed the PalayCheck® (Cruz *et al.*, 2005) recommendations. Thirty days after 50% heading date, the paddies were harvested, and amounts from the three plots (except border rows) were combined as sample source. The composite samples were dried under the sun in net bags until a paddy moisture content of 14% is reached. The rice samples were then cleaned and winnowed properly to remove impurities and dirt.

A hand-operated wooden dehuller with polyurethane rubber was used to remove rice bran from the grain, assisted with a ceramic pincher. The equipment was cleaned and sterilized after every sample manual dehulling to prevent contamination. The brown rice was triple washed, as normally done during household cooking and allowed to dry at room temperature. Rice samples were ground and dried overnight (12 hours) at 60°C. This procedure was adapted from Al-Saleh and Shinwari (2001). The dried and ground samples were weighed at 40 g each and were packed in coin envelopes which were properly labeled, sealed and sent at the Central Analytical Services Laboratory of the National Institute of Molecular Biology and Biotechnology, University of the Philippines Los Baños for Cd and Pb analysis.

Chemical analyses

Cd analysis was done using the protocol of AOAC 965.05 19th edition, while Pb analysis was done using the modified AOAC 972.25 19th edition using Atomic Absorption Spectrophotometry.

Data processing and analysis

Heavy metal concentrations of the investigated rice samples were reported as mean ± standard deviation (SD), as purchased weight. One-way Analysis of Variance (ANOVA) was employed in determining significant differences among the heavy metal concentrations from the three sites of cultivation using the software IBM SPSS Statistics 20.

Further, the estimated daily intake (EDI) and target hazard quotient (THQ) were calculated using the following formulas as developed by the US Environmental Protection Agency (US EPA):

$$\boldsymbol{EDI_i} = \left(\frac{E_f \times E_d \times F_{ir} \times C}{W_{ab} \times T_a}\right) \times 10^{-3}$$

$$\boldsymbol{THQ_i} = \left(\frac{E_f \times E_d \times F_{ir} \times C}{R_fD \times W_{ab} \times T_a}\right) \times 10^{-3}$$

where C is the average concentration of heavy metal (mg/kg, as purchased weight); F_{ir} is the rate of rice consumption (the average F_{ir} for adults is 290 g/day/person as reported on FNRI's FCS), E_f is the exposure frequency (365 days/year), E_d is the exposure duration (68 years, Filipino's life expectancy), R_fD is the oral reference dose (mg/kg/day) (0.1 mg/kg/day for Cd and 0.2 mg/kg for Pb), W_{ab} is the average adult body weight (52.5 and 60.5 kg for women and men, according to the Philippine Dietary Reference Intake 2015, respectively), T_a is the averaging time for non-carcinogens (E_d×365 days/year); and 10^{-3} is the unit conversion factor (Fang *et al.*, 2014).

The Combined Target Hazard Quotient (CTHQ) was calculated using the equation:

$$\boldsymbol{CTHQ} = \sum_{j=1}^{3} THQ_j$$

where j represents the individual heavy metal content namely Cd and Pb. The CTHQ evaluates the risks of the two studied metals together in the brown rice samples. Exposure to two or more pollutants may result in additive effects (Wang *et al.*, 2005 in Cherfi, 2016).

RESULTS AND DISCUSSION

Heavy metal content

Among the three samples, brown rice from Nueva Ecija has significantly highest amount of Cd at 0.037 ppm, whereas Bukidnon brown rice has 0.015 ppm, while no Cd was detected in brown rice from Ilo-ilo (Table 1). Ilo-ilo is a geographic island surrounded by multiple bodies of water with different wetlands as well as coasts and rivers. The abundance of water source in the island could be one factor for the non-detectable quantity of Cd in the brown rice grown in the said area. Nonetheless, the Cd content from all three sites were below the maximum allowable levels for cereals, according to the Joint FAO/WHO Food Standards Programme (FAO/WHO, 2001).

