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World Nutrition Journal Editorial Office

Wisma Nugraha Building, Suite 501, 5th Floor
Jl. Raden Saleh No. 6 Jakarta Pusat

Phone: +622131905330 Email : worldnutritionjournal@gmail.com

Aim and Scope

World Nutrition Journal (abbreviated: W Nutr J) is an international, English language, peer-reviewed, and open access journal upholding recent evidence related to nutrition sciences. The journal accepts manuscripts in terms of original paper, case report, editorial, and letter to editor.

About

World Nutrition Journal was founded in 2016 as the official journal of Indonesian Nutrition Association. It aims to publish high quality articles in the field of community, clinical, and critical care aspects of nutrition sciences

Peer Review Process

All manuscripts submitted will be screened by an editor whether they have the requirements and accordingly to author instructions. Then, the editor will transfer them to Editor-in-chief. Editor-in-chief will choose peer-reviewer for the manuscripts, according to their field of expertise, with help from associate editors and editorial board members. Peer-review process should be based on Committee on Publication Ethics (COPE) standards. This journal uses double-blind review. After the peer review process and the manuscripts are approved, they will be sent back to Editor-in-chief. Afterwards, Editor-in-chief will send the approved manuscripts back to the editor for final editing, layout editing, and proofreading. Finally, the approved and edited manuscripts will be published.

Publication Frequency

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THE IMPORTANCE OF NUTRITION JOURNAL PUBLICATION

Saptawati Bardosono
Chief Editor of World Nutrition Journal

The rapid increase of nutrition journal publications indicate on the urgency of the problems in the world of nutrition. Research scientists had published various condition related to nutritional status and determined the cause of this problem. Expanding populations, newly achieved national freedoms, and the urge for a better life in the developing countries of Asia, Africa, and Latin America, together with food surpluses, enlightened self-interest, and awakened conscience in the more privileged countries of Europe, North America and Oceania have contributed to resurgence of interest in the world of health and nutrition problems.[1]

Indonesian Nutrition Association (registered as *Perhimpunan Nutriti Indonesia*), which was founded in 2011, has been continuing visions and misions to continue and develop the work of late Dr. Iqbal Mustafa, MD, PhD, FCCM. He encouraged the evolution of multidisciplinary Critical Care Systems in developing countries to provide benefit for great number of recipients at the lowest affordable cost. His interest in Nutritional Support had become a foundation to INA.[2] To continue his legacy, INA has financially support the publishing of Journal Critical Care and Shock, the spreading of research to meet the needs of population.

Since 2011, INA has taken an active role in all aspects of education and post-graduate trainings, practices, researches, and publications in nutrition disciplines. It also fosters collaboration among professionals involved in nutrition sciences.

The yearly event held by INA, Nutri Symposium, has been running for 12 years. As part of this activity, we gather all participants to submit oral or poster presentation, which will also be published in form of abstracts in proceeding book. The demand from nutrition researchers and scientists to join the Nutri Symposium as a platform to publish their work has encouraged INA to create an International Journal focus on Nutrition, entitled World Nutrition Journal.

Knowledge of nutrition; which includes community, clinical, and critical care nutrition, is constantly growing. Therefore, the needs of journal submissions and reference searches continue to increase. In this digital era, the web has greatly reduced the barriers for communicating scientific output.[3]

One of many methods of publishing journals online is open access (OA). This is a method of publishing peer-reviewed journals and articles in the internet. This model of publishing can be freely accessed and read.[4] In Indonesia, there are still only few open accessed journal publications, especially regarding nutrition-related knowledge. Considering the importance of journal publications, INA establishes World Nutrition Journal as an open access journal for the expansion of nutrition-related knowledge and acts as a media for authors to publish their researches.

World Nutrition Journal (abbreviated as WNJ) is the peer-reviewed, world scale scientific journal of community, clinical, and critical care nutrition. It is the official journal of Indonesian Nutrition Association. As a brand new journal, WNJ's team have roles to achieve standard requirements of an International Journal. Recently, WNJ's ISSN has been registered from LIPI (*Lembaga Ilmu Pengetahuan Indonesia* or Indonesian Science Institution), and has become a member of Crossref for Metadata and Similarity Check. Our goals are to publish high standard articles which are available to be tracked and cited by International scientists, as well as being indexed in Scopus, a bibliographic database containing abstracts and citations for academic journal articles.

We do realize our first issue of World Nutrition Journal is a starting point and a stepping stone to develop our journal to be more credible and havemore impact factors. We will strive to polish and achieve our targets to be fulfilled for our next edition.

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NITROGEN BALANCE AND ITS RELATION WITH ENERGY AND PROTEIN INTAKE IN CRITICALLY ILL ELDERLY PATIENTS

Niken Churrniadita,[1] Luciana B Sutanto,[2] Rudyanto Sedono [3]

1 Palang Merah Indonesia Hospital, Bogor, West Java, Indonesia

2 Indonesian Nutrition Association, Jakarta, Indonesia

3 Department of Anesthesiology, Cipto Mangunkusumo Hospital, Jakarta, Indonesia

Abstract—**Introduction:**Nitrogen balance in criticaly ill patients has the tendency to be negative due to stress response. In the elderly patients, the metabolic changes risk to worsening nitrogen balance. The aim of this study was to determine nitrogen balance and its correlation with energy and protein intake in critically ill elderly patients within 48 hours in ICU.

Methods: The method was cross sectional, consecutive sampling on 26 critically ill elderly subjects. Inclusion criteria were patients admitted to ICU, age ≥60 years old, male/female, whose family/relatives agreed to join this study. Patients who had urine output <0,5 mL/kg/hours were excluded. Data collected were energy and protein intake, urinary urea nitrogen (UUN), and nitrogen balance during 24 hours I and II of admission.

Results: The median age was 70 (61 – 85) years old, body mass index was 22. 9 ± 2.7 kg/m^2, most of the subjects were surgical patients. In 24 hours I and II, the nitrogen balances were -5.2 (-31.2 − -4.1) and -4.5 ± 4.6 respectively, energy intakes were not significantly different; 78.8 ± 45.0% and 91.1 ± 50.2% respectively, and protein intakes were significantly different; 34.1 + 19.3 g/d and 41.2 + 21.3 g/d respectively. There was positive correlation between nitrogen balance and energy intake; r=0.6 and r=0.5, and also between protein intake; r=0.5 and r=0.4, in 24 hours I and II respectively.

Conclusion: There were significantly positive correlation between nitrogen balance with energy and protein intake.

Keywords: elderly, critically ill, nitrogen balance, energy intake, protein intake

INTRODUCTION

Population of elderly people in the world is growing.[1] In America, half of ICU patients are elderly.[2] In Australia and New Zealand, the proportion of critically ill elderly patients in ICU had been increased 5.6% per year.[3] The prevalence of critically ill elderly patients in ICU of Cipto Mangunkusumo General Hospital in 2011 and 2013 was 19.6% and 21.2% respectively.[4] Morbidity and mortality of critically ill elderly patients are higher than young adult critically ill patients.[3,5,6]

Critically ill is a condition caused by physiologically unstable condition and organ failure that may cause death in several minutes or hours.[7] Nitrogen balance tend to be negative due to stress response. Critically ill elderly patients relatively have low muscle mass[8,9] in conjunction with higher muscle degradation.[10,11]

Energy and protein intake[12] may increase denomination of nitrogen balance in critical illness,[13-16] including elderly patients.[16] Therefore critically ill patients need an adequate nutrition intake in early handling. The ICU of RSUPNCM applies evidence-based nutrition protocol,[17-19] to restore nitrogen balance. A research by Sinaga[14] in 2013 which was done to both young adults and critically ill elderly patients in the first 24 hours of admission in ICU, concluded that patients who had energy and protein intake closer to the protocol target had better nitrogen balance.

Therefore, this study of nitrogen balance and its relationship to energy and protein intake in critically ill elderly patients was conducted in ICU of RSUPNCM. It was expected that the results of this study can be used as an evaluation for the development of nutrition management in ICU of RSUPNCM, and most other ICU. This study was done in 2 days to observe the difference of energy and protein intake between day 1 and 2. Since patients were tend to be more stable in day 2, it was expected that the energy and protein intake would be higher on day 2 compared to day 1.

METHODS

The research was held on December 2013 to June 2014, and had been approved by The Ethics Committee of the Faculty of Medicine, University of Indonesia. The inclusion criteria were male/female critically ill elderly patients, age ≥60 years old, and gained permission by his/her family. The exclusion criteria was urine output <0.5 mL/kg/hour. The patient will be dropped out if being unable to complete the study or had incomplete data.

The research was carried out for eight weeks with 26 subjects were analyzed. There were 33 patients admitted to ICU and approved to participate in the study by patients family. Two subjects did not complete the study, and five subjects had incomplete data.

Characteristic data such as age, gender, BMI, and nutrition intakes were taken from the medical records. Energy intake data was compared to ESPEN guideline (%).[17] Anthropometric measurements of weight and body length were done. Body weights were measured by using bed scale (Seca 984 series, with precision 0.1 kg). Body lengths were measured by using measuring tape, with precision of 0.1 cm. From anthropometric data, body mass index (BMI) and nutrition intakes were calculated. Urinary urea nitrogen (UUN) was measured from 24 hours collected urine. Nitrogen balance was calculated from nitrogen intake (protein intake in gram divided by 6.25) and UUN.

Data were analyzed using Statistical Package for Social Science (SPSS) version 20. The Shapiro-Wills normality test was used to analyze data distribution. Body mass index, energy and protein intake were presented in mean and standard deviation. Age, UUN and nitrogen balance were presented in median (minimum− maximum). T-pair test was used to analyzed the difference between energy and protein intake in 24 hours I and II. The Wilcoxon test was used to analyzed the difference of UNN and nitrogen balance in 24 hours I and II. The Man Whitney test was used to assess nitrogen balance in <50% and >50% target energy intake in 24 hours I and independent t-test in 24 hours II.

RESULTS

Characteristic data is shown in Table 1. Most subjects were digestive surgical patients, who could not receive nutrition therapy aggressively.

Energy and protein intake, UUN, and Nitrogen Balance

The target of energy intake from oral, enteral, and parenteral was referring to ESPEN guidelines for critically ill patients (20 kcal/kgBW/day). As seen in Table 2, the energy intake of subjects in 24 hours I and II was not significantly different. There was significant difference of protein intake between 24 hours I (34.1 ± 19.3 g/d) and II (41.2 ± 21.3g/d), however there were no differences of UUN and nitrogen balance between 24 hours I and II. Carbohydrate, mostly had been given in 24 hours I. Although protein intake was increased significantly in 24 hours II compared to I, UUN was not significantly different between both days. Nitrogen balance was also not significantly different.

Subject's energy intake and nitrogen balance are shown in Table 3. The number of subjects who had target energy intake ≤50% decreased from 8 subjects in day I to 4 subjects in day II. We then divided the subjects into group A (who had received energy intake ≤50% target) and group B (who had received energy intake >50% target). In group A, UUN excretion in 24 hours II was less than 24 hours I, resulting a better nitrogen balance in 24 hours II. In 24 hours II there were extreme values in group B which made nitrogen balance did not differ significantly compared to group A. There was no subject suffered from overfeeding even though their energy intake exceed 110% in group B during 24 hours II. Excessive energy intake was due to the target used for analysis was lower than target of energy intake in clinical usage.

The Correlation between Nitrogen Balance with Energy and Protein Intake

Spearman correlation test was used to analyzed the correlation between nitrogen balance with energy and protein intake in 24 hours I, and Pearson correlation test was used in 24 hours II. The correlation between nitrogen balance with energy intake in 24 hours I and II were significantly positive; r=0.6 and r=0.5. The correlation between nitrogen balance with protein intake in 24 hours I and II were significantly positive; r=0.5 and r=0.4. These results can be seen in Figure 1 to 4.

The correlation was weaker in 24 hours II because there was an extreme values, in which higher energy and protein intakes brought a more negative nitrogen balance. By excluding the extreme values

Table 1 Characteristic of the Subjects

Variable	Results
Gender	
- Male, n(%)	11 (42)
- Female, n(%)	15 (58)
Age (years)	70 (61−85)
BMI* (kg/m2)	22.9 ± 2.7
Diagnosis	
- Nonsurgical, n(%)	6 (23)
- Surgical, n(%)	20 (77)
Nutrition delivery method in 24 hours I	
- Total Enteral, n(%)	18 (69)
- Total parenteral, n(%)	0 (0)
- Enteral and parenteral, n(%)	8 (31)
Nutrition delivery method in 24hours II	
- Total enteral, n(%)	13 (50)
- Total parenteral, n(%)	0 (0)
- Enteral and parenteral, n(%)	8 (31)
- Oral and enteral,n(%)	4 (15)
- Oral	1 (4)

Table 2 Energy, protein intake, and nitrogen balance 24 hours

Variable	24 hours I	24 hours II	δ	p
Energy intake (%)	78.8 ± 45.0	91.1 ± 50.2	12.3 ± 5.1	>0,05
Protein intake (g/d)	34.1 ± 19.3	41.2 ± 21.3	7.1 ± 2	<0.05
UUN *(g/d)	6.3 (1.7−27.2)	6.75 (2.1−23.3)	0.45 (0.4−3.9)	>0.05
Nitrogen balance	-5.2 (-31,2− -4,1)	-4.5 ± 4.6	1.3 ± 1.2	>0.05

Table 3 Energi Intake and Nitrogen Balance in 24 Hours I dan II

Group	Energy Target	n (26)	Energy Intake (%)	p	UUN* (g)	p	Nitrogen Balance (g)	p
24 hours I								
A	≤50%	8	31.9 ± 15.1	<0.001	7.7 ± 8.2	>0.05	-9.7 ± 8.9	<0.05
B	>50%	18	99.6 ± 37.5		7.1 ± 3.3		-4.1 ± 3.4	
24 hours II								
A	≤50%	4	26.7 ± 10.3	<0.005	4.5 ± 2.1	>0.05	-6.9 ± 2.2	>0.05
B	>50%	22	102.8 ± 45.2		7.7 ± 4.4		-4.1 ± 4.8	

*UUN: Urinary urea nitrogen

from analysis, the correlation will be much stronger, with r=0.7 for the correlation between nitrogen balance with energy intake in both 24 hours I and II. The correlation between nitrogen balance with protein intake in 24 hours I and II were r=0.5 and r=0.7. The extreme values was probably due to patient who had second digestive surgical within 14 days who possibly has more severe catabolism.

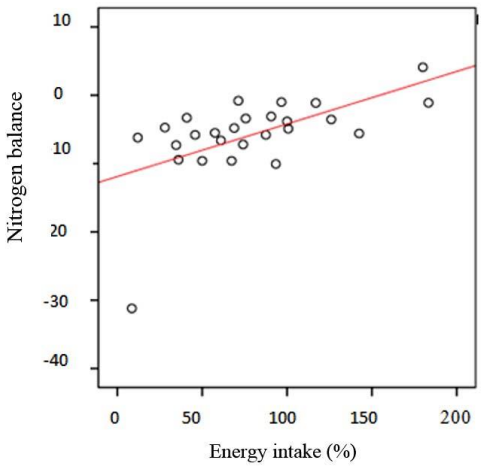

Figure 1. Correlation between energy intake and nitrogen balance in

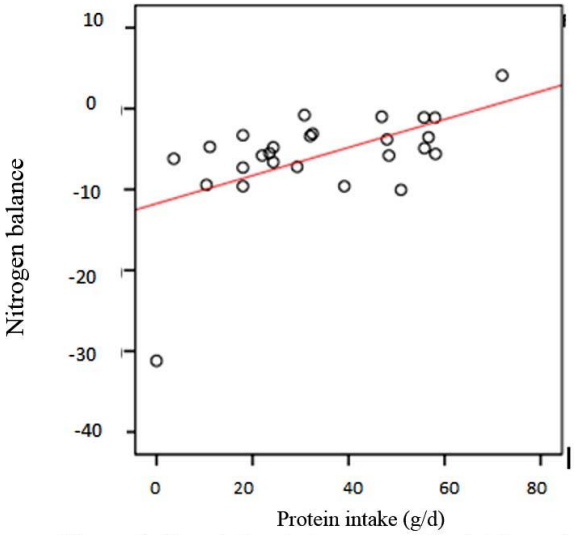

Figure 2. Correlation between protein intake and nitrogen balance in 24 hours I

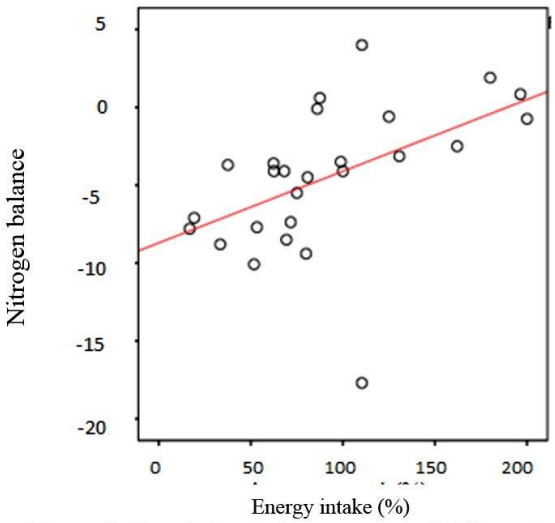

Figure 3. Correlation between energy intake and nitrogen balance in 24 hours II

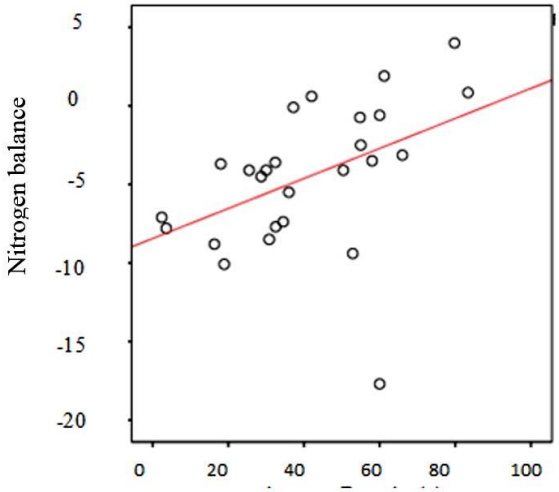

Figure 4. Correlation between protein intake and nitrogen balance in 24 hours II

DISCUSSION

The median age of the subjects was 70 (61-85) years old, UUN is lower in elderly patients which may caused by lower muscle mass.[16] The BMI in this study was in normal category.[20]

Energy Intake

Nutrition given to patients in this research is based on the guidelines: all patients who are expected not be able to receive full oral diet within three days should receive enteral nutrition.[17] Also, if nutrition needs could not be fulfilled with enteral nutrition, or if enteral nutrition is contraindicated or intolerable, then the patient should receive parenteral nutrition within 24 to 48 hours.[18] Therefore, energy intake in 24 hours I and II was similar.

Energy intake in this research was higher than research by Sinaga,[14] 2013 in ICU of RSUPNCM (56.3 $\pm$ 33.9%), which used the same guidelines. At that time, commercial enteral nutrition was not available yet in Nutrition Unit in RSUPNCM, thus complete nutrition enteral feeding could not be given as soon as patients could be fed. Energy intake in O'Meara[21] research was 56.3 $\pm$ 33.9% using Harris-Bennedict equation. Krishnan[22] study based on American College of Chest Physician (ACCP) guidelines (25−27 kcal/kgBW/d) energy intake was 50.6%. Japur[23] research using indirect calorimeter was able to meet 92% energy intake target. A multicenter research by Heyland[24] from

2007 to 2009 concluded that energy intake could only reach 40−50% from calorie target.

The guidelines formula have some variables, such as weight, age, sex, stress factors, and others into account. It is necessary to use an accurate method to calculate target of nutrition intake for critically ill elderly patients. Indirect calorimeter can be used to measure total nutritional daily needs which fluctuating in critically ill patients, and misallocation of formula-based total energy target from variable data inaccuracies can be avoided.

Protein Intake

Based on guidelines, protein needs for critically ill patients is 1.5 to 2 g/kgBW/d.[17] In this study protein intake reached 0.6 g/kgBW/d (34.1 ± 19.2 g/d) in 24 hours I, and 0.7 g/kgBW/d (41.2 ± 21.3 g/d) in 24 hours II. Protein intake in this study was higher than research by Sinaga[14] (23.75 ± 16.87 g/d), but less than Japur[23] (59.37 ± 32.5 g) and Dickerson[16] studies (1.1 g/kgBW/d in elderly and 1.3 g/kgBW/d in adult critically ill). Hoffer[15] reviewed 13 studies published from 1948 to 2012 which strongly suggest 2 to 2.5 g/kgBW/d protein intake for critically ill patients, however obviously most critically ill patients could only take less than half of the common recommendation (1.5 g/kgBW/d).