Table 1. Heavy metal content (cadmium and lead) of sampled brown rice (*N*=3)

Rice sample	*Heavy metal content*† *(mg/kg, AP wt.)*	
	Cadmium	*Lead*
Nueva Ecija	0.037±0.000[a]	1.510±0.475[a]
Ilo-ilo	*ND*[c]	1.863±0.478[a]
Bukidnon	0.015±0.018[b]	1.705±0.241[a]

†Maximum Allowable Levels: Cd – 0.1 mg/kg; Pb – 0.2 mg/kg

ND – not detectable

[a, b, c] Different alphabets denote significant difference at $p<0.05$.

On the other hand, all brown rice samples showed Pb contents that exceeded the maximum allowable level for cereals (FAO/WHO, 2001). Brown rice from Nueva Ecija, Ilo-ilo and Bukidnon contained 1.510 ppm, 1.863 ppm and 1.705 ppm, respectively, which were not significantly different. Xie and colleagues (2016) revealed that

rice has high adsorption capacity for Pb suggesting that the high concentration of Pb identified from the samples were sourced from the contamination of each cultivation sites with Pb.

As the three brown rice samples had the same cultural management, the differences in the amounts of heavy metals found could be attributed to different locations, sources and quality of water, soil and air quality. Xie *et al.* (2016) reported that the bio-concentration ability of Pb and Cd had no difference between conventional and hybrid rice, suggesting soil quality is an important consideration for producing contaminant-free rice.

Another interesting observation from the result is the brown rice from Ilo-ilo for it contained non-detectable amounts of Cd but the highest concentration of Pb among the samples. This indicates that the presence of heavy metals may be independent of one another

Estimated daily intake

The EDI of heavy metals for both men and women were calculated and compared to the maximum levels recommended by FAO/WHO (2001). The values were obtained by assuming that brown rice is consumed regularly as a result of promotion for its consumption. Thus, EDIs was calculated to estimate daily intake of Cd and Pb from brown rice consumption.

EDI of Cd for both men and women were 0.2 μg/kg/day, ND and 0.04 μ/kg/day from Nueva Ecija, Ilo-ilo and Bukidnon, respectively, which accounts for 0.2%, 0.0% and 0.04% of the oral reference dose (R*f*d). EDI for both men and women from different sources are approximately similar due to the low concentration of Cd. However, the EDI of Pb, men were 7.2 μ/kg/day, 8.9 μ/kg/day and 8.2 μ/kg/day, which accounts for 3.6%, 4.45% and 4.1% of the R*f*d, from Nueva Ecija, Ilo-ilo and Bukidnon, respectively. In comparison, women were observed to have higher EDI at 8.3 μ/kg/day, 10.3 μ/kg/day and 9.4 μ/kg/day, accounting for 4.15%, 5.15% and 4.7% of the R*f*d, from Nueva Ecija, Ilo-ilo and Bukidnon, respectively. It is thus observed that women were more likely to have higher intake of these heavy metals. This is a concern in relation to the physiological attribute especially during time of pregnancy since these contaminants can penetrate through the placenta (Zhu *et al.*, 2014), thus can affect the pre-natal environment and development of the fetus.

The estimated daily intake of Cd and Pb showed that daily intakes were lower than the R*f*d, both for women and men. This suggests that consuming brown rice at 290 g, as purchased, is generally safe on a daily basis.

Total hazard quotient (THQ)

Heavy metals are accumulated in the body through chronic consumption, such as a staple food like rice. Thus the THQ was calculated to determine its hazard from chronic consumption. The THQ values for Cd were 0.002 for men and 0.003 for women, and 0.0004 both for men and women, from Nueva Ecija and Bukidnon, respectively. There is a higher THQ value for Pb as compared to Cd due to its higher heavy metal concentration (mg/kg). Specifically, THQ values were 0.036 for men and 0.042 for women, 0.044 for men and 0.051 for women, and 0.044 for men and 0.047 for women from Nueva Ecija, Ilo-ilo and Bukidnon, respectively. It is observed that the calculated THQ were far below the hazard indicator value of 1.0, thus considered generally safe (Figure 1). The Pb content of brown rice poses a higher health hazard risk, as compared to the Cd contaminant.

Combined total hazard quotient (CTHQ)

In consideration of the additive effects of the heavy metals under study, the CTHQ values among three cultivation sites were estimated to be below 1.0, which

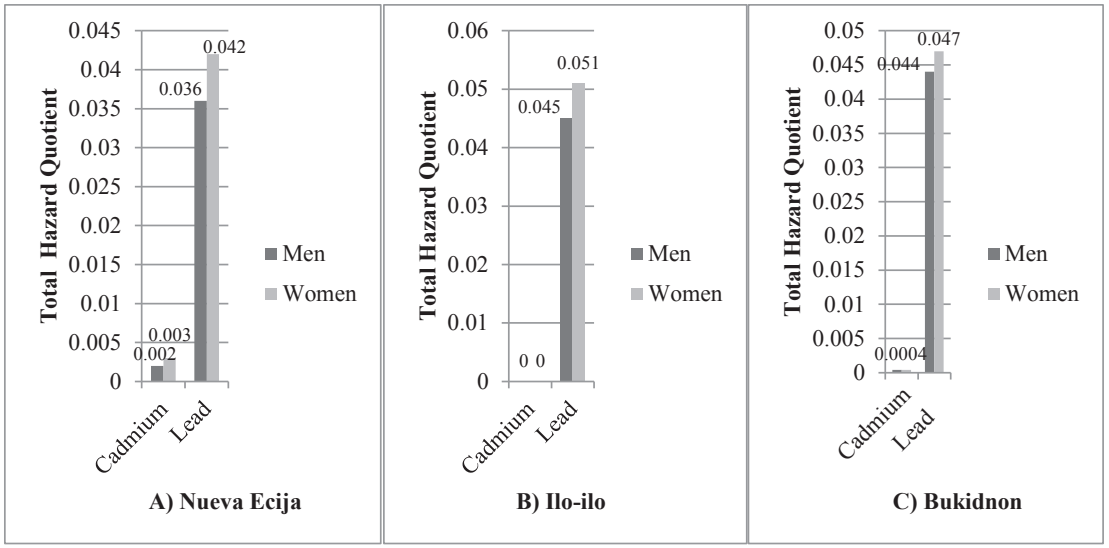

Figure 1. Target hazard quotient (THQ) of brown rice from Nueva Ecija, Ilo-ilo and Bukidnon for men and women.

is considered as generally safe. However, Pb has the greatest contribution in the hazard risk as compared to Cd, and women showed higher CTHQ than men for both metals.

In analyzing the concentration of heavy metals of brown rice from three major producing rice sites in the country, results revealed that Cd concentration was below the maximum level of 0.1 ppm; however, Pb concentration of brown rice from the three cultivated sites was relatively higher, exceeding the maximum level of 0.2 ppm. Nevertheless, based on calculation of EDI, THQ and CTHQ, sampled brown rice is considered as generally safe for consumption.

CONCLUSION

The results showed that the sampled brown rice contain heavy metals but at levels that is still considered generally safe for consumption. It is suggested that analysis for other possible heavy metals such as mercury and arsenic be determined in brown rice consumed in the Philippines.

Authors' contributions

MA Layosa collected the data, wrote the manuscript and conducted the study; LM Atienza supervised the study; ADR Felix helped in the conceptualization and data collection.

Conflict of interest

The authors declare they have no conflict of interest.

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SHORT COMMUNICATION

Knowledge, attitude, and practices regarding food safety among food employees in Ambon City, Indonesia

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ABSTRACT

Introduction: It is estimated that each year, 1.8 million people worldwide die as a result of diarrhoeal diseases attributed to contaminated food. This cross-sectional study was conducted to determine the knowledge, attitude and practices regarding food safety and hygiene among food employees in Ambon Capital City, Maluku Province, Indonesia. **Methods:** A validated questionnaire was self-administered and completed by 135 food employees in small food companies in Jan-March, 2017. The knowledge section consisted of 19 yes-no questions. For knowledge, the score was considered acceptable if total score was >10. Fourteen 4-point Likert-scale questions were constructed for the attitude section, whereby a score of 3.0 and above for each question was considered positive. The practice section consisted of 13 4-point Likert scale items, and a score of ≥3 was considered good practice. The WHO Five Keys to Safer Food Manual was used as reference. **Results:** The respondents had an acceptable level of knowledge about food safety and personal hygiene (mean score=13.08±2.55), a positive attitude (mean score=3.38±0.55) and good practices toward food hygiene measures (mean score=3.98±0.55). A significant correlation was observed between education level, training experience, knowledge, attitude and practices, indicating that having good knowledge and attitude toward food safety could have positive influence on food handling practices. **Conclusions:** It is recommended that regular food safety training and adequate guidelines should be provided to improve food safety practices of food service employees in Ambon City.