By using guidelines for nutritional management, energy intake in ICU of RSUPNCM can achieve the target of 20 kcal/kgBW/day at 24 hours I and II. However, protein intake target according to the recommendations could not be achieved, despite an increase in protein intake on 24 hours II. This was due to the low energy intake target at the beginning of critically ill. Nevertheless, if patient's critical condition can be stabled then energy intake target can be raised, thus protein intake is also expected to raise.

Urinary Urea Nitrogen (UUN)

In acute phase of critical illness, inflammation and immobilization cause great protein degradation.[10] The degradation of body protein can cause the increase of nitrogen excretion.[25]

Urinary urea nitrogen in this study was less than research by Sinaga[14] (8.3 ± 4.4 g/d), and Japur[23] studies (14.7 ± 4.8 g/d). The subjects in those studies were relatively young. According to Dickerson study,[16] aside from less lean muscle mass in elderly, less severity of illness can also lower UUN excretion in critically ill elderly patients. However, this study did not measure the severity of illness which brought a limitation of this study.

Nitrogen Balance

In acute phase of critically ill, nitrogen balance is always negative due to inflammation[26] and immobilization.[11] In Mohil[27] study, nitrogen balance did not seem to be less negative even on the fourth day post operation, eventhough protein intake was raised. It was because the amount of protein (0.2 to 0.8 g/kgBW/d) was not enough to make nitrogen balance better. It was less protein intake than in Hoffer[15] and Dickerson[16] studies. In Dickerson[16] study, nitrogen balance measurement was done on day 5 to 14 post-trauma, therefore it is possible that the influence of inflammation had been reduced at that time. Another possibility is that protein intake in this study (0.6 - 0.7 g/kgBW/d) and Mohil's (0.2 - 0.8 g/kgBW/d) [27] were less than protein intake in Dickerson's[16] study and Hoffer's[15] review.

Energy intake also had an important role to improve nitrogen balance. Kan et al[28] showed evidence that nitrogen balance would become positive on the seventh day of critical illness, if the patients had sufficient energy intake.

The Correlation between Nitrogen Balance with Energy and Protein Intake

In this study, the correlation between nitrogen balance and energy and protein intake in the 24 hours I and II were significantly positive. Dickerson[16] concluded there were no significant difference on correlation between nitrogen balance and protein intake in young adult and elderly critically ill patients. The correlation between nitrogen balance and energy intake in Sinaga's[14] study (subjects; age 48 ± 13.2 years old) had similar value with this study. According to her study the correlation between nitrogen balance and energy intake in 24 hours were not different between young adult and elderly critically ill patients.

In conclusion, there were significantly positive correlations between nitrogen balance with energy and protein intake. Further research is needed to determine nitrogen balance difference in surgical and non surgical patients, and also whether the disease severity affect the nitrogen balance. Periodic examination of nitrogen balance should be considered to assess body response to energy and

protein intake, in order to evaluate nutritional intake adequacy on critical ill elderly patients.

Conflicts of Interest

Authors declared no conflict of interest regarding this study.

Acknowledgment

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THE RELATIONSHIP BETWEEN HYPERGLYCEMIA STATUS AND HIGH GASTRIC RESIDUAL VOLUME STATUS IN ADULT ICU PATIENTS

Pittara Pansawira,[1] Luciana B Sutanto,[1] Dita Aditianingsih[2]
1 Indonesian Nutrition Association, Jakarta, Indonesia
2 Department of Anesthesiology, Cipto Mangunkusumo Hospital, Jakarta, Indonesia

Abstract—**Introduction:** Hyperglycemia commonly occurs in critically ill patients due to metabolic stress. Hyperglycemia can cause gastric motility disturbance in which can cause high gastric residual volume (GRV). The objective of this study is to determine the relationship between hyperglycemia and high GRV status in ICU patients.

Methods: This study used cross sectional method with consecutive sampling of 96 adult ICU patients. Blood glucose level were measured every 12 hours and GRV every 4 hours on first and second day admission to ICU. Data were analyzed with Statistical Package for Social Science Program (SPSS) version 20.0. The relationship between hyperglycemia status and high GRV status were analyzed using Chi-Square test.

Results: Hyperglycemia status was found in 45.8% subjects on day 1 and 35.4% on day 2. High GRV status was found in 28.1% on day 1 and 25% on day 2. There was no significant relationship between hyperglycemia and high GRV status on day 1 and 2 (p=0.34 and 0.81). However, in 12 subjects, we found high GRV occurred 19.67 ± 11.06 hours after the onset of hyperglycemia.

Conclusion: There was no significant relationship between hyperglycemia status and high GRV status, but high GRV could be found later after hyperglycemia.

Keywords: critically ill patients, hyperglycemia, gastric residual volume

INTRODUCTION

Hyperglycemia commonly occurs in critically ill patients due to metabolic stress, even though without history of diabetes.[1–3] The prevalence of hyperglycemia varies in many researches. Van den Berghe *et al*[4] found 12% of critically ill patients had blood glucose level >200 mg/dL. Another study by Cely *et al*[5] found 38% of critically ill patients had blood glucose level >150 mg/dL and 23% of them had >200 mg/dL. Unfortunately, data about prevalence of hyperglycemia in critically ill patients has not been found in Indonesia yet.

Based on the Normoglycemia in Intensive Care Evaluation-Survival Using Glucose Algorithm Regulation (NICE-SUGAR), hyperglycemia in critically ill is defined by blood glucose level >180 mg/dL.[6] It occurs due to insulin resistance and the increase of gluconeogenesis in responds to inflammation.[1,3] It can also occur due to excessive intake and corticosteroids therapy.[1,7]

Hyperglycemia may results in gastric motility disturbance which alter gastric emptying and, therefore, can cause high gastric residual volume.[8,9] A study by Nguyen *et al*[10] shows high gastric residual volume occurred more often in patients who had hyperglycemia. The cut off points of high gastric residual volume varies from >150 mL to >500 mL.[11–13] In Intensive Care Unit of Cipto Mangunkusumo Hospital, the cut off point used is >125 mL every 4 hours.

Prevalence of high gastric residual volume varies from 32–60%.[13,14] A study by Junizar[15] in Cipto Mangunkusumo Hospital ICU found 23.3% of critically ill patients had high gastric residual volume. This condition can hinder adequate enteral feeding[14] and increase the risk of aspiration[16] which results in increase of mortality.[17,18] Other causes of high gastric residual volume are low perfusion,[19] sympathetic response during metabolic stress,[20] low potassium level,[9] opioids and cathecolamine therapies.[14,21]

There are only few studies regarding the relationship between hyperglycemia in critically ill patients and high gastric residual volume. However, there is still no research data regarding this issue in Indonesia. Therefore, this study was conducted in ICU of Cipto Mangunkusumo General Hospital, Jakarta, Indonesia. The results of this study was expected to be able to estimate the percentage of hyperglycemia and high gastric residual volume for

additional knowledge in nutrition therapy for critically ill patients, especially in Indonesia.

METHODS

The study was done in May to August 2014 in Cipto Mangunkusumo General Hospital, Jakarta, Indonesia, and had been approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia. The inclusion criteria were male and female, age 18–59 years old, had nasogastric or orogastric enteral tube applied, and consented by their family or relatives to participate in this study. Any patient who had surgical procedure involving removal of pyloric sphincter was excluded.

Data were collected within the first and second 24 hours of admission in ICU. Subjects who had earlier enteral tubes removal, left ICU (move to ward) or passed away before data collection was completed were dropped out from this study. The data was collected in the first and second 24 hours of admission in ICU to avoid large number of drop outs, loss to follow up, and too much data variation because the staying time differs greatly in this ICU.

The sample size was determined with the formula below and subject was recruited consecutively. According to formula below, minimum number of subject should be recruited was 96 patients.

$$n = \frac{Z_\alpha^2 \text{ x } PQ}{d^2}$$

- n = number of subject
- $Z\alpha$ = cut off point for statistical significance = 1.96, for α= 0.05
- P = percentage of high gastric residual volume in critically ill patient with hyperglycemia. P was assumed to be 50% = 0.5[22]
- Q = 1 – P
- d = acceptable drop out rate in clinical research = 10%

The data collected were age, gender, weight, BMI, nutritional status, calorie intake, diagnosis, serum potassium level, MAP status, use of opioids and catecholamines from ICU medical charts. Blood glucose levels were measured by glucometer every 12 hours starting from admission until 48 hours during ICU stay. Gastric residual volume was measured every 4 hours (gastric residue was collected in an empty container and its volume was measured). All parameters measured were divided into two categories: hyperglycemia (if there was at least once blood glucose level >180 mg/dL) and euglycemia (if blood glucose levels were ≤180 mg/dL); high gastric residual volume (if there was at least once gastric residual volume >125 mL found) and normal (if gastric residual volume was ≤125 mL in each measurement); hypokalemia (serum potassium level was <3.5 mmol/L measured during this study) and normo/hyperkalemia (serum potassium levels were normal or >5.5 mmol/L); low MAP status (if there was at least once MAP level <65 mmHg) and normal MAP status (if MAP levels were ≥65 mmHg); "yes" (if any opioid medication or catecholamine was used) and "no" (if it was not used).

Data were analyzed using Statistical Package for Social Science (SPSS) version 20.0. The Kolmogorov-Smirnov test of normality was used for age, weight, BMI, and calorie intake. Numerical data that are normally distributed will be shown in mean ± standard deviation (SD); and in median (minimum–maximum value) if otherwise. Categorical data will be shown in distribution and frequency. The relationship between hyperglycemia and high gastric residual volume were analyzed using Chi-Square test.

RESULTS

Within 10 weeks of the study, 104 subjects were collected. There were 96 subjects analyzed. There were 8 subjects who were dropped out from this study: 2 subjects had their enteral tube removed and 6 subjects passed away before 48 hours.

Baseline characteristic of the subject are shown in Table 1 and 2. Percentage of hyperglycemia and high gastric residual volume status are shown in Figure 1. Analysis of relationship between hyperglycemia and high gastric residual volume of this study is shown in Table 3. There was no significant relationship between hyperglycemia status and high gastric residual volume in the first and second 24 hours of admission.

Tabel 1 Baseline Characteristic of The Subjects

Characteristic	Results
Age (years old)	39 (18–59)
Gender, n (%)	
Male	51 (53.1%)
Female	45 (46.9%)
Weight (kg)	60 (30–90)
BMI (kg/m^2)	23.06 ± 4.29
Nutritional status, n (%)	
Underweight	13 (13.5%)
Normoweight	36 (37.5%)
Preobese	18 (18.8%)
Obese I	23 (24%)
Obese II	6 (6.3%)
Diagnosis, n (%)	
Surgical	69 (71.9%)
Medical	27 (28.1%)

Additional analysis was done to determine the possibility of time needed to develop into high gastric residual volume from the time hyperglycemia was detected. There are 12 subjects who had high gastric residual volume after hyperglycemia was detected. The analysis showed the mean time measured was 19.67 ± 11.06 hours.

Tabel 2 Calorie Intake, Potassium Level Status, MAP Status, Use of Opioids and Cathecolamines on Day 1 and 2 Admission in ICU

Characteristic	Day 1	Day 2
Calorie intake (kcal/kg/day)	11.16 ± 6.14	17.45 ± 7.18
Potassium level status, n (%)		
Hypokalemia	29 (30.2%)	24 (25.3%)
Normokalemia	60 (62.5%)	65 (67.7%)
Hyperkalemia	7 (7.3%)	7 (7.3%)
MAP status, n (%)		
Low	45 (46.9%)	24 (25%)
Normal	51 (53.1%)	72 (75%)
Opioids, n (%)		
Yes	33 (34.4%)	30 (31.3%)
No	63 (65.6%)	66 (68.8%)
Catecholamines, n (%)		
Yes	49 (51%)	47 (49%)
No	47 (49%)	49 (51%)

DISCUSSION

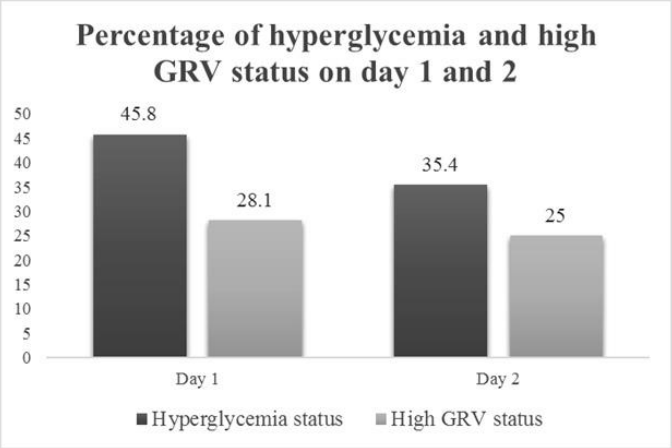
According to several studies, age,[23,24] gender,[25] BMI,[26] and calorie intake[27,28] may affect blood glucose level or gastric residual volume. However in this study, none of those characteristics affected blood glucose level or gastric residual volume (data not shown). Calorie intakes were still below target recommended by ESPEN guidelines.[29] Almost all subjects (98.96%) of this study received intermittent enteral feeding with slow drips. The nutrition composition given was a standard formula complied to ESPEN guidelines.[30] Therefore, calorie intakes were considered not affecting the outcomes of this study. Other factors (potassium level, the use of opioid, and catecholamine medications) also did not affect gastric residual volume in this study (data not shown).

Blood Glucose Level dan Gastric Residual Volume Status

There was a significant decrease of hyperglycemia percentage (>10%) in second day of admission in ICU compared to first day. This could mean that hyperglycemia was promptly managed. In this ICU, management of hyperglycemia is done with strict glucose control by insulin starting from blood glucose >180 mg/dL. This cut off point is based on ASPEN Clinical Guidelines.[31]

The proportion of hyperglycemia in this study is lower compared to other studies by Van den Berghe et al[4] (12%) and Cely et al[5] (38%). This can be due to higher blood glucose cut-off point in both studies (>200 mg/dL).

There was no significant decrease of subjects with high gastric residual volume status in this study. This number also does not differ significantly compared to study by Junizar et al[15] (23.3%). The number of subjects with high gastric residual volume in this study is lower compared to studies by Mentec



GRV: gastric residual volume

et al[14] (32%) and Metheny et al[16] (72.8%) which can be due to different cut-off point and frequency of measurement used in both studies.

showed critically ill patients who are intolerant of enteral feeding had a trend for higher blood glucose levels on admission and had greater variation in blood glucose levels, especially 24 hours prior to enteral feeding intolerance. However, in this research, the analysis can not be concluded due to lack of numbers of subject. Therefore, further research with case control or longitudinal method is needed to determine this issue.

No relationship between hyperglycemia and high gastric residual volume in this study could be due to the length of study time, blood glucose level measurement frequencies and management of hyperglycemia in subjects. Longer time is probably needed to observe the effect of hyperglycemia to gastric residual volume. In this study, blood glucose

Table 3 The Relationship Between Hyperglycemia Status and High Gastric Residual Volume Status

Blood glucose level status	Gastric residual volume							
	24 hours				48 hours			
	High	Normal	Total	p	High	Normal	Total	p
24 hours								
Hyperglycemia	10	34	44	0.28	13	31	44	0.34
Euglycemia	17	35	52		11	41	52	
48 hours								
Hyperglycemia	-	-	-	-	9	25	34	0.81
Euglycemia	-	-	-	-	15	47	62	

The relationship between hyperglycemia and high gastric residual volume

The results showed no significant relationship between hyperglycemia and high gastric residual volume in both first and second 24 hours of admission in ICU. These results were similar with a study by Mentec et al[14] which found no significant difference of blood glucose level between subjects with high and normal gastric residual volume. The result of this study differs from a study by Nguyen et al[10] which found high gastric residual volume was associated with hyperglycemia. The difference of the results may due to difference in the studies objectives and blood glucose measurement frequencies.

Additional analysis was done to determine the possibility of time needed to develop into high gastric residual volume from the time hyperglycemia was detected. The analysis of 12 subjects with high gastric residual volume occurred after hyperglycemia showed the mean time measured was 19.67 ± 11.06 hours. A study by Nguyen et al[10] level measurements done other than every 12 hours were not included in the analysis. Therefore, there were possibly more subjects who had hyperglycemia. In addition, the prompt management of hyperglycemia in this study possibly hindered the high gastric residual volume to develop. For those reasons, further research using case control or longitudinal method is needed. Using more frequent blood glucose level measurements or continuous blood glucose monitoring is suggested in further research.

There are limitations to this study. This study did not examine disease severity and more specific diagnosis of the subjects in which would possibly affect gastric emptying rate. Data were only collected within two days, therefore it may be too short to observe the effect of hyperglycemia to gastric residual volume. Blood glucose level was measured every 12 hours, therefore any hyperglycemia occurred beyond those time could be missed. The design of this study could also become a limitation. We used cross sectional study, whose

data was divided categorically, to determine the relationship because high gastric residual volume was expected to happen concurrently with hyperglycemia onset or after hyperglycemia occurred. Diabetic patients were not excluded, which could also affect the outcome of this study. Gastric emptying disorder frequently occurs in patients with chronic diabetes mellitus.

In conclusion, there was no significant relationship between hyperglycemia status and high gastric residual status in this study. Further research with more frequencies of blood glucose measurements or using continuous blood glucose monitoring is needed to better determine the hyperglycemia status in critically ill patients. Further research is also needed to determine whether episodic or chronic hyperglycemia can cause high gastric residual volume much later, and also observation on the average time from the beginning of hyperglycemia to develop into high gastric residual volume is needed.

Conflicts of Interest

Authors declared no conflict of interest regarding this study.

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THE EFFECTS OF SPECIFIC ORAL NUTRITIONAL SUPPLEMENTS COMPARED TO TEA WITH SUGAR ON GASTRIC EMPTYING TIME, BLOOD GLUCOSE LEVEL, AND HUNGER RESPONSE IN HEALTHY ADULTS

†Stella E Bella,[1] Luciana B Sutanto,[1] Jacob Pandelaki[2]

1 Indonesian Nutrition Association, Jakarta, Indonesia

2 Dept. of Diagnostic Radiology, Faculty of Medicine Universitas Indonesia, Cipto Mangunkusumo Hospital Jakarta, Indonesia

Abstract—**Introduction:** The objective of this study was to compare gastric emptying time, hunger response, and blood glucose level after drinking specific oral nutritional supplements (S-ONS) to tea with sugar in healthy adults.

Methods: This study was a clinical, parallel, random allocation, and single-blind trial. This study was conducted at Cipto Mangunkusumo General Hospital, Jakarta. The subjects were 12 healthy adults divided into 2 groups: intervention group (n = 6) and control group (n = 6). Patients in the intervention group received 200 mL S-ONS (200 kcal, 18% protein, 20% fat and 62% carbohydrate) and control group received 200 mL of tea with 10 grams of sugar (40 kcal). Gastric volume was measured using 2D-sonography every 30 minutes. Blood glucose level was measured using blood peripheral sample. Hunger response was measured using visual analog scale (VAS).

Results: Gastric emptying time in the intervention group was <90 minutes, and in the control group <60 minutes. Blood glucose level was increased in the intervention group and decreased in control group. Hunger response was decreased in intervention group and increased in control group.

Conclusion: Gastric emptying time after either drinking S-ONS and drinking tea with sugar in healthy adults subjects were less than 2 hours. Patients receiving S-ONS had significantly higher blood glucose level and were less hungry than the control group.

Keywords: ONS, gastric emptying time, blood-glucose level, hunger response

INTRODUCTION

Fasting before medical procedures, such as elective surgery, abdominal ultrasound scanning, endoscopy scanning, and intubation, is standard protocol to ensure that the stomach is empty and avoid pulmonary aspiration.[1] Preoperative guidelines state that the minimum duration for fasting before surgery is two hours for clear fluids and six hours for light meal.[2-4] The guidelines do not include the provision of oral nutritional supplements (ONS), which is more complete in nutritional content, able to meet nutritional needs, and able to reduce hunger as well as maintain blood glucose level.[5] The stress of surgery may be tolerated by children when they are fed up to 2 h before elective surgery.[6] A case report by Sutanto *et al*, 2009[7] concluded that the preoperative nutrition management of patient with ONS resulted in more satisfaction, less hunger, less thirst, and reduced postoperative nausea and vomiting compared to the patient not receiving ONS during the preoperative period.