Keywords: Knowledge, attitude, practice, food safety, personal hygiene

INTRODUCTION

Foodborne related diseases are among the leading causes of morbidity and mortality worldwide (Centers for Disease Control and Prevention, 2015). According to the foodborne outbreak database published by Centres for Diseases Control and Prevention (CDC), 37% of the foodborne disease outbreaks reported in 2010 were associated with food handling process. It has been estimated that each year 1.8 million people die as a result of diarrhoeal diseases and most of these cases can be attributed to contaminated food or water (Chapman *et al.*, 2010). A meta-analysis study highlighted that proper food preparation can prevent most foodborne diseases (Soon, Baines & Seaman, 2012).

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The World Health Organization (WHO) has long been aware of the need to educate food handlers about their responsibilities for food safety. In the early 2006, WHO developed the Five Keys to Safer Food Manual to provide guideline of food handling and inform more details on the reasoning behind the suggested measures. The aims were to improve the knowledge and skills as well as reapplication of practical food safety among food handlers (World Health Organization, 2006).

Past studies have shown that food employees lack food safety knowledge and follow improper food safety practices. A study by Webb *et al.* (2015), showed that food service workers did not always wash their hands, and 22% did not change gloves between touching raw meat and ready-to-eat (RTE) food. More striking findings were that 33% of food service workers did not wear gloves when touching RTE food, and only 47% used a food-grade thermometer to check the temperature of cooked food for doneness (Webb & Morancie, 2015).

Previous studies have shown mixed results when examining whether increased knowledge leads to better food safety attitudes, practices, and behaviours. Adesokan, Akinseye & Adesokan (2015) found that enhancing knowledge can change behaviours and practices, while Meysenburg *et al.* (2014) argued that improving knowledge through training alone may not result in behavioural changes. Rowell *et al.* (2013) found significant discrepancies between self-reported food safety knowledge and food safety practices. Mizanur *et al.* (2012) identified a number of factors, which affected employees' food safety behaviour. These included time pressure, equipment and resource availability, management and co-workers' attitude to food safety, and food safety education and training. A study in Malaysia in 2014 argued that food safety improvement requires more than food safety training and that training should be multidimensional (Sani & Siow, 2014).

Research studies on knowledge, attitudes and practices regarding food safety among food employees in the meat processing industry in Indonesia are limited. The objectives of the present study were to determine food employees' knowledge, attitudes and practices (KAP) regarding food safety in Ambon city, Maluku province.

MATERIALS AND METHODS

The study was conducted among 135 food employees from various small food companies in Ambon City. The respondents were selected through purposive sampling with technical assistance from the staff of National Agency of Drugs and Food Control regional office. A self-administered questionnaire modified from previous studies was used (Tokuça, Berberoğlua, Bilgeb & Dedelera, 2009; World Health Organization, 2006). Content validation of the questionnaire was done by cross-reference and verification by food safety experts. Reliability of the questionnaire was tested among pharmacy students in Gadjah Mada University with Cronbach's alpha for each set of the questions range within the acceptable limit (>0.7). The assessments evaluated the knowledge, attitude, and practice of the food employees on food preparation, reheating food, food storage, working area, handling raw and cooked food and others.

The respondents' socio-demographic characteristics, such as gender, age, educational level, work duration and certification grades were collected during the study. The age groups were classified according to less than 30 years old and more than 30 years old, have "low educational level" (received

education up to secondary level) and "high educational level" (that received education after their secondary level), "working experience" (work for five year and more, and working for less than five year), and small industries certification (yes or no).

Knowledge section consisted of 19 questions. Respondents were required to choose 'yes' or 'no' answers for this section. Fourteen questions were constructed for attitude section. The respondents were required to choose one of the four options provided which were 'strongly agree', 'agree', 'disagree' and 'strongly disagree'. For knowledge, the score was considered acceptable if its value was above 10. The attitude mean-average score was considered positive if a score of 3.0 and above was achieved. The practice section consisted of 13 4-point Likert scale items. The marks were converted to poor (marks below 3) and good practice (3 and above). The WHO Five Keys to Safer Food Manual was used as reference (WHO, 2006).