Oral nutritional supplement could be used as a nutritional choice in preoperative fasting guidelines, therefore the gastric emptying rate after drinking ONS needs to be determined. Research on gastric emptying rate after drinking 200 mL of specific ONS (S-ONS) with a formula was initiated by Sutanto *et al* in 2010.[5] They found that the gastric emptying rate after drinking 200 mL of S-ONS with a specific formula was less than two hours. The purpose of this study was to compare the rate of gastric emptying, hunger, and blood glucose levels after consuming either S-ONS and tea with sugar.

METHODS

The study design was a clinical, parallel, random allocation, and single-blind trial. Data was collected in Cipto Mangunkusumo General Hospital, Jakarta, from May - June 2011 after the proposal was approved by the Ethical Clearance Research Committee of Faculty of Medicine University of Indonesia.

The target population was healthy adult patients and the subjects were those who met the

study criteria. Inclusion criteria were 19-50 years old and agreed to join the study by signing the informed consent. Exclusion criteria were patients with nausea and vomiting, or on antiemetic and/or promotility drugs, with high blood pressure and/or diabetes, pregnancy and lactation.

Twelve healthy subjects were allocated by block randomization into two groups, intervention and control groups. The intervention group received 200 mL S-ONS (200 kcal, 18% protein, 20% fat, and 62% carbohydrate) and the control group received 200 mL of tea with 10 grams of sugar (40 kcal). Gastric emptying of subjects in both groups were measured using a 2D-ultrasound. This technique has been used,[7] validated and recommended for gastric emptying assessment.[8] Gastric volume was determined by using π r2 L formula, the same method used in a study by Sutanto *et al*.[7] To ascertain the stomach was empty before measurement, subjects were required to follow the fasting guidelines, ate last main meal six hours before and drink clear fluid two hours before examination.[3] The initial examination was considered as empty stomach if the gastric volume was less than 80 mL.[11] Measurement of the gastric volume was done every 30 minutes until the stomach was considered to be empty again.

Blood glucose level was measured at the initial and end when measuring gastric volume. Blood sample was taken from peripheral vein. Hunger response was taken at the same time as blood glucose measurement, using visual analog scale (VAS) 0-100 mm interview.

RESULTS

Both groups had no significant differences in characteristics, as seen in Table 1. The gastric volume was measured using 2D-sonography every 30 minutes, gastric emptying time of intervention group was <90 minutes, and control group was <60 minutes, therefore gastric emptying time for both groups were less than 2 hours, as seen in Table 2.

Blood glucose level of the intervention group was increased, while control group was decreased. Subjects received S-ONS had significantly higher blood glucose level than control group, as shown in Table 3.

Hunger response in the intervention group was decreased, while control group was increased. Subjects received S-ONS were significantly less hungry than control group, as shown in Table 4.

DISCUSSION

The gastric emptying time of all subject were less than two hours; gastric emptying time of the intervention group was longer than control group, 90 and 60 minutes respectively. This result was consistent with the study by Sutanto *et al*, 2010.[5] Their study used the same amount and characteristics of ONS as this study although the subject was different, i.e. healthy pregnant patients with a gestational age of greater than 37 weeks. It showed that gastric emptying after drinking 200 mL S-ONS in pregnant women were as fast as non pregnant healthy adults.

The higher calories cause longer gastric emptying time. A study by Gentilcore *et al*, 2006[14] showed that gastric emptying rate was lower in patients after having higher calories. In that study, one group consumed 500 mL beef broth soup (12 kcal) and the other group consumed 300 mL of dextrose 25% (314 kcal). The results of this study was consistent with Gentilcore[14] in which gastric emptying time of the intervention group was longer than control, in which the intervention group received 200 kcal S-ONS and the control group received 40 kcal.

Table 1. Characteristics of intervention and control groups.

Variable	Intervention (n=6)	Control (n=6)	*p*	CI 95%
Age (years)	23.17 ± 3.82	29.00 ± 5.66	0.067*	-12.16-1.60
BMI (kg/m^2)	21.75 ± 1.65	20.83 ± 1.60	0.351*	-1.17-3.00

(*p <0,05 significant, unpaired t-test)

Table 2. Mean initial and end gastric volume, and gastric emptying time of intervention and control groups

Variable	Intervention (n=6)	Control (n=6)	*p*	CI 95%
- Initial gastric volume (mL)	34.67 ± 15.68	51.33 ± 12.51	0.071*	-35.03-1.69
- Gastric emptying time (minutes)	90	60		

(*p <0,05 significant, unpaired t-test)

Table 3. Mean initial and end blood glucose level of intervention and control groups.

Variable	Intervention (n=6)	Control (n=6)	*p*	CI 95%
Initial blood glucose level (mg/dL)	82.83 ± 6.58	88.50 ± 2.95	0.096*	-12.65-1.31
End blood glucose level (mg/dL)	98.83 ± 10.42	86.17 ± 4.17	0.030*	1.68-23.65

(*p <0,05 significant, unpaired t-test)

Table 4. Mean initial and end VAS score of intervention and control groups.

Variable	Intervention (n=6)	Control (n=6)	*p*	CI 95%
- Initial VAS score (mm)	68.17 ± 6,05	66.33 ± 7,78	0.659*	-7.21-10.87
- End VAS score (mm)	60.00 ± 4,33	69.83 ± 6,18	0.011*	-16.80- (-2.86)

(*p <0.05 significant, unpaired t-test)

The amount of calories had an impact on the subjects' blood glucose level and hunger response. At the end, mean blood glucose level of the intervention group was increased (at minute 90), while control group decreased (at minute 90). Compared to the control group, mean blood glucose level of the intervention group was significantly higher. It meant that S-ONS was better than tea with sugar in controlling blood glucose level in patients who would have medical procedure.

Hunger response was decreased from the basal value in the intervention group, while it was increased in the control group. At the end, hunger response was lower in the control group. Compared to the control group, the intervention group receiving 200 mL S-ONS had lower hunger response.

Theoretically, the lower blood glucose level will decrease glucose supply for brain metabolism, this will stimulate hunger and increase peristalsis.[15] The results of this study corresponded to that theory, the control group with lower blood glucose level had

longer VAS which described higher hunger response.

We concluded that by giving S-ONS before medical procedures would help control blood glucose level and prevent hunger. Specific Oral Nutritional Supplements should be considered as a medical drink prior to a medical procedure that requires preoperative fasting. Furthermore, S-ONS could be given 2 hours before medical procedure because gastric emptying time was less than 2 hours. Further studies should be carried out so that S-ONS can be included in the existed standard protocol.

Conflicts of Interest

The authors declare no conflict of interest regarding this research.

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ASSOCIATION OF SERUM ADIPONECTIN LEVELS WITH METABOLIC SYNDROME RISK FACTORS IN MALAY ADULTS

Nur Firdaus Isa[1,3], Laila Ruwaida Mohd Zainuddin[2], Wan Manan Wan Muda[3], Hamid Jan B. Jan Mohamed[3]

1 Department of Biotechnology, Kulliyyah of Science, IIUM, Kuantan, Pahang, Malaysia.
2 School of Nutrition and Dietetics, Faculty of Health Sciences, Universiti Sultan Zainal Abidin (UniSZA), Gong Badak Campus, 21300 Kuala Terengganu, Terengganu, Malaysia.
3 Nutrition and Dietetics Programme, School of Health Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

Abstract— **Introduction:** This study aimed to investigate the relationship between serum adiponectin and metabolic syndrome in adults living in rural Malaysia.

Methods: A total of 299 Malay adults (men=124; women = 175) with a mean age 48.8 (11.7) years were recruited. Measurements for waist circumference and blood pressure were taken before drawing an overnight fasting blood samples. Biochemical tests for triglycerides, HDL cholesterol, glucose and serum adiponectin concentration were measured.

Results: Our results show that the adiponectin level in the subjects with metabolic syndrome was significantly lower than those without metabolic syndrome ($p < 0.05$). Among the metabolic syndrome risk factors, adiponectin level was significantly associated with hypertriglyceridemia and reduced HDL cholesterol ($p < 0.001$).

Conclusion: The outcome from this study which highlights the association of hypoadiponectinemia with risk factors of metabolic syndrome in Malay adults, suggests that the reduced level of adiponectin may play a pivotal role in the development of metabolic syndrome in this ethnic group.

Keywords: *adipokines, metabolic syndrome, obesity, dyslipidaemia, Asian*

INTRODUCTION

Metabolic syndrome is an emerging public health concern worldwide. The risk factors of metabolic syndrome are central obesity, hypertriglyceridaemia, reduced high-density lipoprotein cholesterol (HDL cholesterol), hypertension, and hyperglycemia.[1] Based on the International Diabetes Federation (IDF) definition, the presence of central obesity with any two of the other risk factors is qualified for the diagnosis of metabolic syndrome.[2]

A comprehensive overview on metabolic syndrome pandemic in the America, Europe and India indicates that more than 20% of the adult populations have metabolic syndrome.[1] Several South East Asian countries such as Indonesia, Singapore and Thailand are actively involved in evaluating the metabolic syndrome.[3-7] There is growing interest in assessing whether metabolic syndrome is a health threat in our population. This is based on the accumulating reports documenting the occurrence of metabolic syndrome in Malaysia in various locations and sociodemographic background.[8-11] Since the metabolic syndrome pandemic is observed in diverse population therefore there is a pressing need to investigate the mechanism underlying metabolic syndrome as well as searching for robust and sensitive biomarkers to serve as a diagnostic tool for early detection of metabolic syndrome.

The underlying mechanisms for development of metabolic syndrome remain plausible. The accumulated evidence suggests that the reduced adiponectin concentration (hypoadiponectinemia) plays pathophysiologic role of metabolic syndrome. The inverse correlation between visceral adiposity and adiponectin concentration suggests that hypoadiponectinemia is unequivocally associated with central obesity, a principal feature for metabolic syndrome.[12-14]

Adiponectin, a type of adipose-specific serum protein can be found extensively in serum ranging from 1.9–17.0 μg/ml.[15] It is expressed inversely to total fat, and acted not only as anti-diabetic but also as anti-inflammatory and anti-atherogenic agent.[16] In the last few years, a growing body of evidence suggests that adiponectin is a potential biomarker for the metabolic syndrome and its risk factors. The present study was designed to examine the association between serum

adiponectin level and metabolic syndrome or risk factors in the Malay adults in Malaysia.

METHODS

Study subjects

This was a cross-sectional study involving a total of 299 Malay adults recruited from October 2008 to August 2009. This study was approved by the Human Research Ethics Committee of Universiti Sains Malaysia (USMKK/PPP/JEPeM [205.4.(1.3)]. Written informed consent was obtained from all of the subjects. Each subject was interviewed using a structured questionnaire on demographic characteristics such as age, sex, and personal medical history. A qualified nutritionist measured height, weight and waist circumference using a portable bodymeter (Seca 206, Germany), body composition monitor (Tanita BC545, Japan) and non-elastic measurement tape (Seca 201, Japan). After 15 minutes of rest, blood pressure was measured twice by a trained laboratory technician using an automatic blood pressure monitor (Omron SEM-1, Japan). The general characteristics of the subjects were previously described.[8]

Biochemical Tests

Peripheral venous blood samples were collected after an overnight fasting. Metabolic syndrome biomarkers such as fasting blood glucose, triglycerides and high-density lipoprotein cholesterol were assessed with commercially available reagents (Randox, UK) by using Vitalab Selectra E chemistry analyzer (Vitalab, Netherlands). Serum adiponectin concentration was determined by using the Human Adiponectin Enzyme-Linked Immunosorbent Assay (ELISA) kit (Chemicon, USA).

Definition of Metabolic Syndrome

The following risk factors and criteria were used: central obesity (waist circumference; men ≥ 90 cm, women ≥ 80 cm) plus any two of the following: (1) raised triglycerides (> 1.7 mmol/L (150 mg/dL) or specific treatment for this lipid abnormality); (2) reduced HDL-cholesterol (men <1.03 mmol/L (40 mg/dL) or women <1.29 mmol/L (50 mg/dL) or specific treatment for this lipid abnormality); (3) raised blood pressure (≥ 130/85 mm Hg or treatment of previously diagnosed hypertension); (4) raised fasting plasma glucose (≥ 5.6 mmol/L (100 mg/dL) or previously diagnosed type 2 diabetes).

Statistical analyses

Subjects with missing information in socioeconomic, anthropometric measurements and blood analyses were excluded from statistical analyses. Each variable was examined for normality of frequency distribution based on the histogram. Normally distributed data were expressed as mean (standard deviation or SD) and skewed data were expressed as median (Interquartile Range or IqR). The Mann-Whitney *U* test was conducted to study association of adiponectin concentration and metabolic syndrome or risk factors. The two-way ANOVA analysis of variance was applied to explore the mean differences of adiponectin concentration on status of metabolic syndrome or risk factors. Although this test requires the dependant variables to be normally distributed, however it is robust against violations of the assumption of the normal distribution (17). A 2-tailed value of $p < 0.05$ was considered significant. All statistical analyses were performed using the Predictive Analytics Software (PASW Statistics 18.0) (SPSS Inc., Chicago, IL, USA).

RESULTS

A total of 299 Malay adults mean (Mean age ± SD: 48.8 ± 11.7) of which 124 were men (49.8 ± 11.7) and 175 were women (48.6 ± 11.8) were recruited in this study. Clinical characteristics of metabolic syndrome among the subjects *i.e* the mean (SD) for waist circumference, systolic and diastolic blood pressures, glucose, triglycerides and HDL cholesterol, and median (IqR) for adiponectin concentration are presented in Table 1.

Table 1. General characteristics of the study subjects (n=299)

Characteristics	Mean	SD
Age (years)	48.8	11.7
Waist Circumference (cm)	83.8	11.3
Systolic Blood Pressure (mmHg)	134.8	23.1
Diastolic Blood Pressure (mmHg)	79.1	11.2
Glucose (mmol/L)	5.7	2.3
Triglycerides (mmol/L)	1.4	0.9
HDL cholesterol (mmol/L)	1.2	0.3
	Median	**IqR**
Adiponectin (μg/ml)	6.5	5.7

The association of adiponectin level with metabolic syndrome were examined using Mann-Whitney *U* test (Figure 1). The median (IqR) of adiponectin concentration among those with and without metabolic syndrome were 5.8 (3.7) μg/ml and 7.2 (6.8) μg/ml, respectively. The adiponectin level among those with metabolic syndrome was significantly lower than those without metabolic syndrome ($p = 0.004$).

DISCUSSION

The total serum adiponectin level observed in the Malay population is comparable with a study conducted among the Japanese, but lower than in other populations (Table 3). The adiponectin concentration reported among the Malays with metabolic syndrome was significantly lower than those without metabolic syndrome; this finding is in agreement with other reported studies.[18,19]

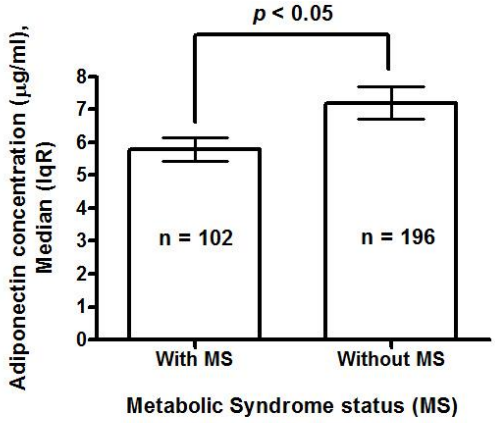

Figure 1. The median (IqR) of adiponectin concentration among subjects with and without metabolic syndrome (Mann-Whitney *U* test)

The association of adiponectin concentration with the individual metabolic syndrome risk factors is presented in Figure 2 and Table 2.

Subjects with high level of triglyceride (5.2 μg/ml) had significantly lower level of serum adiponectin than subjects with lower triglycerides (7.7 μg/ml) ($p < 0.001$).

The Malay subjects with hypertriglyceridemia or reduced HDL cholesterol had significantly lower concentration of serum adiponectin concentration than those without them, respectively. The result is in accordance with

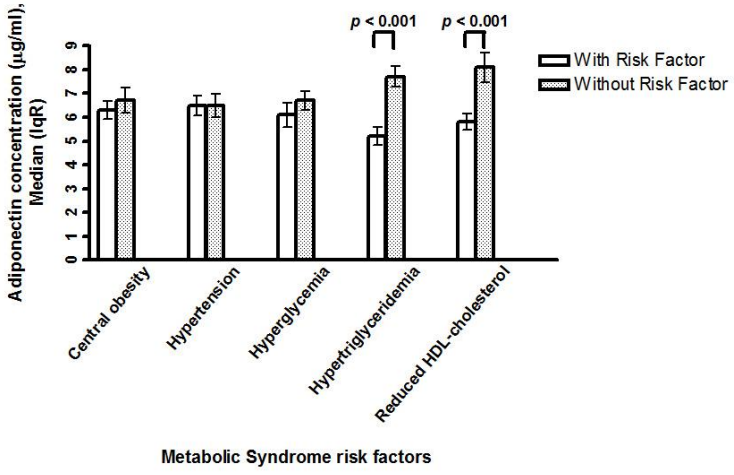

Figure 2. The median (IqR) of adiponectin concentration based on metabolic syndrome risk factors (Mann Whitney *U* test)

Similarly, subjects with reduced HDL cholesterol (5.8 μg/ml) had significantly lower concentration of serum adiponectin concentration than subjects with elevated HDL cholesterol (8.1 μg/ml) ($p < 0.001$).

previous reports from various population such as Caucasian[24] and Japanese[25] and large-scale

Table 2. The association between metabolic syndrome risk factors with the adiponectin concentration

Risk Factors	Status	n (%)	Adiponectin (µg/ml) Median (IqR)	Z-stat[a]	*p*-value
Central obesity	With	146(48.7)	6.3 (4.6)	-0.931	0.352
	Without	153 (51.3)	6.7 (6.6)		
Hypertension	With	168(56.0)	6.5 (5.4)	-0.731	0.465
	Without	131 (44.0)	6.5 (5.6)		
Hyperglycemia	With	98(31.2)	6.1 (4.9)	-1.157	0.247
	Without	205 (68.8)	6.7 (5.8)		
Hypertriglyceridemia	With	87 (28.9)	5.2 (3.4)	-5.488	**<0.001**
	Without	212 (71.1)	7.7 (6.4)		
Reduced HDL cholesterol	With	196 (65.4)	5.8 (4.6)	-3.215	**<0.001**
	Without	103 (34.6)	8.1 (6.3)		

[a]Mann-Whitney *U* test

nationwide studies such as Rancho Bernardo Study[26] and KORA Survey 2000.[27] Although the exact crosslink between adiponectin and lipid metabolism remains unknown, it was proposed that adiponectin acts with HDL cholesterol in reverse cholesterol transport and involved in hepatic lipase activity.[28] HMW is thought to be the active form of the adiponectin, and is implicated in the progression of metabolic syndrome.[30,31] Lara-Castro and the group reported that the reduced concentration of HMW adiponectin was associated with measures of central fat distribution, rather than the total adiponectin that were commonly investigated by many researchers.[32] As findings from this study found no significant association between adiponectin and central obesity, it was proposed that adiponectin, specifically the total adiponectin measured during the course of the study, was not responsible for the association with central obesity in Malay population. Perhaps future investigation of HMW would provide more conclusive perspective.

Table 3. Adiponectin concentration of Malay population and other populations

Population	Scope of the Study	Study Subjects	Adiponectin Concentration (µg/ml)
Malays This study	Metabolic Syndrome	Men and Women	6.5
Indonesian[7]	Metabolic Syndrome	Men	3.1
Japanese (20)	Healthy Young Adults	Men	6.4
Non-Hispanic White (21)	Pre-menopausal	Women	12.4
Hispanic (21)	Pre-menopausal	Women	9.5
Taiwanese (22)	Hyperglycemia	Men and Women	7.8
African Americans(23)	Insulin Resistance	Men	12.9
		Women	14.3

The finding from this study whereby hypoadiponectinemia is significantly associated with hypertriglyceridemia and reduced HDL cholesterol, suggests that an increased in adiponectin level could have protective effect via promoting HDL cholesterol and reducing the triglycerides.

We found no significant association between adiponectin with central obesity which appears to be in contradiction with the previous reports.[24,29] Such conflicting finding could be attributed by the adiponectin multimeric forms. Serum adiponectin exists as a trimer; low molecular weight (LMW) hexamers, medium molecular weight (MMW) and high molecular weight (HMW) multimerics. The

Circulating adiponectin levels have been studied for their role in different ethnic groups which suggests that adipose tissue mass or distribution may be attributed by ethnic variation.[33] Since the present study displayed no significant association between

adiponectin and central obesity, further studies on the influence of Malay ethnic factor perhaps could explain the discrepancy.

The adiponectin concentration found in Malay subjects with hypertension was similar to those without hypertension. To our knowledge there are limited studies associating adiponectin and hypertension as compared to other risk factors. Besides, a number of studies purposely examined hypoadiponectinemia and hypertension in selected patients with other abnormalities for example in patients with essential hypertension; a form of high blood pressure with no identifiable cause or among obese participant.[34,35]

The association of hypoadiponectinemia and type 2 diabetes has been demonstrated as early as 2000.[36] Adiponectin is regarded as an insulin sensitizer that enhances insulin sensitivity by increasing fatty acid oxidation and inhibiting the hepatic glucose production.[37] Several studies carried out in diverse population show that diabetic subjects experience hypoadiponectinemia as compared to their nondiabetic counterparts.[38-40] We found no significant difference of the adiponectin level between hyperglycemic and non-hyperglycemic in our studied subjects. Increasing the sample size and expanding this cross-sectional study to a cohort study with longer follow-up may fill in the gaps.

Here we report the association of adiponectin levels in the Malay ethnics in Malaysia and its association with metabolic syndrome risk factors. The study population was selected from a rural area, thus the result may not be generalised to Malays living in urban Malaysia due to differences in socioeconomic status. A nationwide multicentre study involving not only Malay ethnics but also Indian Malaysian and Chinese Malaysian may provide perspective into the role of adiponectin in the development of metabolic syndrome in Malaysian. Studies have suggested that adiponectin can be a promising therapeutic agent for metabolic syndrome and its risk factors. Increasing circulating adiponectin concentration or enhancing adiponectin signalling through its receptors could promisingly tackle the root that cause the metabolic syndrome.[41] Although the claims excited many researchers, future investigations taking the ethnic differences into account, are required to support the matter.

Conflicts of Interest

The authors declare there is no conflict of interest regarding this research

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EFFECT OF GREEN TEA INGESTION ON POSTPRANDIAL TRIGLYCERIDE LEVELS IN YOUNG WOMEN

Trismiyanti,[1] Ida Gunawan,[1] Tri Juli Edi Tarigan[2]

1 Department of Clinical Nutrition, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

2 Division of Endocrinology, Department of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

Abstract— **Introduction:** High blood triglyceride (TG) level is a risk factor for cardiovascular disease. Green tea, as a beverage, may reduce postprandial blood TG level through inhibition of fat absorption in the intestinal lumen. The aim of this study was to assess the difference of postprandial blood TG level changes between treatment group (high-fat meal and green tea beverage containing 738 mg of catechins) and control group (high-fat meal and plain water containing 0 mg of catechins).

Methods: The study was a randomized, single-blind, parallel-group clinical trial including 40 healthy young women. Blood was collected immediately before the meal and then at 2 and 4 h thereafter of each group. Method of TG measurement: GPO-PAP, using Beckman CX 5-CE machine and Good's buffer reagent.

Results: Postprandial blood TG level at 4 h in the treatment group was significantly lower compared to the control group (1.00 ± 0.27 vs. 1.22 ± 0.34 mmol/L, $p = 0.03$). The change in blood TG level from baseline to 4 h postprandial was also significantly lower in the treatment group compared to the control group (0.21 ± 0.14 vs. 0.37 ± 0.26 mmol/L, $p = 0.02$).

Conclusion: It was concluded that green tea ingestion during high fat meal suppressed postprandial elevation of TG 4 hour after meal.

Keywords: *green tea, triglyceride, postprandial, catechin, high fat meal*

INTRODUCTION

Cardiovascular diseases are major causes of death in the world. It was estimated that in 2015 the number of deaths caused by cardiovascular diseases would reach 20 million. One of the main risk factors for cardiovascular disease is dyslipidemia.

Dyslipidemia is a lipid metabolism disorder signed by either increased or decreased blood lipids.[3] The main type signs of lipid disorder are increased of total cholesterol level, low density lipoprotein cholesterol (LDL-C), and triglyceride (TG), as well as decreased of high density lipoprotein cholesterol (HDL-C). The causes of dyslipidemia include imbalanced diet, high-fat meals, inadequate dietary fiber intake, and inadequate physical activity.[4,5]

Tea is a popular beverage in Asia and the second most consumed beverage worldwide after water. Among all types of tea, green tea has become well-known due to its beneficial health effects. Green tea contains catechins, which has antioxidant, antiinflammatory, anticarcinogenesis, antihypertension, antiobesity, antidiabetic, antiplatelet, vasodilation, and hypocholesterolemic effects.

Human and animal studies have shown that green tea catechins may lower blood cholesterol and TG level by inhibiting the synthesis of pancreatic lipase, which plays a role in digestion and absorption of fat, therefore fat absorption is inhibited and cholesterol is excreted through feces.[15,16] Green tea catechins disturb fat emulsification process, causing bigger size of fat droplets, therefore inhibiting fat digestion.

METHODS

Green tea beverage

Five bags of green tea were brewed using 300 mL of hot water at 90°C for 3 minutes. The catechins content was analyzed at laboratory. The catechins content was 3691.45 ppm = 369.145 mg/100 mL = 738.29 mg/200 mL. This study used 200 mL of the green tea beverage containing 738 mg of green tea catechins to be compared to plain water (0 mg of catechins) as the control.

Subjects

After obtaining ethic approval from the Research Ethics Committee of Faculty of Medicine, Universitas Indonesia, recruitment for the study subjects was announced to all female undergraduate students aged of 19−24 years undergoing clinical education at Tangerang Regency General Hospital in Banten. Forty subjects were recruited for the study; each had a normal BMI according to the BMI cut-offs in Asian and Pacific populations (18.5−22.9 kg/m^2),[22] had normal blood pressure (<120/80 mm Hg),[23] had fasting blood TG level of <1.70 mmol/L,[24] and gave written consent to participate in the study. Exclusion criteria included smoking, alcohol consumption, pregnant or lactating, history of cardiovascular disease, diabetes mellitus, kidney disease, or liver disease, consumption of any supplement or food containing catechins, consumption of any drug that may affect lipid metabolism, i.e. fibrates, statins, progestins, steroids, and a history of gastritis after ingesting tea.

Study design and experimental protocol

This study was a randomized, single-blind, parallel-group clinical trial to compare the change of blood TG level between groups of 2 and 4 h after the ingestion of 200 mL of green tea beverage containing 738 mg of green tea catechins, and control group of 200 mL of water ingestion. The subjects were divided into two groups (treatment and control groups) through block randomization, resulting in 20 subjects in each group.

In the morning after 12 h fasting overnight, blood samples were collected from the subjects. The subjects were then given 200 mL of green tea beverage or 200 mL of hot water. No sugar or artificial sweetener was added to either beverage. The two beverages both were served with a high-fat meal (>35% of total energy from fat), which consist of margarine and granulated sugar smeared on sliced white bread (consisted of 46 g of carbohydrate, 23.5 g of fat, and 7 g of protein for a total energy of 1.77 MJ (or 422 kcal)). The meal and the beverage were consumsed and finished within 10 minutes. Blood samples were collected at 2 and 4 h after the meal. All blood samples were sent to the laboratory for TG level assessment.

Statistical analysis

Result data were analyzed using the Statistical Package for Social Science (SPSS) 11.5.

Shapiro-Wilk test was used to assess the distribution of the data. All data are normally distributed; therefore all results are presented as mean ± SD. Unpaired t-test was used to compare the data between the two groups. Significant difference was determined if p <0.05.

RESULTS

Subject characteristics

All 40 subjects were included in this study. There was no significant difference in age or BMI between control and treated groups (Table 1).

Every subject ingested ≥95% of the whole meal and beverage served. The meal and the beverage were well-tolerated by the subjects.

Triglyceride

There was no difference in fasting blood TG level between control and treatment groups, showing that the two groups were similar at baseline. Triglyceride level at 2 h postprandial showed no significant difference between treated and control groups. However, at 4 h postprandial, there was a significant differences in blood TG level and blood TG level change between treated and control groups (Table 2).

DISCUSSION

This study aimed to explore the effect of ingesting 200 mL of green tea beverage containing 738 mg of green tea catechins compared to plain water on postprandial blood TG levels in young women. This study was conducted as an effort to find out a way to prevent cardiovascular diseases in the scope of nutrition studies.

The study used young subjects to ensure normal body metabolism and normal *lipoprotein lipase* activity.[6] Only female subjects were recruited because elevation of postprandial blood TG level may be influenced by sex, i.e. elevation of postprandial TG level in men would be higher than in women.[6,27] In addition, subjects with normal BMI were chosen because it was found that in overweight and obese subjects blood TG level was still high even after 10–12 h postprandial.[27]

Blood samples for the TG level assessment were collected after the subjects fasted for 12 h in order to obtain endogenous blood TG levels (not influenced by the previous meal).[24] The postprandial blood TG levels were assessed twice (at 2 h and 4 h postprandial) to adequately predict the peak of the postprandial TG level. After 2 h postprandial, triglyceride-rich chylomicrons are already in the circulation.[28,29] Whereas, the time to reach the peak of blood TG level varies between 2 and 7 h postprandial.[26,27]

In individuals ingesting 15−30 g of fat, 450−900 mg of green tea catechins are needed to suppress postprandial hypertriglyceridemia.[19] According to a meta-analysis by Zheng et al.[31], the dose of catechins used in the present study is still safe and does not cause adverse effects.

In this study, there were no significant differences in the blood TG level and the change in blood TG level at 2 h postprandial between the control and treatment groups. This is in accordance with the study by Koutelidakis et al.[32] that reported a significant difference at 3 h postprandial. It is probably because at 2 h postprandial the catechins has not reached the intestine.

At 4 h postprandial, the blood TG level and the change in blood TG level in the treatment group were significantly lower than those in the control group. This corresponds with the results of previous studies.[30,32,33] Green tea catechins are assumed to be able to affect the size of fat droplets, resulting in inefficient fat emulsification process.[33] In addition, green tea catechins may inhibit the synthesis of pancreatic lipase, which plays a part in fat digestion and absorption.[15,16]

Table 1. Subject characteristics

	Control (n = 20)†	Treatment (n = 20)‡	*p*
Age (year)	20.6 ± 1.7	20.5 ± 1.8	0.938
BMI (kg/m^2)	20.8 ± 1.3	20.8 ± 1.3	0.921

†, ‡ results are presented as mean ± SD

Table 2. Fasting and postprandial blood triglyceride levels (mmol/L)

	Control (n = 20)§	Treatment (n = 20)¶	*p*
Fasting	0.85 ± 0.24	0.80 ± 0.26	0.572
2 h postprandial	1.03 ± 0.29	0.99 ± 0.35	0.668
4 h postprandial	1.22 ± 0.34	1.00 ± 0.27	0.033
Change at 2 h postprandial	0.18 ± 0.15	0.19 ± 0.22	0.906
Change at 4 h postprandial	0.37 ± 0.26	0.20 ± 0.14	0.019

§, ¶ results are presented as mean ± SD.

In conclusion, the results of the this study suggest that green tea ingestion can lower the elevation of blood TG level after a high-fat meal in young women, and the effect was reached at 4 h postprandial. Further studies are needed to characterize the effect of green tea ingestion on postprandial blood TG level in male subjects and also in subjects with dyslipidemia.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding publication of this paper.

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THE EFFECT OF VITAMIN C AND E COMBINATION ON SPERM QUALITY AND CEMENT 8-OHdG LEVEL OF SMOKE EXPOSED RATS

Eviana B. Sutanto,[1] Taufiq R. Nasihun,[1] Israhnanto Isradji,[1] Luciana B. Sutanto [2]
1 Sultan Agung Islamic University (UNISSULA), Semarang, Indonesia
2 Indonesian Nutrition Association, Jakarta, Indonesia

Abstract—**Introduction:** Cigarette smoke causes oxidative stress which results in reduced sperm concentration, motility and morphology, also increased levels of 8-OHdG as a marker of DNA damage. Vitamin C and E have potential role in repairing spermatozoa damages. The aim of this study was to determine the effect of vitamin C and E combination on sperm quality and cement 8-OHdG level of smoke exposed rats.

Methods: This study used a post test only control group design among 18 male Wistar rats subject, aged 8 week, 150-200 grams body weight (BW). The subject was randomly divided into 3 groups, K1: control, K2: cigarettes smoke exposed, K3: cigarettes smoke exposed and given a combination of 0.045 mg/gBW vitamin C and 0.036 IU/gBW vitamin E per oral. Analysis was done on day 21 using one-way ANOVA and post-hoc LSD for sperm concentration, motility and morphology; using Kruskal-Wallis and Mann-Whitney tests for cement 8- OHdG levels.

Results: The lowest sperm concentration was found in K2 (K2 32.59 million/mL, K1 47.91 million/mL, K 339.43 million/mL); the lowest normal sperm motility was found in K2 (K 238.97%, K 164.57%, K3 51.43%); the lowest normal sperm morphology was found in K2 (K2 27.56%, K 138.36%, K 331.18%); and the highest cement 8- OHdG level was found in K2 (K2 20.18ng/mL, K1 3.43ng/mL, K3 5.28ng/mL).

Conclusion: Combination of vitamin C and E can improve sperm concentration, motility and morphology and decrease cement 8-OHdG levels of smoke exposed rats.

Keywords: Sperm quality, 8-OHdG, vitamin C, vitamin E.

INTRODUCTION

Smoking is associated with increased levels of reactive oxygen species (ROS) which also causes increased oxidative stress level.[1] Under normal conditions, there is a balance between ROS and antioxidants. If the balance is disturbed, it will cause oxidative stress. Oxidative stress may exceed the antioxidant repair capacity in seminal plasma and can be toxic to sperm, which causes structural damage to spermatozoa.[1,2] Hydrogen peroxide is a major product of ROS which, in high concentrations, causes lipid peroxidation which causes cell death.[3] In addition to damaging cell structure, ROS can also damages DNA molecule through single and double chain DNA termination, deletion and modification of nucleoside.[4] Antioxidants act as free radical scavengers which protect spermatozoa from the ROS effects. Non-enzymatic antioxidants, such as vitamin C, vitamin E, pyruvate, carnitine, and glutathione, have the ability to compensate the damage to sperm cytoplasmic enzyme; an enzyme which functions in the endogenous defense and repair mechanisms.[3] Combination of vitamin C and vitamin E aims to prevent the formation of free radicals. Vitamin E is a tocopheroxyl radical which can be reduced by vitamin C back into tocopherol.[5] Researches on the role of vitamin C in cigarette smoke exposed rats showed positive effect for the quality of spermatozoa and the mean score of spermatogenesis.[6-7] Studies of vitamin E showed positive effect on spermatogenesis and sperm function in rats who suffered from spinal cord injuries.[8-9] Until now, there has not been any experiment which suggest that combination between vitamin C and vitamin E can provide protective effects on the fertility of smokers with sperm quality and levels of cement 8-hydroxy-2'-deoxyguanosine (8-OHdG) as indicators.

Globally, there were approximately 1.8 billion smokers and 80% of them live in low-income countries.[10] In Indonesia, 34.8% or 57.6 million adult population are smokers. Data obtained from National Socioeconomic Survey, Baseline Health Research and the Adult Global Tobacco Survey showed an increase in the number of smokers in Indonesia from 53.9% in 1995 to 67.0% in 2011. In 2008, Indonesia became a country with the third largest cigarette

consumption in the world. According to the data, prevalence of male smokers account for 67% of overall men population, which means the number reached around 57.6 million men.[11] Data from WHO noted that the biggest prevalence of smokers are young adult males in reproductive ages (46% of smokers age 20-39 years old). Moreover, in 30-50% of couples with infertility, the most common cause is abnormal seminal components. Several studies from multiple centers around the world had been researching the effects of smoking on cement quality, especially in heavy smokers and long-term smokers. The results showed that smoking resulted in decrease of cement parameters, such as viability, concentration, motility and morphology.[1,12,13] Other studies showed the occurrence of sperm DNA damage, such as a marked increase in 8-OhdG level; a parameter of DNA damage caused by oxidative stress, which can be caused by cigarettes.[2,3,14]

Research by Claudia et al,[6] concluded that vitamin C can improve sperm quality (motility, concentration and morphology) of male rats exposed by cigarette smoke. Research by Intania[7] also showed that vitamin C increased the average score of spermatogenesis in male rats exposed to cigarette smoke. Therefore it is concluded that administration of vitamin C may affect the sperm of smoke-exposed rats. Vitamin C has been known as an antioxidant supplement, but its effect on 8-OHdG is still controversial. Fraga et al,[15] reported that intake of high doses of vitamin C has protective effects against the formation of 8-OHdG in human cement DNA. On the other hand, Loft et al,[16] stated that vitamin C intake did not correlate with urinary excretion of 8-OHdG. In the study conducted by Inoue et al,[14] increased levels of 8-OHdG among smokers abstinent participant that were given extra vitamin C for 14 days did not show statistically significant differences with ones who received placebo. Research conducted by Subekti et al,[8] concluded that administration of vitamin E can improve spermatogenesis in cigarette smoke exposed rats, but did not show a significant increased in sperm count.[17] The study by Wang et al[9] in rats with spinal cord injury, vitamin E demonstrated a benefit in improving sperm function. Bruno et al,[18] concluded that smoking caused an increased need of vitamin E due to the reduction of vitamin E plasma level. Research by Ummi Kalsum et al,[19] using a combination of vitamins C and E on monosodium glutamate exposed rats showed an improvement in weight and testical volume, seminiferous tubule diameter and number of spermatogenic testes.

The process of smoking itself is divided into two phases: gas and particulate phase. Smoke cigarettes produce 4,000 kinds of substance through the process of hydrogenation, pyrolysis, oxidation, decarboxylation and dehydration. Elements which have the greatest impact on health are nicotine, tar generated in the particle phase, and carbon monoxide produced in the gas phase.[20] Carbon monoxide (CO) will be broken down by hemeoxygenase enzyme (HO) into a pro-oxidant environment which initiates the formation of ROS.[21] ROS is a collection of various radical form. The most numerous form of ROS in spermatozoa are O_2- (superoxide), H_2O_2 (hydrogen peroxide) and OH^- (hydroxyl ions).[2] Pro-oxidant that exceeds the healing ability of antioxidants in cells will cause oxidative stress. This situation can cause cell damage as a result from the decomposition of important molecules such as DNA, proteins, and lipids. DNA is the main target of damage due to oxidation reaction by endogenous oxidant. DNA damage occurrence can be measured by levels of 8-OHdG.[14]

Therefore, this study was done to determine whether the combination of oral vitamin C and E can improve sperm quality and sperm 8-OHdG levels in smoke exposed rats.

METHODS

This study used a post test only control group design. The study was conducted in the Laboratory of Food and Nutrition Studies Center, Gadjah Mada University. A total of 18 rats (*Rattus norvegicus*) Wistar strain male 8 week old, weight 150-200 grams, divided into 3 groups randomly, K1: control, K2: cigarettes smoke exposed, K3: cigarettes smoke exposed and given a combination of 0.045 mg/g BW vitamin C and 0.036 IU/g BW vitamin E per oral for 14 days. On day 21, all mice were terminated. Sample of epididymises were retrieved by cutting the epididymises from the caudal until their ampullae. Epididymises taken were placed inside the petri dishes containing 0.5 mL physiologic saline fluid. Sperm samples were retrieved by the means of epididymal massage.

Analysis was done using one-way ANOVA and post-hoc LSD for sperm concentration, motility

and morphology; using Kruskal – Wallis and Mann – Whitney tests for cement 8- OHdG levels.