Data were analysed using SPSS software version 16. Chi-square test was used to determine the relationship between the socio-demographic characteristics of the food employees and their knowledge-attitude-practice (KAP) level. Logistic regression was used to determine the predictor variables for food employees KAP level. This survey was reviewed by Medical and Health Research Ethics Committee (MHREC) Universitas Gadjah Mada with reference number UGM/MHREC/317/2017.

RESULTS AND DISCUSSION

Demographic characteristics of respondents

More than half of the respondents were male (55.6%) with 61.5% aged <30 years. Majority of the respondents passed junior high school (65.9%). It was found that 67.4% of the respondents had working experience <5 years, while 52.6% have not attended any training related to food safety. The majority (67.4%) had certification for working in the small food industry. This finding revealed the need for relevant training including in food safety among food processing workers.

Knowledge about food safety

Mean score for knowledge was 13.08±2.55 (max score was 19), indicating that the food employees had an acceptable level of knowledge on food handling. However, more than half of the respondents (57.7%) had a low level of knowledge (score <10). Only one third of the respondents knew the answer for questions about cross-contamination (39.3%), temperature and time control (40.0%), as well as the procedures in handling food (24.0%). Most of them (99.2%) knew that it was necessary to always wash their hands when handling foods and remove their personal effects when processing food (81.8%). These results were similar to the findings of a in Ghana where they also found that >90% of their respondents believed that the use of protective clothing, gloves and proper storage of foodstuffs were vitally important in reducing food spoilage and health hazards to consumers (Akabanda, Hlortsi & Owusu-Kwarteng, 2017). Stratev *et al.* (2017) in Bulgaria also reported that their participants answered correctly to questions on washing of hands. In contrast, a study by Harrison *et al.* (2013) indicated a lack of knowledge about microbial food hazards in the majority (67–78%) of their respondents.

Attitude on food safety

This survey found the food employees obtained a mean score of 3.97±4.67 (max score was 4), indicating that the food employees had a positive attitude regarding the importance of safe food handling. Most respondents agreed

that washing hand before handling raw or cooked foods reduces risk of food poisoning. However, they obtained a low score for the question on using different cutting boards for raw and cooked foods to avoid contamination. This indicates a potential problem arising from cross contamination of food-borne pathogens.

Tokuça *et al.* (2009) found that almost all (93.2%) of their food workers were aware of the danger of touching food with cut hands or fingers. A significant result from Aziz & Dahan (2013) was that 99% of their food employees said they did not touch food with cuts on their hands or fingers. This study found high proportion of the respondents was unsure about checking and discarding food that were beyond its expiry date. Food employees should be adequately trained to increase awareness and improve food handling behaviours (Ansari-Lari, Soodbakhsh & Lakzadeh, 2010; Worsfold & Griffith, 2010).

Food safety practice

Personal hygienic practice is extremely important to ensure delivery of safe food to consumers. The respondents' responses in terms of practices are summarised in Table 1. Overall, the respondents obtained a mean score of 3.98±0.55 out of a maximum of 10 for practices in personal hygiene and food safety, indicating that the respondents showed poor personal hygiene practices whereby they failed to maintain safe practices, such as removing personal effects (e.g. rings, necklaces, hairpins) when processing foodstuffs, and using caps, masks, protective gloves and adequate clothing. Lubran *et al.* (2010) found similar results in their study whereby only half of the street