RESULTS

The result of this study is shown in Table 1. According to Table 1, the result of this study showed that the highest sperm concentration was K1 (47.91 ± 0.51), followed by K3 (39.43 ± 0.22) and K2 (32.59 ± 0.47). Group with the highest sperm motility percentage was K1 (64.57 ± 0.97), followed by K3 (51.95 ± 0.73) and K2 (38.97 ± 1.03). Group with the highest sperm morphology percentage was K1 (38.36 ± 1.94), followed by K3 (31.18 ± 0.80) and K2 (27.56 ± 1.41). Given the data were normally distributed (Shapiro-Wilk, p>0.05) and homogenous (Levene test, p>0.05), with one way ANOVA test for statistical analysis (p = 0.000).

The lowest levels of 8-OHdG was K1 (3.43 ± 0.33), followed by K3 (5.28 ± 0.42), and lastly, K2 (20.18 ± 1.46). Given the data were normally distributed (Shapiro-Wilk, p > 0.05) and was not homogeneous (Levene test, p = 0.001), and the statistical analysis used was Kruskal – Wallis test (p = 0.001).

To analyze which groups have significant differences in terms of concentration, sperm motility

Table 1. Mean Value of Body Weight, Sperm Concentration, Motility, Morphology, and 8-OhdG level.

Variable	Group			P
	K_1 (n=6) ($x \pm SE$)	K_2 (n=6) ($x \pm SE$)	K_3 (n=6) ($x \pm SE$)	
BW (g) before	194.83±4.79	190.00±3.51	186.5±1.75	0.284
after	213.67±4.05	208.17±3.10	207.17±1.42	0.300
Concentration (million/ml)	47.91±0.51	32.59±0,47	39.43±0.22	
Shapiro-wilk	0.240	0.537	0.535	
Levene test				0.117
Statistic analysis	*One way ANOVA*			0.000
Motility (%)	64.57±0.40	38.97±0,42	51.95±0.30	
Shapiro-wilk	0.837	0.210	0.814	
Levene test				0.705
Statistic analysis	*One way ANOVA*			0.000
Morphology (%)	38.36±0.79	27.56±0.58	31.18±0.33	
Shapiro-wilk	0,121	0,725	0,771	
Levene test				0,200
Statistic analysis	*One way ANOVA*			0,000
8-OhdG (ng/ml)	3.43±0,13	20.18±0,60	5.28±0.17	
Shapiro-wilk	0.435	0.692	0.855	
Levene test				0.001
Statistic analysis	*Kruskal-Wallis*			0.001

Note: K_1 : control; K_2 : cigarettes smoke exposed; K_3 : cigarettes smoke exposed and given a combination of 0.045mg/g BW vitamin C and 0.036IU/g BW vitamin E for 14 days.

and morphology, post-hoc LSD test is performed, which is shown in Table 2. The results of post-hoc LSD test on sperm concentration, motility and morphology shows the results differ significantly (P <0.05) in all groups: K1 compared to K2 and K3; K2 compared to K3.

Table 2. Post-hoc LSD test on sperm concentration, motility and morphology

Group	Variable					
	Sperm Concentration		Motility		Morphology	
	Mean Diff (χ)	P	Mean Diff (χ)	P	Mean Diff (χ)	P
K_1>< K_2	15,313	0,000	25,595	0,000	10,795	0,000
K_1>< K_3	8,476	0,000	12,613	0,000	7,186	0,000
K_2>< K_3	-6,846	0,000	-12,981	0,000	-3,618	0,001

As for levels of 8-OhdG, Mann-Whitney test was performed which results are shown in Table 3. The results of Mann-Whitney test on levels of 8-OHdG indicated that the results differ significantly (P <0.05) in all groups.

Table 3. Mann-Whitney test on 8-OhdG levels

Group	Mean Diff (χ)	P
K_1>< K_2	-16,747	0,004
K_1>< K_3	-1.847	0,004
K_2>< K_3	14,900	0,004

DISCUSSION

This study results showed that K1 (control group) have better sperm indicator levels than K2 group which get 4 cigarettes worth of smoke exposure a day for 14 days. There were impairments in concentration, motility and morphology of spermatozoa and an increased level of 8-OHdG cement in K2 compared to K1. This result proved that cigarette smoke caused increased oxidative stress, which then caused excessive lipid peroxidation, resulting in DNA damage and cell apoptosis on spermatozoa. Spermatozoal plasma membrane largely consists of PUFA, which is susceptible to ROS impact. Lipid peroxidation in spermatozoal plasma membrane would result in a formation of leakage in membranic structure (cellular and organelles) and function (transport process, ions and metabolites regulation, and also signal transduction by the receptor). These conditions cause intracellular ATP to disappear rapidly, causing damage to axon, and decreasing viability of spermatozoa, therefore resulted in decrease of sperm motility.

Another theory states that H_2O_2 can diffuse through plasma membrane into cell and inhibit enzymatic activity of G6DP (glucose 6-phosphate dehydrogenase), which leads to decrease of NADPH ability simultaneously with the gluthation oxidase. These leads to decreasing defensive ability of antioxidants and peroxidation of membranes.[2,22] Another target of damage caused by oxidative stress in response to cigarette smoke exposure is the DNA. ROS induces a modified form of the DNA bases guanine primarily through lipid peroxides and alkaloxile radicals. Oxidative stress also causes increase of single and double chain DNA termination. ROS can also induce -SH groups in proteins and DNA, which then would alter the structure and function of spermatozoa, thereby increasing susceptibility to macrophages. The results of DNA base guanine oxidation can be observed in the form of 8-OHdG.[12,14,22] This result is similar to a research done by Batubara et al,[23] which provided cigarette smoke exposure as much as 1, 2, 3, and 4

cigarettes a day for 30 days. The study showed impairment in concentration, motility and morphology of spermatozoa compared to those not receiving cigarette smoke exposure, which concluded that more amount of cigarette smoke exposure caused greater impairment of concentration, motility and morphology of spermatozoa. Likewise, in the study conducted by Somwanshi et al,[12] which compared 50 cement samples of non-smokers to smokers. The study result showed significant decrease in number and motility of spermatozoa of smokers.

Levels of 8-OHdG in the K2 group showed significant increase compared to group K1. This is consistent with a similar study conducted by Inoue et al,[14] which compared the levels of 8-OHdG between smokers and non smokers. The results showed levels of 8-OHdG were significantly higher in smokers compared to nonsmokers.

Group K3; namely groups that received exposure to cigarette smoke 4 cigarettes a day and given vitamin C 0.045 mg/gBW and vitamin E 0.045 mg/gBW per oral, showed better concentration, motility and morphology of sperm, and cement 8-OHdG level compared to group K2. This result proved that administration of vitamin C and E as antioxidants can prevent cell damage caused by smoking. Furthermore, external provision of vitamin C and E prevented decrease of internal vitamin C and E antioxidant level in seminal plasma due to cigarette smoke exposure.[24] This suggests that simultaneous administration of vitamin C and E have protective effect against cigarette smoke exposure. The result was similar to other studies that used combination of vitamin C and E in rats (*Mus musculus*) that were given monosodium glutamate (MSG). The study showed positive effect of the combination of vitamin C and E which recovered subject's seminiferous tubules volume and the number of spermatogenic testes.[19] In another study, administration of selenium and vitamin E to prevent testicular tissue damage due to cigarette smoke exposure can further reduce degeneration of testicular tissue.[25] Research by by Moslemi et al,[26] which administered a combination of selenium and vitamin E in 690 men with infertility, demonstrated beneficial and protective effect of vitamin E especially in sperm motility. Other research, which administer only vitamin C to male rats (*Mus musculus L*) after cigarette smoke exposure, showed that vitamin C can improve sperm quality.[6] In vitro study proved that vitamin E can protect sperm from damage caused by oxidative stress, loss of motility, and morphological changes.[22]

In conclusion, combination of vitamin C and E can improve sperm concentration, motility, and morphology, and also decrease cement 8-OHdG levels of smoke exposed rats.

Conflicts of Interest

The authors of this paper declare there is no conflict of interest.

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CORRELATION BETWEEN SERUM ZINC LEVEL AND ERYTHROCYTE SUPEROXIDE DISMUTASE (SOD) ACTIVITY IN NON-FRAIL AND FRAIL GERIATRIC PATIENTS

Rosnah Pinontoan,[1] Savitri Sayogo,[1] Arya Govinda Roosheroe[2]

1 Department of Clinical Nutrition, Faculty of Medicine, Universitas Indonesia

2 Division of Geriatry, Department of Internal Medicine, Cipto Mangunkusumo Hospital, Jakarta, Indonesia

Abstract—**Introduction:** Frailty syndrome (FS), as a health problem in elderly, can interfere with the quality of life. Many factors play roles in its occurrence, one of which is oxidative stress. Superoxide dismutase (SOD), in which one of its components is zinc (Zn), is one of the endogenous antioxidants that plays a role in preventing oxidative stress. This study was aimed to know the correlation between serum zinc level (sZn) and erythrocyte SOD (eSOD) activity in non-frail and frail geriatric patients.

Methods: This was cross-sectional study with consecutive sampling method conducted in the Geriatric Clinic Cipto Mangunkusumo hospital, from August through September 2014, on 60 patients (30 patients for each group of non-frail and frail). Data were collected from interviews, physical measurements, and venous blood sampling.

Results: Among 90% of the subjects had low Zn intake, at the same time, the mean of sZn was 8.41 ± 1.36mol/l and 93.3% of the subjects had experienced Zn deficiency. The mean of eSOD activity was 1526 ± 508U/gHb, and there was no significant difference between the groups. There were no significant correlation between sZn and eSOD activity both in the overall subject (r = -0.04; p = 0.076), as well as the non-frail group (r = -0.099; p=0.604) and frail group (r = 0.01; p = 0.957).

Conclusion: Serum zinc level did not have any significant correlation with the eSOD activity, neither on overall or both non-frail and frail groups.

Keywords: geriatric patient, serum zinc level, erythrocyte SOD activity, frailty syndrome

INTRODUCTION

Aging process is a natural process with the downward trend in the functional capacity, which occurs continuously at the molecular, cellular, and organ level. Lowering in the body functional capacity affects the occurrence of certain health problems, such as the frailty syndrome.[1,2]

Frailty syndrome is a clinical syndrome caused by the accumulation of the aging process, physical inactivity, weight loss, inadequate chronic intake, lifestyle, and unhealthy environment.[1] These factors are interrelated and endwith decreasein muscle and bone mass.[3,4] Sarcopenia plays an important role in the occurrence of frailty syndrome; which is marked with a condition of loss of skeletal muscle mass and strength due to the aging process.[3,5] Pathogenesis of sarcopenia is multifactorial, including malnutrition, nutritional oxidative stress, inflammation, hormonal changes, physical inactivity, and genetic factors.[5]

Naturally, the human body produces reactive oxygen species (ROS) which are prooxidants and on the other hand has a defense mechanism to cope with the damage that is caused by free radicals through the work of antioxidants, either endogenous or exogenous.[6] In the aging process, the ability of antioxidant defense mechanism is decreased, caused by a decrease in endogenous antioxidants, one of which is the enzyme superoxide dismutase (SOD); and contributes to the occurrence of sarcopenia.[5,6]

Zinc is one of the minerals that plays an important role in maintaining metabolic homeostasis and antioxidant mechanisms,[7,8] which is also an important part in a variety of protein structures of the body, including the role of zinc in the SOD to maintain the stability of the structure, especially isoform CuZnSOD.[9]

Various studies concerning the correlation between serum zinc level and eSOD activity showed no consistent results. A weak negative correlation between serum zinc level and eSOD activity was shown in few healthy elderly subjects. Zinc supplementation increases eSOD activity.[10] Nonetheless, other studies in healthy elderly subjects showed no correlation between zinc level and SOD activity.[7]

Most studies were done in healthy elderly subjects. There are still no data concerning the correlation between zinc level and eSOD activity in elderly patients with frailty syndrome, especially in Indonesia. This study aimed to determine the correlation between serum zinc level (sZn) and erythrocyte SOD (eSOD) activity in non-frail and frail geriatric patients.

METHODS

Design and Subjects

This is a cross-sectional study aimed to determine the correlation between serum zinc level and erythrocyte SOD activity in non-frail and frail geriatric patients, which was conducted at Cipto Mangunkusumo Geriatric Clinic from mid-August 2014 through mid-September 2014. The Ethics Committee from the Faculty of Medicine, Universitas Indonesia, had approved this study. The number of subjects, (this study used $r = 0.5$; $\beta = 0.1$; and $\alpha = 0.05$), including 10% chance of drop-out was 30 for each non-frail and frail group.[11] A total of 60 geriatric patients, who participated in this study, signed an informed consent, and completed the study.

Frailty syndrome status is determined based on the index Frailty 40 items by research assistants of the Geriatric Division of Internal Medicine Department of Faculty of Medicine of Universitas Indonesia. The inclusion criteria were all elderly patients in the geriatric clinic who were given an informed consent and agreed to participate in this study. The exclusion criteria were as follows: having acute infection, intestinal disorder, and cognitive status disorder by Mini Mental State Examination scores <24.

Study Measurements

The design was cross sectional study, which was done in the geriatric clinic in Cipto Mangunkusumo General Hospital, Jakarta, Indonesia. Data were collected consecutively from August to September 2014, by trained research assistants. Data werecollected from interviews for demographic characteristics, anthropometric measurements including body weight and knee height, evaluation of food intake with 3 x 24 hours estimated food record method for macronutrient assessment, and semiquantitative food frequency questionnaire (FFQ) for zinc intake assessment. Volumes and portion sizes for 3 x 24 hours food record and semiquantitative FFQ were estimated using photographs and food models. Intake data were analyzed using Nutrisurvey 2007 program for Windows operating system.

Nutritional status was evaluated based on body mass index (BMI) for Asia-Pacific population.[12] Body mass index was calculated as weight (kg) divided by height (m^2). Weight was measured with minimum clothing using platform SECA, meanwhile height was determined by knee height using knee height measuring instrument.[13]

Venous blood sampling was performed for serum zinc level and erythrocyte SOD activity. Serum zinc levels was determined by atomic absoprtion spectrophotometry method, while erythrocyte SOD activity was determined by using colorimetry method.[14] This study used cut-off values of 10.1 μmol/l serum zinc deficiency level for females and 10.7 μmol/l for males. While erythrocyte SOD activity normal range was 1102–1601 U/g Hb.

Statistical Analysis

All data were analyzed using Statistical Package for Social Science (SPSS) version 20 for Windows operating system. Kolmogorov-Smirnov normality test was done to the numeric data of the total subjects, while Shapiro Wilk test was done to numeric data of each group (frail and non-frail group). For data which were normally distributed according to normality test, Pearson bivariate analysis was done to determine the correlation between serum zinc level and eSOD activity. If not normally distributed, Spearman rank test was used. Significance level used was $p<0.05$.

RESULTS

All subjects completed all procedures used in the study, the characteristics of the demographic and nutrition status data is shown in Table 1. The mean age was 72.6 ± 6.5 years with the highest percentage in the age range 70–79 years (55.0%). Mean BMI was 24.41 ± 4.69 kg/m², with only 3.3% of the subjects classified as underweight and most of the subjects (61.7%) were in the overweight category, meanwhile obese II subjects (10%) were only found in the frail group. There were significant differences in BMI between the groups, which was 2.791 kg/m² (CI95% = -5.132 – (-0.450) higher in the frail group.

Tabel 1. Demographic, Nutrition, Macronutrient and Zinc Intake Status of the Subjects

Characteristic	Frequency (n)	Percentage (%)
Age (year)		
60–69	19	31.7
70–79	33	55.0
>80	8	13.3
Gender		
Male	26	43.3
Female	34	56.7
Nutrition Status (BMI)		
Underweight	2	3.3
Normal Weight	21	35.0
Overweight		
High Risk	16	26.7
Obese I	15	25.0
Obese II	6	10.0
Nutrient Intake		
Energy		
<90% TER*	31	51.7
90–110% TER	14	23.3
>110% TER	15	25.0
Protein		
<10% TER	18	30.0
10–15% TER	24	40.0
>15% TER	18	30.0
Fat		
<20% TER	1	1.70
20–30% TER	27	45.0
>30% TER	32	53.3
Zinc		
< Indonesian RDA** 2013	54	90.0
Indonesian RDA 2013	3	5.0
> Indonesian RDA 2013	3	5.0

*TER = total energy requirement; **RDA = recommended daily allowance

Table 2. Characteristic of Subject's Serum Zinc level and Erythrocyte SOD Activity Based on Frailty Status

	Subject		
	Non-*Frail* (n=30)	*Frail* (n=30)	Total (n=60)
sZn Level			
Normal	3 (10%)	1 (3.3%)	4 (6.7%)
Deficiency	27 (90%)	29 (96.7%)	56 (93.3%)
eSOD Activity			
High	13 (43.3%)	14 (46.7%)	27 (45%)
Normal	10 (33.3%)	11 (36.7%)	21(35%)
Low	7 (23.3%)	5 (16.7%)	12 (20%)

Median energy intake was 86.97 (51.8–58.84)% total energy requirement (TER), low energy intake was found in 51.7% of the subjects. While the median intake of protein was 12.14 (3.39–50.66)% TER, there was no difference in energy and protein intake between the two groups. Despite 51.7% of the subjects had low energy intake, 53.3% of the subjects had fat intake >30% TER, with a mean value of fat intake of 32.5 ± 8.19% TER, with no differences

Table 3. Correlation between Serum Zinc Levels and Erythrocyte SOD Activity

Subject	r	p
Overall (n=60)	-0,04	0,076
Non-Frail	-0,099	0,604
Frail	0,01	0,957

neither between the frail nor non-frail group nor among BMI groups.

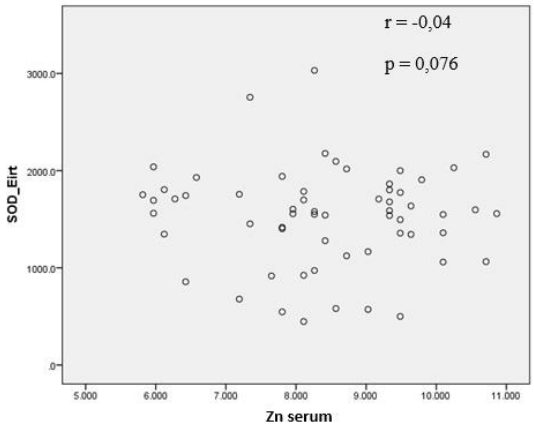

Figure 1. Correlation between sZn level and eSOD Activity in Subjects

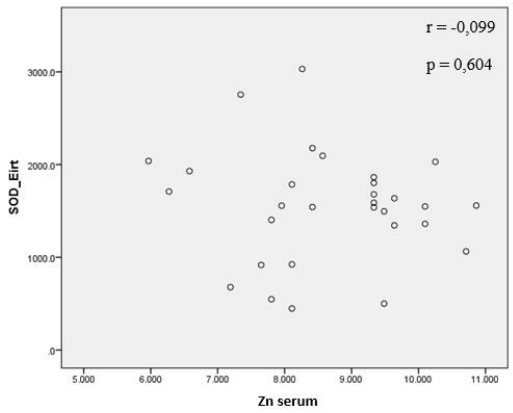

Figure 2. Correlation between sZn level and eSOD Activity in Non-frail Group

Median of zinc intake, determined by semiquantitative FFQ method, was 4.99 mg (2.50–15.76mg), when compared with the Indonesian *recommended daily allowance* (RDA) 2013, 90% of the subjects had zinc intake below the recommendation. The median intake of zinc in both groups was not significantly different, although the intake of the frail group (4.60 (2.50–11.0) mg) was lower than the non-frail (6.52 (2.79–15.8) mg). The characteristic of subjects based on energy, protein, fat, and zinc intake is shown in Table 1.

Mean sZn level was 8.41 ± 1.36 µmol/l, which 93.3% of the subjects categorized as zinc deficient (45% in non-frail group vs 48.3% in frail group). The mean of eSOD activity was 1526 ± 508 U/gHb, while the mean eSOD activity of both groups was 1551 ± 599 U/gHb and 1501 ± 405 U/gHb. There were no significant differences, neither in the mean of sZn level (p = 0.174) nor eSOD activity (p = 0.707) between both groups.

There was no significant correlation found among age with sZn level and eSOD activity of the subjects, both overall as well as per group. There was no significant correlation between sZn level and eSOD activity, neither overall nor per group (Table 3).

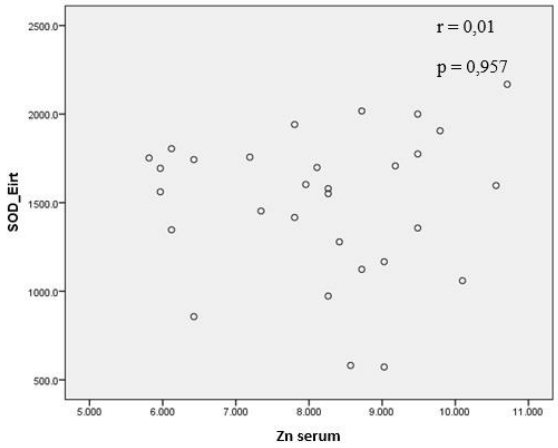

Figure 3. Correlation between sZn level and eSOD Activity in Frail Group

DISCUSSION

In this study, 61.7% of the subjects were classified as overweight, even 10% of subjects were obese II. The elderly rarely perform regular minimum recommended physical activity; moderate intensity for 5 x 30 minutes at least per week; only 22% of the elderly who have continuous sports activities.[15] Sedentary lifestyle often occur in the elderly, due to physiological changes, such as the decreased organ capacity, and reduced muscle strength.[15,16]

The difference in mean BMI between the non-frail vs frail group is 2.79, which was higher in the frail group. Other physiological changes in the elderly, like body composition changes, increased fat

and visceral fat mass, decrease in muscle mass and body metabolic rate, were found.[15] Excessive fat mass and loss of muscle mass, a condition called sarcopenic obesity,[15] together with excess body weight causes a decrease in muscle strength, functional capacity in daily activities.[4,15] Overweight and sedentary lifestyle accelerate the occurence of sarcopenia, and ultimately increase the incidence of frailty syndrome.[15]

Food record concluded 51,7% of the subjects had low energy intake, it should be considered that elderly intake of food is reduced up to 25% compared to adults, caused by the physiological changes in the elderly.[15,17,18] In this study, 30% of the subjects had low protein intake, and we also should be concerned in terms of the quality of protein which is consumed by the subjects. Essential amino acids in animal protein is more complete than vegetable protein, also vegetable protein is bound to carbohydrates therefore it is more difficult to digest due to the limitations of the human digestive enzymes.[19] Although 51.7% of the subjects had low energy intake, on the other hand 53.3% of the subjects had fat intake >30% TER and only 1 subject had a low fat intake (<20% TER). Overweight in the elderly was not caused by a high intake of fat, but it is referring to the lack of the subject's physical activity (in this study we did not explore it further). The main therapy in obesity is weight loss without causing a decrease in muscle mass and increased risk of frailty, with physical exercise and adequacy of dietary protein, vitamins, and minerals, that without causing any increase in the amount of calories.[20]

In this study, 93,3% of the subjects had zinc deficiency, which is in line with the most of the subjects (90%) had a lower zinc intake compared with Indonesian RDA 2013 (13 mg/day for males and 10 mg/day for females). The result is consistent with several studies in other countries which were done in elderly subjects. A cross sectional study by Mariani et al[21] in 981 healthy elderly subjects showed 69% of the subjects had zinc deficiency (plasma zinc level <10 μM). A study by Sfar et al[7] showed a prevalence of zinc deficiency in elderly (age >75 years old) of 65%.[7] The study also showed that the mean plasma zinc level in healthy adults (age 30–40 years old) was 11.75 ± 1.65 μmol/L.

The subjects are not used to consume animal foods, either meat or seafood which are food sources of zinc. Degenerative disease suffered by the subjects (dyslipidemia, hypertension, diabetes mellitus, and hyperuricemia) is also an obstacle for the subjects to consume these foods. Zinc is found in all organs, tissues, and body fluids, as well as having various biologic functions. Homeostasis of zinc keeps plasma zinc level in a small range, therefore a decrease in plasma/serum zinc concentration serum showed zinc deficiency in individuals, especially in relation to long term low zinc intake.[22] In case of low zinc intake, enzymes containing zinc in plasma and metallothionin will be releasing zinc into the pool of zinc, and zinc will subsequently be distributed to other parts that are more in need of zinc.[23]

There are only few studies concerning SOD activity in elderly patients with frailty syndrome. A study in mice with knocked out SOD1 gen (SOD1KO) found that they had increased inflammation and sarcopenia, and also showed characteristics similar to frailty syndrome in humans which includes: weight loss, weakness, low physical activity, and exhaustion. The SOD1KO mice showed alterations in pathways that have been proposed to play roles in the patophysiology of frailty syndrome, which are oxidative stress, mitochondrial dysfunction, and cell senescence.[24] In the present study, there was no differences in erythrocyte SOD activity between the non-frail and frail groups. Meanwhile, 45% of subjects had high erythrocyte SOD activity, and 20% had low erythrocyte SOD activity. High activity of erythrocyte SOD is a response to high pro oxidant/ROS stimulation in the cells, as well as vice versa.[25] High pro oxidant can be due to various conditions, such as aging process, inflammation, anemia, free radicals, and obesity.[25–29] In this study, 33.3% subjects had altered kidney function, which may be caused by anemia in chronic disease and thus contribute to increased pro inflammation. Another factor that may increase ROS level in cells is obesity.[29] In this study, mean BMI was higher in the frail compared to the non-frail group (p=0.02). In fact, 20% subjects in the frail group had class II obesity (BMI ≥30 kg/m^2).

This study did not show any significant correlation between serum zinc level and erythrocyte SOD activity. An in vitro experiment study in tissues, which were made to be zinc deficient, showed altered antioxidant defense mechanism in terms of decreased SOD activity.[30] The result of the in vitro study was different from our study, which may be

due to compensation mechanism from free zinc pool and bound zinc which maintain zinc-related enzyme function. Studies in animal or human subjects still have not shown consistent results in the correlation between zinc status and SOD activity.[7,31–34] The inconsistent result may be due to insufficient number of subjects.[21] A study by Mariani et al[21] with a greater number of subjects (n = 981) showed weak yet significant negative correlation between zinc level and SOD activity ($r = -0.1$; $p < 0.01$). However, other studies involving other trace element status (e.g Cuprum) showed correlation with SOD activity.[32]

Activity of SOD is influenced by many factors, such as free radicals, inflammation due to degenerative disease, and obesity. These confounding factors of SOD activity were not controlled in this study. Otherwise zinc as part of the structure of the CuZnSOD enzyme, in which the role of zinc is a stabilizer of the structure, can be replaced by other metals when the body is deficient. The role of zinc could be replaced by cuprum (Cu) or cobalt (Co),[35] it was probably the reason why the SOD activity can be increased even in the case of zinc deficiency, as it did in the subjects of this study.

In conclusion, in this study serum zinc level did not significantly correlate with erythrocyte SOD activity both overall and per group.

Study Limitation

This study had several limitations. Because it was a cross sectional design, the direction of the reported associations cannot be established. A cross sectional study does not have controls, and is susceptible to bias and confounding factors.

Dietary assessment is challenging in elderly, their abilities to record and remember their diet is limited. Under reporting of dietary analysis, which was performed by semi quantitative FFQ and 3 x 24 hours estimated food record, may be another limitations in this study. Using serum zinc level may not reflect total body zinc level, due to serum zinc level only shows less than 0.1% of body zinc.

The measurement of a single endogen antioxidant activity, which is erythrocyte SOD, was performed in this study, while other major endogenous enzymes were not examined. Fluctuations in the activity of endogen antioxidant enzymes are responsive to pro oxidant stimulation in the body, so that the level of other endogen antioxidants and oxidative stress markers need to be assessed. Further research with larger number of subjects and also considering other endogen antioxidants, oxidative stress marker, and trace elements may be needed to determine the correlation between zinc and SOD activity in elderly patients, especially in frailty syndrome.

Conflicts of Interest

The authors of this paper declare there is no conflict of interest.

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BONDING DEVELOPMENT BETWEEN PARENTS AND CHILDREN THROUGH PLAYING TOGETHER TO IMPROVE HAPPINESS

Saptawati Bardosono,[1] Rini Hildayani,[2] Dian Novita Chandra,[1] Yulianti Wibowo,[3] Ray W. Basrowi[3]
1 Department of Nutrition, Medical Faculty Universitas Indonesia, Cipto Mangunkusumo Hospital
2 Psychology Faculty, Universitas Indonesia
3 Nestle Nutrition Institute Indonesia

Abstract—**Introduction:** This study aims to use the Indonesian translation of modified couple satisfaction index (CSI) tool to evaluate parental happiness scores at before and after participating playing together activities that was designed for bonding development between parents and children.
Methods: By using a serial pre-post intervention experimental design, to assess a minimal of 263 couples of mothers and fathers having under five-year-old child at before after the intervention, and two weeks after. The intervention was playing activities in an interactive playground spent by parents and child for 1.5–2 hours that were located in Medan, Jakarta, Surabaya and Makassar. The validated CSI semi-structured questionnaire (by using Cronbach's alpha of 60% or more) was used to collect data to explore factors contributing to parents' happiness. A multiple measure analysis was used to analyse the parents' happiness improvements.
Results: The CSI tool was validated with the Cronbach's alpha of 0.87 and 0.84 for father and mother, respectively. This study could recruit a total of 370 couples, however only 282 couples completed all data collection. There are 11 items asked for parents' happiness, 3 items regarding to factors contributing to parents' happiness, and 6 items to assess parents' satisfaction. There are improvements in all items by proportions, and the total score of parents' feelings about their relationships are significantly increased by time ($P<0.001$).
Conclusion: The quality of playing together between parents and child can increase family happiness at both short and longer-periods.

Keywords: bonding development, parents and child, family happiness

INTRODUCTION

There is an indication that being a parent is associated with higher well-being or so called happiness. One main source of parents' happiness is their children. However, there are factors contributing to parents' happiness, among others are purpose or meaning in life, human needs, positive emotions, and social roles that have positive effects. On the opposite, negative emotions, financial strain, sleep disturbance and strained partner relationships contribute as negative effects to parents' well-being.[1]

Happiness itself is a culturally constructed concept that is defined internalized and socialized accordingly.[2] Therefore, there is a need to use an assessment tool of happiness that is culturally specific. There are several tools available, and among others is the Subjective Happiness Scale (SHS) by Lyubomirsky and Lepper (1999) which is the most widely used short self-report to measure happiness across age. However this study decide to use the couples satisfaction index (CSI),[3] the previous scale seems too general, while the CSI is more promising to measure one's satisfaction in a relationships, such as mother and/or father relationship to partner and/or under-five year old child.

In our knowledge, there is no study exploring the relationships between family happiness and child nutritional status yet. However, happy families are far more likely to be characterized by good family relationships that include mutual understanding and respect between parents and children.[4] This condition could also be applied to family and child eating behavior. This study aims to use the Indonesian translation of modified CSI to evaluate parental happiness scores at before and after participating playing together activities that designed for bonding development between parents and children.

METHODS

Study design

This study implemented a serial pre-post intervention experimental design to evaluate parents' happiness at before and after the intervention, and two-weeks after the intervention that indicated the

long-term effect. A preliminary study was conducted prior to the main study to validate the Indonesian translation of the modified CSI, and exploring factors contributing to parents' happiness and their satisfaction toward the intervention.

Type of activity applied for parents and child in this study was playing together in a playground. The activities was designed to encourage both parents and child to play/spend time together which enable to stimulate bonding and happiness between them. Playing activities provided in the playground namely fishing, feeding animals (cat, rabbit, deer and bird), taking picture with animal, snow playground and playing in the giant slide. There is no mandatory on the selection of activities should be followed by the parents and child in the playground, however the average time of playing among families was about 1.5-2 hr. Some families were able to participate in all activities provided but some were not. The CSI questionnaire was filled in by the parents at before and after playing in the playground.

Place and Time

The preliminary study was done in Jakarta to prepare the instrument for the main study (1st of March 2016) while waiting for the formal release to ethics' letter. While the main study was conducted afterward in several big cities of Indonesia (March–May 2016), i.e. Jakarta (5th of March and 28th of May), Medan (14th of May), Surabaya (19th of March), and Makassar (16th of April).

Population and Subjects

For the preliminary study, thirty mothers and fathers having children aged 1-5 years who lived in Jakarta and willing to join this study were asked to participate. While for the main study, mothers and fathers having children aged 1-5 years who were willing to participate in this study and sign the informed consent were included, and those could not finish the study protocol were not be included in the analysis.

Sample Size Calculation

For the preliminary study, a minimal of thirty eligible parents (mother and fathers) were sufficient to validate the Indonesian modified CSI in determining parents' happiness and exploring factors contributing to it. For the main study, minimal sample size was calculated to compare two or more paired means of SHS score. By using 95% confidence interval and 90% of power to detect 10% of score improvement and assuming of standard deviation of 50%, then a minimal sample size of each 263 mothers and fathers were needed. Consecutive sampling was used to get the eligible subjects in each location. By allowing 40% of drop out or no response rate, then this study should get a minimal 300 mothers and 300 fathers to be included in the analysis.

Data Collection

The English version of modified the Couples Satisfaction Index (CSI) was first translated into Bahasa Indonesia and then was back translated into English at before being used for the validation/preliminary study. After screening of the subjects for the preliminary study then a minimal of 30 mothers and 30 fathers were asked to fill in the Indonesian version of modified CSI, and followed by filling in a semi-structured questionnaire to explore factors contributing to parents' happiness.

After validating the Indonesian version modified CSI, then the modified CSI was used to for data collection in the main study at before and after participating to the playing together activities, and was repeated after two weeks later. Besides, a satisfactory tool was also employed to evaluate parents' satisfaction toward the intervention.

The intervention was a playground set-up in a special area in the mall, which included several activation sections. In each section visited, parents should accompany and play together with their child. The following are variables understudy: **Parents' happiness**, is defined as a mental or emotional state of well-being defined by positive or pleasant emotions ranging from contentment to intense joy, that can be evaluated by using modified the Couples Satisfaction Index (CSI) tool and graded from score 0 to 6 and/or 0 to 5. **Factors contributing to parents' happiness** are including several conditions that could have positive and negative effect to parents' happiness, categorized to 1 as having the positive or negative factor and 0 as else.[1] **Parents' satisfaction**, is defined as a happy or pleased feeling because of something done or happened.

Data Management and Analysis

Subjects' characteristics were analyzed descriptively by using central tendency and/or frequency distribution (n, %). Validation of CSI tool used Cronbach's Alpha of 60% or more to determine that the tool is valid. And, a dependent-t test and/or multiple measure analyses were used to evaluate the score improvements at before and after the intervention.

Ethical Consideration

Ethical clearance approval was received from the Ethical committee Faculty of Medicine Universitas Indonesia, i.e. No. 275/UN2F1/ETIK/2016 by April 4, 2016.

RESULTS

The preliminary study has done a translation and back-translation of the instrument used in this study by a sworn formal translators. Furthermore, by using 30 subjects of fathers and mothers of under-five children, we did a reliability test and had a good reliability for both father and mother with a Cronbach's Alpha of 0.87 and 0.84, respectively, showing that the tool is valid.

This study could get 370 subjects of each mother and father who had children age 1-5 years old to participate at baseline, as shown in Figure 1. However, the response rate after the two weeks intervention, in average was 76.2% (282 subjects of father and mother), i.e. 67.4%, 86,8%, 84.9% and 85.7% in Jakarta, Medan, Surabaya and Makassar, respectively. The response rate is still higher than the expected of 60% and still fulfilled the minimal sample size of this study (i.e. 263 pairs of father and mother).

Table 1 shows the socio-demographic characteristics of the subjects in total and by the study locations. In general, the child sex was comparable, with a high education parents, permanent job for father and approximately one-third of the mothers are working. Thus, the family income is found to be sufficiently high and more than half of the families owned their house.

Furthermore, Table 2 shows that most of the subjects has a nuclear family type (75%), had approximately five years of marriage and mostly brought their owned first born child to the playground intervention place (72%). They mostly have one child of their own (56%).

Almost all of the parents perceived no problem in taking care of their child. While the main child caregiver is mother (80%), as shown in Table 3.

Parents' happiness

By participating in the playground intervention program, Figure 2a and 2b show that there are more than 20% increased proportions on several aspects of happy characteristics during and after the intervention both for fathers and mothers. There are 11 items (item A to item K) to measure parents' happiness, i.e. changing in perfect degree of happiness (in %) at before and after attending the playground (item A), changing in the approximate extent of always agree between parents and the child for the amount of time spent together (in %) at before

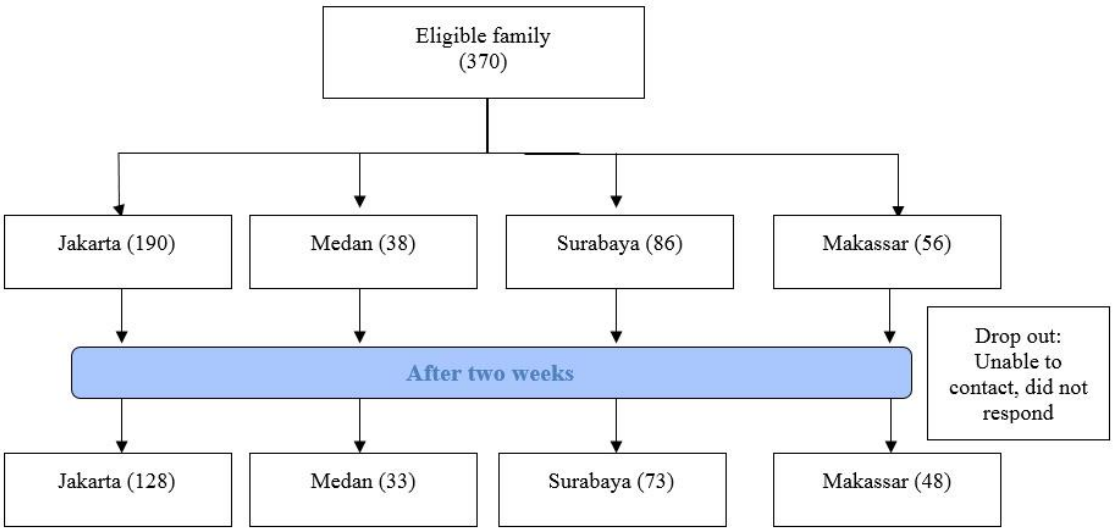

Figure 1. The study workflow

and after attending the playground (item B), changing in the approximate extent of always agree between parents and the child for the affection demonstrated each other (in %) at before and after attending the playground (item C), changing in the strong connection that is completely true between parents and the child for the affection demonstrated each other (in %) at before and after attending the playground (item D), changing in the strong relationship that is completely true between parents and the child for the affection demonstrated each other (in %) at before and after attending the playground (item E), changing in the relationship that is completely true between parents and the child that make them happy (in %) at before and after attending the playground (item F), changing in a warm and comfortable relationship that is completely true between parents and the child (in %) at before and after attending the playground (item G), changing in feeling like part of a team that is completely true between parents and the child (in %) at before and after attending the playground (item H),

Table 1. Socio-demographic characteristics distribution of the subjects (n=370)

Variables	Jakarta 1 n=100	Surabaya n=86	Makassar n=56	Medan n=38	Jakarta 2 n=90	Total n=370
Age, year						
Child	3.6 ± 1.8	3 (1–5)	2 (1–5)	2.6 ± 1.0	3.5 (1–5.5)	3 (1–5.5)
Father	33 (25–50)	34.3 ± 5.8	31.5 (2–46)	33.6 ± 5.5	35 (25–55)	33 (21–55)
Mother	31 (22–46)	30.9 ± 5.0	29.11 ± 4.12	30.7 ± 4.7	32.4 ± 4.7	31 (20–46)
Child gender, n (%)						
Boys	47 (47)	56 (65.1)	26 (46.4)	20 (52.6)	38 (42.2)	187 (50.5)
Girls	53 (53)	30 (34.9)	30 (53.6)	18 (47.4)	52 (57.8)	183 (49.5)
Education level, n (%)						
Father						
Less than Junior high school	-	-	1 (1.8)	-	-	1 (0.3)
Junior high school	-	2 (2.3)	5 (8.9)	-	1 (1.1)	8 (2.2)
Senior high school	18 (18)	33 (38.4)	21 (37.5)	9 (23.7)	14 (15.6)	95 (25.7)
Higher education	82 (82)	51 (59.3)	29 (51.7)	29 (76.3)	75 (83.3)	266 (71.9)
Mother						
Less than Junior high school	1 (1)	3 (3.5)	5 (8.9)	1 (2.6)	3 (3.3)	1 (0.3)
Senior high school	17 (17)	32 (37.2)	25 (44.6)	11 (28.9)	12 (13.3)	97 (26.2)
Higher education	82 (82)	51 (59.3)	26 (46.4)	26 (68.4)	75 (83.3)	260 (70.3)
Type of job, n (%)						
Father						
No regular job	-	1 (1.2)	1 (1.8)	1 (2.6)	-	3 (0.8)
Labor	1 (1)	3 (3.5)	1 (1.8)	1 (2.6)	-	6 (1.6)
Vendor	4 (4)	11(12.8)	7 (12.5)	6 (15.8)	6 (6.7)	34 (9.2)
Private employee	78 (78)	53 (61.6)	28 (50)	16 (42.1)	62 (68.9)	237 (64.1)
Government official	7 (7)	5 (5.8)	8 (14.3)	7 (18.4)	7 (7.8)	34 (9.2)
Others	10 (10)	13 (15.1)	11 (19.6)	7 (18.4)	15 (16.7)	56 (15.1)