Table 1. Food safety and personal hygiene practices

No	*Item (N=135)*	*Mean±SD*
1	I wash my hands before and during food preparation	4.37±0.55
2	I clean surfaces and equipment used for food preparation before re-using on other food	4.32±0.74
3	I use separate utensils and cutting-boards when preparing raw and cooked food	4.21±0.65
4	I remove my personal effects (e.g., rings, necklaces, hairpins) when process foodstuffs	3.09±0.74
5	I use caps, masks, protective gloves and adequate clothing reduce the risk of food poisoning	3.37±1.59
6	I consume food or beverages (e.g., coffee) inside processing areas	3.71±0.59
7	I will take leave when I am sick, or have a fever or cold	3.90±0.67
8	I separate raw and cooked food during storage	4.18±1.33
9	I check that meats are cooked thoroughly by ensuring that the juices are clear or by using a food grade thermometer	4.27±0.76
10	I reheat cooked food until it is piping hot throughout	4.32±1.63
11	I thaw frozen food in the refrigerator or other cool place	4.63±0.67
12	After I have cooked a meal, I store any left-overs in a cool place within two hours	3.51±0.78
13	I check and throw away food beyond its expiry date	3.72±1.08
14	I wash fruits and vegetables with safe water before eating/serving them	4.21±0.74
	Overall practice score (Mean score±SD)†	3.98±0.55

†The score scale ranges from 1 to 4 Likert scale

vendors (53.7%) in the Philippines knew that wearing accessories could cause bacterial contamination. According to the Codex Alimentarius Commision (2013), improper food handling is a major cause of foodborne diseases and poor hand hygiene is an important risk factor in the occurrence of food contamination. Food employees should always wash their hands at every stage of food production, particularly before handling foods, after eating, after touching contaminated materials, and after using the washroom.

Although most of respondents in this study said that they always wash their hands with soap and water, but not many of them were observed to do so in actual practice. As a matter of fact, handlers who directly prepare foods should wash their hands thoroughly using soap under hot running water and dry with a single-use towel; hand sanitisers may be used as a proper step in hand washing before wearing waterproof gloves. We recommend guidelines on food handling be disseminated among the small food companies.

Relationship between independent variables and food handling practice

The relationship between socio-demographic factors, food handling KAP are summarised in Table 2. The result of the correlation coefficient between educational level, training experience, and food handling KAP was significantly

Table 2. Relationship between independent variables and food handling practice

Variable	*Practice in food handling*		X^2	*p-value*
	Negative	*Positive*		
Gender			3.164	0.075
Male	39(52.0)	36(48.0)		
Female	22(36.7)	38(63.3)		
Age			2.469	0.650
<30	39(47.0)	44(53.0)		
>30	22(42.3)	30(57.7)		
Educational level			6.930	0.008*
Low	33(71.7)	13 (28.3)		
High	33(37.1)	56 (62.9)		
Small industry certificate			2.491	0.110
With certificate	34(37.4)	57(62.6)		
No certificate	20(45.5)	24(54.5)		
Working experience			2.303	0.316
<5 years	39(42.9)	52(57.1)		
>5 years	23(52.3)	21(47.7)		
Training experience			4.436	0.035*
Yes	26(36.6)	45(63.4)		
No	35(54.7)	29(45.3)		
Knowledge			7.837	0.005*
Good	19(33.3)	38(66.7)		
Poor	45(57.7)	33(42.3)		
Attitudes				
Positive	24(32.4)	50(66.6)	14.730	0.000*
Negative	40(65.6)	21(34.4)		

*$p<0.05$

positive ($p<0.05$), while other characteristics (gender, age, working experience, certification status) and food handling KAP were not significant. These findings indicate that food safety knowledge and training of the food employees could influence their attitude and practices in food safely. However, these results are in contrast with other studies which found that although food service employees had good knowledge of food safety, they rarely applied this knowledge when handling foods (Rowell *et al.*, 2013).

Several limitations were noted in this study. We relied on the use of a self-administered questionnaire that depended on the honesty of the food employees in answering the questions. As the study only focused on selected small food companies, these results should not be generalised to the entire Ambon city. More studies on a larger sample size should be conducted involving collaboration of the Ministry of Health and National Food and Drug Control Agency.

CONCLUSION

The study reported findings on the knowledge, attitude and practices regarding food safety among food employees working in small companies. Food safety training and guidelines should be provided to improve the food safety practices of food service employees in Ambon City.

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Authors' contributions

SAK, RSP, and JS performed in conception and design, acquisition of data analysis and interpretation of data. SAK and JS were drafting the article or revising it critically. Three authors were approved the final version to be published.

Conflict of interest

The authors declare no conflict of interest.

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