Table 1. (continued) Socio-demographic characteristics distribution of the subjects

Variables	Jakarta 1 n=100	Surabaya n=86	Makassar n=56	Medan n=38	Jakarta 2 n=90	Total n=370
Mother						
No regular job	8 (8)	9 (10.5)	15 (26.8)	7 (18.4)	4 (4.4)	43 (11.6)
Vendor	3 (3)	5 (5.8)	4 (7.1)	3 (7.9)	4 (4.4)	19 (5.1)
Private employee	39 (39)	33 (38.4)	6 (10.7)	6 (15.8)	28 (31.1)	112 (30.3)
Government official	5 (5)	7 (8.1)	6 (10.7)	3 (7.9)	5 (5.6)	26 (7.0)
Others	45 (45)	32 (37.2)	25 (44.6)	19 (50.0)	49 (54.4)	170 (46.0)
Family income, n (%)						
<500,000 IDR	-	-	1 (1.8)	-	1 (1.1)	2 (0.5)
500,000 – <1,000,000 IDR	1 (1)	3 (3.5)	-	2 (5.3)	-	6 (1.6)
1,000,000 - <2,000,000 IDR	-	18 (20.9)	12 (21.5)	4 (10.6)	3 (3.3)	37 (10.0)
≥2,000,000 IDR	99 (99)	65 (75.6)	43 (76.8)	32 (84.2)	86 (95.6)	325 (87.8)
House ownership, n (%)						
Owner	63 (63)	44 (51.2)	24 (42.9)	23 (60.5)	52 (57.8)	206 (55.7)
Rent	15 (15)	19 (22.1)	7 (12.5)	9 (23.7)	16 (17.8)	66 (17.8)
Stay over	21 (21)	23 (26.7)	22 (39.3)	5 (13.2)	21 (23.3)	92 (24.9)
Others	1 (1)	-	3 (5.4)	1 (2.6)	1 (1.1)	6 (1.6)

[a] mean ± SD or mean(min-max)

changing in imagining another person to make as happy that is completely true as their child (in %) at before and after attending the playground (item I), changing in rewarding completely (in %) in the relationship with the child at before and after attending the playground (item J), and changing in satisfaction completely (in %) with the child at before and after attending the playground (item K).

Factors contributing to parents' happiness

Based on the subjective semi-quantitative score on the quality of relationships between fathers and mothers to the child, there are varied findings on the changing in the proportion toward better relationships, especially for fathers (Figure 3). There are three indicators being compared during the study, i.e. changing in extremely good score (in %) in relationship with the child at before and after attending the playground, changing in more often (in %) in enjoying the companionship with the child at before and after attending the playground, and changing in more often (in %) in having fun together with the child at before and after attending the playground. The finding shows that more often in enjoying the companionships (score 1 and score 2) with the child are both decreased, for father and mother. More often having fun together (score 3) is slightly increased for mother but decreased for father.

Parents' satisfactions

Further more, based on the first impression and immediate feeling about how the father and mother feel about the relationship with the child, Figure 4 show slightly increased on their feelings (less than 10%). The six satisfaction indicators used were as follows: changing in interesting score (in %) in the feeling about the relationship with the child at before and after attending the playground, changing in good score (in %) in the feeling about the relationship with the child at before and after attending the playground, changing in full score (in %) in the feeling about the relationship with the child at before and after attending the playground, changing in

Table 2. Family status characteristics distribution of the subjects (n=370)

Variables	Jakarta 1 n=100	Surabaya n=86	Makassar n=56	Medan n=38	Jakarta 2 n=90	Total n=370
Family status, n (%)						
Nuclear	77 (77)	57 (66.3)	30 (53.6)	34 (89.5)	79 (87.8)	277 (74.9)
Extended	23 (23)	29 (33.8)	26 (46.4)	4 (10.5)	11 (12.2)	93 (25.1)
Length of marriage	5.5 (2–20)	5 (1–20)	5 (2–14)	6 (2–14)	5.0 (2.5–15.0)	5 (1–20)
Length of having the child	3 (0–8)	3 (0–13)	2 (1–12)	2.5 (1–9)	3.3 (1.0–5.5)	3 (0–13)
Child status, n (%)						
Own child	100 (100)	86 (100)	56 (100)	37 (97.4)	90 (100.0)	369 (99.7)
Adopted child	-	-	-	1 (2.6)	-	1 (0.3)
Child rank, n (%)						
First	58 (58)	62 (72.1)	44 (78.6)	20 (52.6)	72 (80.0)	272 (73.5)
Second	32 (32)	18 (20.9)	10 (17.9)	14 (36.8)	16 (17.8)	77 (20.8)
Third and more	10 (7)	6 (7.0)	2 (3.6)	4 (10.5)	2. (2.2)	21 (5.7)
Number of children, n (%)						
1	58 (58)	50 (58.1)	29 (51.8)	15 (39.5)	58 (64.4)	210 (56.8)
2	32 (32)	28 (32.6)	24 (42.9)	18 (47.4)	26 (28.9)	128 (34.6)
3 and more	10 (10)	8 (9.3)	3 (5.4)	5 (13.1)	6 (6.6)	32 (8.6)

friendly score (in %) in the feeling about the relationship with the child at before and after attending the playground, changing in hopeful score (in %) in the feeling about the relationship with the child at before and after attending the playground and changing in enjoyable score (in %) in the feeling about the relationship with the child at before and after attending the playground.

However, Figure 5 shows that in term of overall scoring, the total score of the feeling about their relationship are significantly increased by time, both for father and mother (P<0.001).

Table 3. Child caring characteristics distribution of the subjects (n=370)

Variables	Jakarta 1 n=100	Surabaya n=86	Makassar n=56	Medan n=38	Jakarta 2 n=90	Total n=370
Problems in child caring, n (%)						
Father	98 (98)	83 (96.5)	55 (98.2)	38 (100.0)	90 (100.0)	364 (98.4)
Mother	96 (96)	82 (95.3)	56 (100)	38 (100.0)	89 (98.9)	361 (97.6)
Main childcare provider, n (%)						
Father	1 (1)	1 (1.2)	-	3 (7.9)	1 (1.1)	6 (1.6)
Mother	72 (72)	64 (74.4)	51 (91.1)	32 (84.2)	76 (84.4)	295 (79.7)
Grandparents	18 (18)	14 (16.3)	4 (7.1)	2 (5.3)	11 (12.2)	49 (13.2)
Others	9 (9)	7 (8.1)	1 (1.8)	1 (2.6)	2 (2.2)	20 (5.4)

DISCUSSION

We understand that the majority of children in the developing world, including Indonesia are not able to reach their full developmental potential as the results of poverty, undernutrition, micronutrient deficiencies, and learning environments as well as parental home stimulation activities that do not provide enough responsive stimulation and nurturance which affecting of their wellbeing or happiness.[5–7] Exploration on the happiness indicators

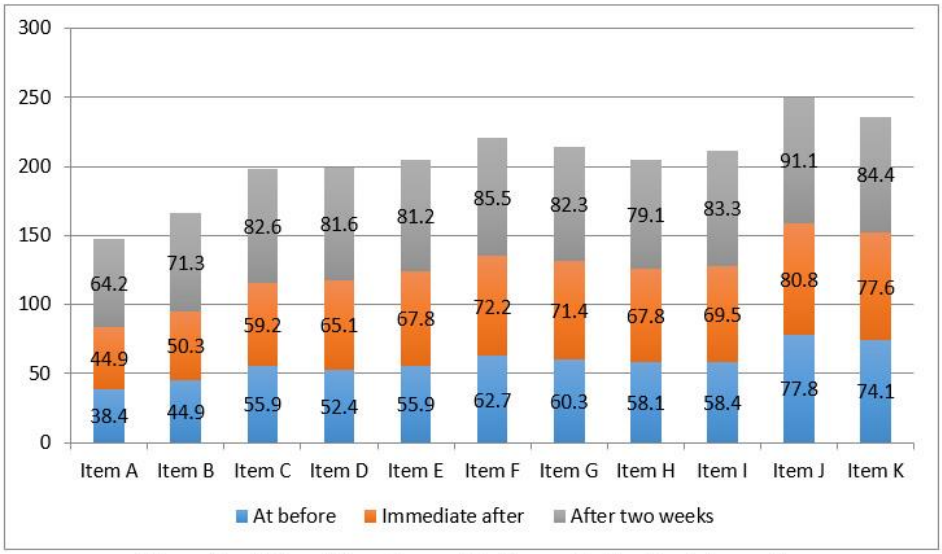

Figure 2a. Fathers' happiness at before and after the intervention

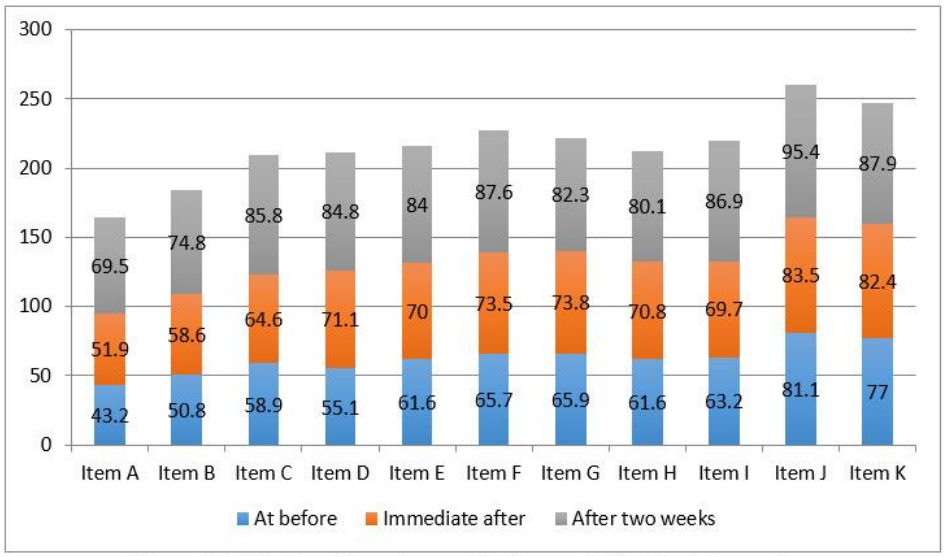

Figure 2b. Mothers' happiness at before and after the intervention

of the Malaysians and Indonesians, revealed that there are 12 happiness indicators in which the first mentioned is family from the others, i.e. career, interpersonal and social relationships, self-growth/self-autonomy, wealth, recreation, needs, education, absence from negative feelings, national prosperity, health, religion, and basic needs.[5]

This study is the first study to explore the bonding (strong connection) development between parents and children through playing together in improving family happiness in Indonesia, represented by parents and children in several big cities such as Jakarta, Surabaya, Medan and Makassar. We found that based on the total score in the feelings about the relationships of parents, both fathers and mothers with the child, there were score increments between before, immediate after, and after the two weeks attending the playground activities/stimulation. These increments in the total score, in details are contributed from the score increments (more than 10%) of the proportions on degree of happiness, spending time together, showing affection, strong connection, strong, happy, warm-comfortable relationships, a solid team, make them feel more happy, rewarding, satisfaction relationships with the child, as reported by both father and mother. It is well defined that the first few years of life are particularly important in which vital development occurs in all domains through brain development. Child's development, besides of genetic inheritance, it is affected by both psychosocial and biological factors. Therefore, early undernutrition, iron-deficiency, environmental pollutions, stress, poor stimulation and social

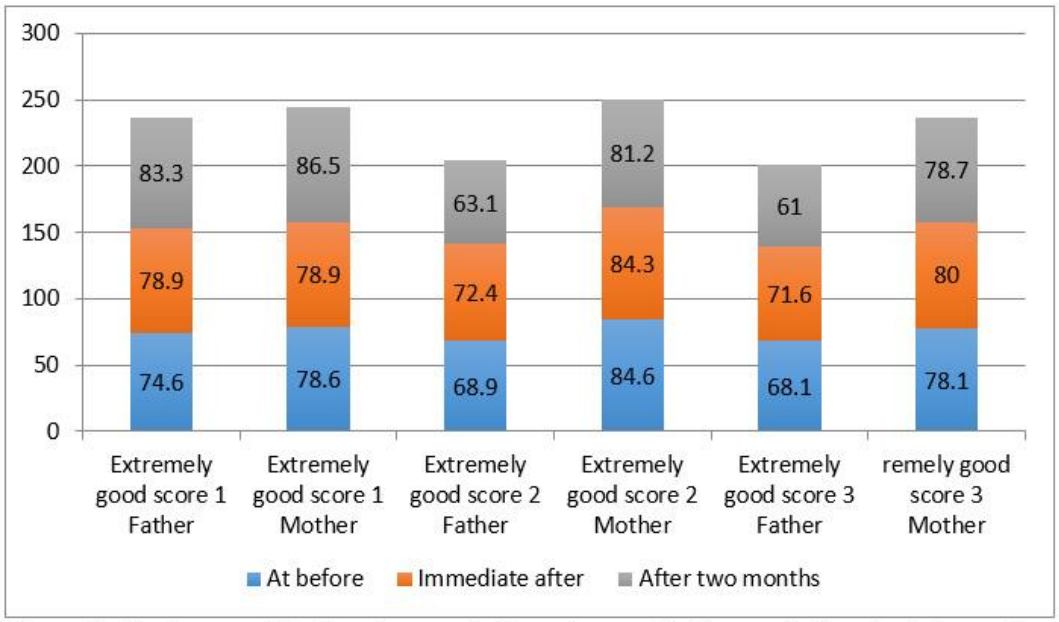

Figure 3. Factors contributing to parents' happiness at before and after the intervention

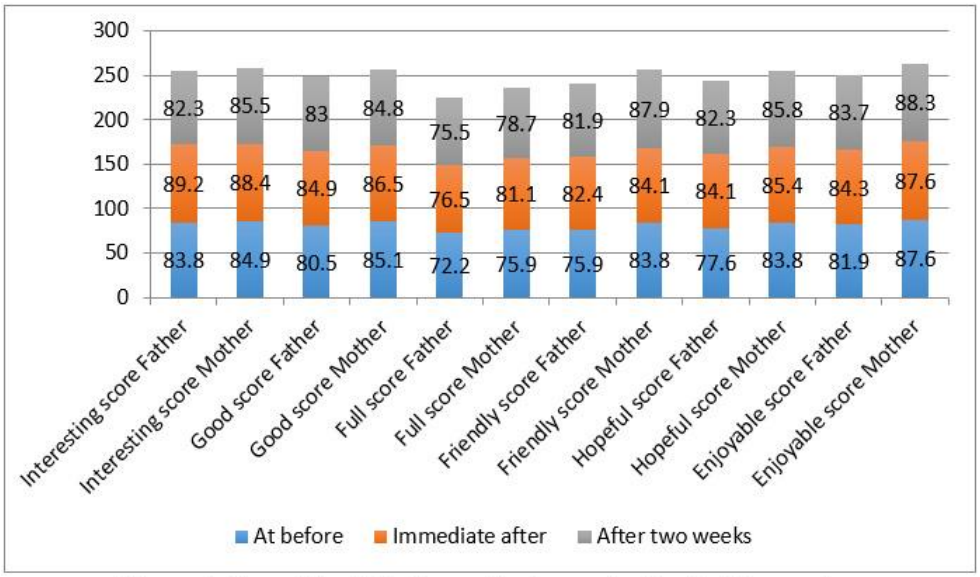

Figure 4. Parents' satisfactions at before and after the intervention

interaction can affect brain structure and function, and have lasting cognitive and emotional effects, such as happiness.[8] A happy family is a good environment for child development and growth. Happy family can be created if the parents and the child are happy.

Happiness in the family is like a circle. It depends on the happiness of every member of the family, i.e. father, mother and the child. Thus, parents will be happy as long as the child happy. Vice versa, the child can be happy if the parents who care for and nurture them is happy. A feeling of happiness that is always embedded in the family can create a healthy emotion that is good for the child development. However, in most Asian countries, especially those with the Confucian cultures, they score very low in happiness survey. There are some speculate reasons, such as environmental disruption, excessive competitiveness, repressive education, excessive conformity, negative attitudes towards enjoyment, and the emphasis on outward appearance.[9] Besides, family happiness is influenced by four broad factors, i.e. family type (nuclear or extended, married, single or separated, etc), family processes (inter-generational history of family relationships, attitudes to parenting and family roles, etc), individual characteristics (personality traits, such as positive and negative emotionality as well as

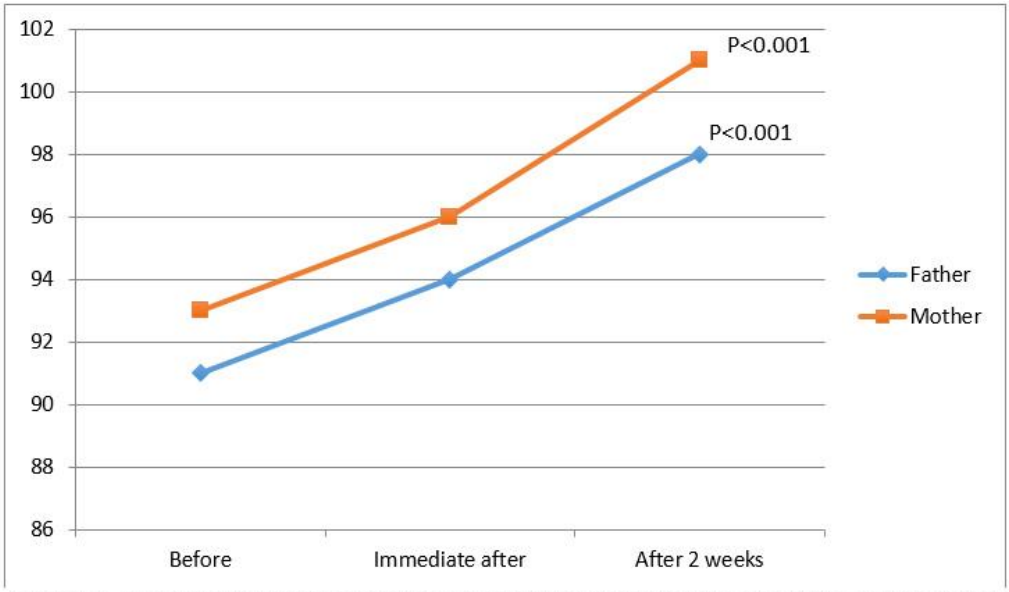

Figure 5. Changing in total score (in median value) in the feeling about the relationship with the child at before and after attending the playground

psychological independence and interdependence), and family circumstances (life events, education, social class, hours worked, etc).[10] However, demographic variables related to the family, i.e. number of siblings, age of parents, and marital status of parents) were only weakly, or not at all, associated with children's happiness. While social relationships are significantly correlates and predictors of happiness.[11]

Related to fathers' involvement, it revealed that children, since infants of highly involved fathers, as measured by amount of interaction, including higher level of play and caregiving activities, are more cognitively competent at 6 months and score higher on the Bayley Scales of Infant Development. By one year they continue to have higher cognitive functioning, are better problem solvers as toddlers, and have higher IQ's by age three. When compared with mothers, fathers' talk with toddlers is characterized by more "wh" questions, such as what, where, etc that requires children to assume more communicative responsibility in the interaction. This encourage toddler to talk more, use more diverse vocabulary, and produce longer utterances when interacting with their fathers. Father involvement is positively correlated with children's overall life satisfaction and their experience of less depression, less emotional distress, less expressions of negative emotionality, such as fear and guilt, less conduct problems, greater sense of social competence, higher levels of self-reported happiness, fewer anxiety symptoms, and lower neuroticism.[12] However, in relation to emotional intelligence, parental bonding style of fathers has an effect only to the level of child motivation and empathy, while the parental bonding style of mothers affects child self-awareness, self-regulation, motivation, empathy and social skills. We are aware that high level of emotional quotients may contribute to child development.[13] Thus, the children need both parental bonding style from fathers and mothers. And, playing together has been proven can improve family happiness as it can increase the affection, bonding (strong connection), warmth, compactness, and makes the relationship between parents and child more enjoyable. When playing, child becomes happy and relaxed, and so the brain will in a happy state as well. And, when the brain is in the positive state, then through a brain and gut cross talk, child digestion will be healthier. And, vice versa, a healthy digestive system can help the child to enjoy playing and absorb the benefits properly, so they could be happier.

However, we have to ensure that the child can play comfortably, and absorb the benefits of playing to the fullest, then parents should provide the child with nutrition that are good for their digestion, such as energy (carbohydrates, essential amino-acids, omega-3 fatty acids and water), vitamins (A and B's), and minerals (zinc). Besides, it is also important for parents to maintain the hygiene while playing with the child by ensuring the child has a good immune system. The largest immune system is

in the gut. Thus, one of the most important steps to take is to maintain a healthy digestive system of the child, such as providing probiotics intake from the child meals and drinks.[14,15]

However, this study also found that the opportunity to be together in the playground did not significantly prove yet to increase the level of scores on their extremely good relationship, more often enjoying being together and having fun together. Actually, by doing activities like playing together, parents and the child can interact and communicate each other. Interaction and communication are the most important basic elements in increasing happiness, besides can show their affection to one another.[16] It is also related to the finding in this study that did not significantly changes on the scores for being more interesting, having good, full, friendship, hopeful, and enjoyable feeling about the parents' relationships with the child after attending the playground. Besides the limited available time provided, unfortunately most parents were not familiar with existing parenting programs.[17] Actually, while playing together, the connection or relationship between parents and the child can grow stronger and in returns can create a feeling of belonging (family as one unity or a team). Besides, playing activities can also be utilized to optimize child's growth and character development. This study has a limitation for not trying to elaborate the bonding development but more to identify parents' emotional perception toward happiness in a short (i.e. after playing together) and in a longer time (i.e. after two weeks).

In conclusions, it is not the playground itself as the only type of stimulation, or the length of playing time spent together, but the quality of playing together between parents and the child that can increase family happiness at immediate after and even weeks after, because parents feel happy when seeing their child happy by doing any playing activity.

Conflicts of Interest

The authors of this paper declare there is no conflict of interest regarding this research.

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EFFECT OF RICE BRAN OIL ON THE LIPID PROFILE OF MILD-MODERATE HYPERCHOLESTEROLEMIC MALE AGE 19-55 YEARS OLD

Noor Diah Erlinawati,[1] Samuel Oetoro,[2] Dwirini R Gunarti[3]

1 Department of Nutrition, Faculty of Medicine, University of Bengkulu, W.R. Supratman, Bengkulu, Indonesia
2 Department of Nutrition, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia
3 Department of Biochemistry, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

Abstract—**Introduction:** Adult individuals in Indonesia showed changes in diet and lack of physical activity, therefore increasing the risk of hypercholesterolemia and obesity. One of nutritional therapy for hypercholesterolemia is rice bran oil, which contains active substances that works synergistically in controlling lipid profile. The substances are gamma-orzanol, phytosterols, and vitamin E isomers (tocotrienol and tocopherol).

Methods: A clinical trial was conducted for 4 weeks in City Hall of Jakarta on 20 males, age 19–55 years old, with mild-moderate hypercholesterolemia, to assess lipid profile improvement of the supplementation of rice bran oil 45 ml/days compared to 15 ml/days without changing eating patterns.

Results: After 4 weeks of intervention, there was significant difference in total cholesterol level between both groups ($p = 0.049$). In the group that received 45 ml/days of rice bran oil, total cholesterol level decreased 14%, and in the group of 15 ml/days total cholesterol level decreased 7.8%. The reduction of LDL and triglycerides and the increase of HDL were not significantly different between both groups ($p > 0,05$). There was no significant changes of body weight in both groups.

Conclusion: This study showed that consumption of 45 ml/days rice bran oil led to better improvements in lipid profiles compare to 15 ml/days. It has been demonstrated that gamma oryzanol and plant sterols in rice bran oil have the capability to remove cholesterol from bile salt micelles, thus decreasing cholesterol absorption in intestine.

Keywords: rice bran oil, gamma-oryzanol, hypercholesterolemia, lipid profile

INTRODUCTION

Cardiovascular disease has a tendency to increase in developing countries as a result of lifestyle changes.[1] According to WHO data of non-communicable diseases in year 2010 in Indonesia, the prevalence of elevated cholesterol level was 35.1% and was higher in women (37.2%) than in men (32.8%).[2] Male adults are at risk for suffering hypercholesterolemia and central obesity due to lifestyle changes.[3,4] This condition may lead to increased risk of metabolic syndrome, hypertension, diabetes mellitus, obesity and other chronic diseases.[4–6]

Nutritional therapy became the first pillar as part of lifestyle intervention in hypercholesterolemia. Phytosterol, which is one of functional food, has been recommended by the National Cholesterol Education Program (NCEP) for controlling lipid profiles.[7] Rice bran oil contains gamma-oryzanol, phytosterols, and vitamin E (tocotrienols and tocopherols), which are known as unsaponifiables fractions. Gamma-oryzanol consists of ferulic acid and phytosterol esters, which are structurally and functionally similar to cholesterol. Both substances can reduce the absorption of cholesterol in the intestine as competitive inhibitors. Unsaponifiable fractions of rice bran oil act synergistically in controlling blood lipid profile. The composition of fatty acids in rice bran oil are closest to the recommendation of the American Heart Association (AHA).[8–13]

There were some studies done by Raghuram[14], Kuriyan[15], Rajnarayana[16] and Eady[13] which showed rice bran oil effect in improving lipid profile in hypercholesterolemic patients. Rice bran oil products are currently widely recommended by clinicians because of their benefits of preventing cardiovascular diseases. Earlier studies mostly used 300 mg/day dose of gamma-oryzanol or 50 grams of rice bran oil, which were included in the daily diet or cooking oil.[13,17] However no studies regarding the effective amount or dose of rice bran oil in daily consumption without changing food intake on lipid profiles of hypercholesterolemic patients has been done yet. This study aimed to assess the lipid profile

improvement after intake of different amount of rice bran oil without changing the eating patterns.

METHODS

Subjects

A total of 26males with hypercholesterolemia, age 19-55 years old, were recruited for the study. Inclusion criteria were cholesterol levels 200-300 mg/dl and body mass index 20-30 kg/m^2. The exclusion criteria were any consumption of cholesterol-lowering drugs, steroids and other drugs that affect lipid metabolism; smoking more than 10 cigarettes/day; taking weight loss program or consuming appetite inhibitor drugs; taking supplements containing phytosterol; have gastrointestinal, thyroid, heart, liver and kidney diseases which are determined by medical records; in hormonal therapy that affects the metabolism of fat, and suffering from type 2 diabetes mellitus. Subjects agreed to sign the informed consent and they were assured that all information will be confidential. Medical Ethics Commitee from University of Indonesia has approved the study protocol.

Clinical Study

This study was a randomized, single-blind, parallel design conducted at City Hall of Jakarta in April through May 2015. Subjects who met the study criteria were interviewed to complete the demographic data, physical activity, food intake using semiquantitative FFQ, and anthropometric measurements. Before and after 4 weeks of intervention, subjects were asked to complete food intake data of an estimated 3 days of food intake record of their usual dietary pattern. They were asked to record their food intake of 3 non-consecutive days : 2 weekdays and 1 day off or non working day, prior to rice bran oil treatment. They were also given a leaflet to help their estimation of food portion sizes.

The subjects were randomly assigned to 1 of 2 groups. One group was assigned to consume 45 ml/day rice bran oil (n=13), while another was 15 ml/day rice bran oil (n=13). Rice bran oil used for this study was purchased from the market. The oil were manufactured by Pietro Coricelli S.p.a., Italia. The oil was measured and packed into smaller bottles, which were then given to the subjects.. The subjects were asked to consume two bottles of rice bran oil everyday. Compliance was assessed by facilitated researchers. The oil could be consumed with lunch and dinner or be drunk directly. Subjects were asked to not changing their daily diet.. Each subject was asked to consume the rice bran oil once a week until the end of treatment and also be interviewed to determine any complaints while taking the oil.

Laboratory analysis

Subjects were asked to fast for at least 10 hours prior to blood sampling. Laboratory tests for lipid profile were performed twice, before and after 4 weeks of intervention.. During blood sampling, 3.5 ml of blood was drawn for the test. Fasting blood samples were collected into tubes and were placed for 30−40 min. Afterwards, blood samples were centrifuged at 1300−3000g for 20 min, and then the blood serum were taken for subsequent analysis in laboratory. Total cholesterol and triglycerides serum level were measured by enzymatic methods in an automated biochemistry analyzer. HDL-C values were measured by precipitation and direct methods. LDL was estimated with the Friedewald equations.[18] Lipid assays were standardized by the Center for Disease Control and Prevention.[19]

Statistical analysis

Data were analyzed using SPSS version 20 for Windows. Unpaired t-test or Mann-Whitney test was used to compare the data from before and after the study intervention, $p < 0,05$ indicated a statistical difference data from the food intake analyzed with Nutrisurvey 2007 for windows.

RESULTS

From 26 who initially agreed to participate, 20 subjects completed the 4-week study.. One subject developed illness unrelated to the study and 5 refused to continue the study protocol. Rice bran oil consumption compliance was reported to be greater than 90% as assessed by self-completed check sheets. Baseline characteristics of the subjects are shown in Table 1.

The mean age of the 45 ml/day group and 15 ml/day group were 48.90±5.26 and 45.70 ± 6.82 years old, respectively. Most of the subjects in this

Table 1. Baseline Characteristic of The Subjects (n=20)

Variables	45 ml/day RBO group (n=10)	15 ml/hari RBO group(n=10)
Age	48,90±5,26	45,70±6,82
Height,cm	164,70±6,18	164,40±6,13
Weight,kg	75,06±12,90	73,07±11,54
BMI,kg/m^2	27,63±4,20	26,97±3,65
Energy,kkal	2205,25±193,53	2071,07±254,28
Carbohidrate,g	281,2±28,02	266,92±37,24
Protein,g	91,86±13,21	97,22±16,96
Fat,g	78,78±11,01	68,73±13,67
Fiber,g	11,06±0,96	12,25±3,18
Total cholesterol,mg/dL	242,30±33,66	228,80±16,96
LDL cholesterol, mg/dL	173,70±46,34	157,10±25,41
HDL cholesterol, mg/dL	40,70±7,02	45,70±10,06
Trigliseride, mg/dL	176,50 (84-308)	161,00 (97-427)

Values are expressed as mean±SD, median(min-max)

study were classified as in both groups. Physical activity of 45 ml/day group were mostly mild to moderate, and 15 ml/day RBO group were mostly moderate. Most subjects did not have family history of hypercholesterolemia. Also, most of the subjects were non-smokers. The two groups had similar characteristics in terms of age, education level, nutritional status, physical activity, family history of hypercholesterolemia, and smoking history (data not shown).

There was no significant difference in food intake between both groups before treatment. Composition of food intake in 45 ml/day RBO group was 51% carbohydrate, 17% protein and 32% fat. Composition of food intake in a 15 ml/day RBO group was 52% carbohydrate, 18% protein and 30% fat.

Average calorie intake after 4 weeks of intervention in 45 ml/day RBO group was 2387 kcal/day with composition of 46% carbohydrates, 16% protein and 43% fat. Whereas in 15 ml/day RBO group, calorie intake was 2224 kcal/day, with the composition of 48% carbohydrates, 18% protein and 37% fat. There was significant difference in total fat intake after intervention (p=0,014). However, there was no significant difference in energy intake, carbohydrate, protein and fiber after intervention between the two groups. Change of total fat intake (p=0.007) and percentage of fat intake (p=0,003) before and after treatment were also significantly different. Food intake analysis and changes are shown in Table 2.

The decline in serum cholesterol after 4 weeks of intervention was significantly different between both groups (p=0,049). The average changes of cholesterol levels in 45ml/day RBO group were -37,50 (-53,00−-9,00) and in 15ml/ day RBO group was -10,50 (-74,00−4,00). Decrease of LDL and triglycerides level were greater in 45 ml/day RBO group compared to the other group. Levels of HDL increased greater in 45 ml/day RBO group compared to the other group. However, LDL, HDL and triglyceride levels after treatment and changes during treatment were not statistically significant difference between both groups. Lipid profile examination results are shown in Table 3. Body weight was measured before and after treatment to ensure the safety of RBO intervention for 4 weeks. There was no difference of body weight before and after treatment in both groups.

DISCUSSION

To our knowledge, this was the first study in Indonesia which compared intervention of rice bran

Table 2. Average Daily Intake of Energy, Protein, Fat, Carbohydrate and Fiber at Baseline, after Intervention and The Changes during Intervention

Variables	45 ml/day RBO group (n=10)			15 ml/day RBO group (n=10)		
	Baseline	Week 4	Change (Δ)	Baseline	Week 4	Change (Δ)
Energy,kkal	2205,25±193,53	2205,25±193,53	182,73±79,78	2071,07±254,28	2224,86±317,38	153,79±130,30
Carbohydrate,g	7,35±6,24	275,76±20,89	-5,44±16,98	266,92±37,24	267,45±32,69	0,53±23,94
Protein,g	91,86±13,21	94,18±14,15	2,31±7,81	97,22±16,96	103,09±18,53	4,80(0,20−21,50)
Fat,g	78,78±11,01	115,16±12,05*	36,38±5,8*	68,73±13,67	91,56±23,44	22,82±12,03
Fiber,g	11,06±0,96	11,48±0,73	-0,42±0,84	12,25±3,18	12,32±3,08	0,40(-2,30−1,40)

Values are expressed as mean ± SD, and median (min-max). Values with asterisk (*) are significantly different from 15ml/day RBO group, p <0,05.

oil with different doses without any change in daily diet to determine the effect of unsaponifiable fractions in both groups. Most of the study subjects were obese with mild-to-moderate physical activity. Lack of physical activity stimulates escalation of lipid profiles and blood glucose which independently affect central obesity.[20] Higher fat and lower fiber intake compared to recommendations can cause hypercholesterolemia and weight gain. Nutritional recommendation for dyslipidemia suggested that fat intake should be around 20-30% of total calories and fiber around 30 g/day.[4,7] Consistent to that, food intake data of the subjects in this study showed that unbalanced nutritional intake contributes to hypercholesterolemia and obesity.

In this study, intervention with additional rice bran oil (RBO) to the daily diet that contains high fat would affect the food intake proportion of the subjects. There was significant difference in fat intake (p = 0,014) between the two groups. Change of fat intake in 45 ml/day RBO group was 15% and in 15 ml/day RBO group was 10%. This result shows eventhough the 45 mL/day RBO group consumed as much as three times the amount of RBO compared to 15 mL/day group, however, the fat intake difference between both groups was only 14 gram or 5%. This may happened because the subjects who consumed oil 45 ml/day felt full and satisfied faster and longer, which then reduce the consumption of carbohydrates and fat.

Tabel 3. Lipid Profile of Research Subjects (n=20)

Variables	45 ml/day RBO group (n=10)			15 ml/day RBO group (n=10)		
	Baseline	Week 4	Change (Δ)	Baseline	Week 4	Change (Δ)
TC (mg/dL)	242,30±33,66	207,90±29,07	-37,50 (-53,00−-9,00)*	228,80±16,96	210,70±26,75	-10,50 (-74,00−4,0
LCL-C (mg/dL)	173,70±46,34	157,10±38,58	-16,6±11,18	157,10±25,41	151,60±24,59	-5,5±19,36
HDL-C (mg/dL)	40,70±7,02	45,90±4,77	5,20±4,52	45,70±10,06	47,50±12,17	1,80±4,39
TG (mg/dL)	176,50 (84-308)	148,50 (104-294)	-37,70±37,65	161,00 (97-427)	158,00 (105-367)	-20,5±35,43

Values are expressed as mean ± SD, and median (min-max). Values with asterisk (*) are significantly different from 15ml/day RBO group, p <0,05.. TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDLC, high density lipoprotein cholesterol; TG, triglyceride.

This study results showed improvement in lipid profiles, decrease in total cholesterol, LDL and triglycerides as well as increase of HDL level, which are consistent with the previous studies. In several studies previously, gamma oryzanol, phytosterols and vitamin E in rice bran oil have been reported in some previous studies to have hypocholesterolemic properties.[14,15,21] Effective dose of gamma-oryzanol itself is still unclear until now. Most earlier studies used doses of gamma-oryzanol 300 mg/day or, in the form of rice bran oil, 50 grams/day which was included in the daily diet or cooking oil.[13,17]

Research by Eady et al.[13] used 20 grams of RBO spread (118 mg of phytosterols and 30 mg of gamma-oryzanol) and showed significant improvements in lipid profiles. It showed that administration of small quantities of gamma-oryzanol still gave positive results on the lipid

profile. A study by Berger et al,[22] which compare the intervention of gamma-oryzanol 50 mg/day and 800 mg/day in the same amount of RBO (50 grams), showed decreased levels of lipid profile, however the differences were not statistically significant. Adding 18 g/day of rice bran oil (gamma-oryzanol 40 mg/dl) combined with diet for 5 weeks in diabetic significantly lowers total cholesterol and tends to decrease LDL level.[23] Earlier studies showed that a dose of 0.8-1 g/day of phytosterols can lower LDL level by 5%.[13] Related to the doses of RBO, this study found that administration 45 ml/day RBO led to greater improvements in lipid profiles. It is likely influenced by the amount of unsaponifiable fractions greater in administration of 45 ml/day. According to the data from the RBO manufacturer, there is around 33 mg gamma oryzanol in 15 ml RBO. Therefore, in this study, subjects consumed approximately 33 mg gamma oryzanol in 15 ml/day RBO group and 100 mg gamma oryzanol in 45 mg/day RBO group, which both still led to improvement in lipid profile.

Gamma-oryzanol inhibits HMG-CoA reductase enzyme which then lower the level of cholesterol in the liver, therefore, increasing hepatic LDL receptors and the uptake of LDL in the blood. The decrease of LDL level was influenced gamma-oryzanol and phytosterol contained in the rice bran oil. The two substances help to increase the expression of hepatic LDL receptors which facilitate reduction in LDL cholesterol, and increase the expression of CYP7A1 which facilitate the catabolism of cholesterol. Phytosterols and gamma-oryzanol have also been shown to inhibit the absorption of cholesterol in the body, thus increasing the fecal excretion of cholesterol. [17,24,25]

The percentage of increase of HDL level in 45 ml/day RBO group was 14%, indicating that there was clinically significant increase of positive effect eventhough not statistically significant. Many previous studies regarding administration of RBO in humans had not shown changes in HDL levels. The result of this study is different compared to Berger et al,[22] which showed no significant difference in HDL levels between the group who received low dose compare to high dose of gamma oryzanol. In this study, greater improvement of other lipid profiles indirectly lead to improvements in HDL value, although not significantly. For safety reason, subjects' body weight were measured before and after treatment. The result showed there was no significant body weight difference in both groups after 4 weeks intervention.

There were some limitations in this study, such as possible errors in recording food intake. Furthermore, the analysis and composition of rice bran oil, factor storage, packaging, and environmental factors, which may influence the effective nutrients in rice bran oil, were not included in the data. This study was only done in male subjects in one community, thus the results of this study generally only could be used in individuals with similar characteristics.

In conclusion, this study showed that consumption of rice bran oil 45 ml/day led to better improvements in lipid profiles compared to 15 ml/day. Unsaponifiables fractions of rice bran oil had a role in controlling lipid profiles in hypercholesterolemic subjects. Rice bran oil in small dose (15 ml/day) still improved lipid profiles in hypercholesterolemic males. This study is a preliminary study with a short duration of time, therefore larger sample size and a longer intervention time to observe changes in body composition and lipid profile in the hypercholesterolemic patients is needed. Moreover, to assess the RBO effect in more diverse subject is necessary.

Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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