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The study of rice bran oil emulsions beverage intervention on inflammation status among metabolic syndrome adult subjects

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Abstract. The inhibition of the oxidation reaction through dietary therapy is expected to prevent from the emergence of metabolic disorder e.g. obesity-related complications, insulin resistance, and dyslipidemia. One of the food commodities that can be utilized is rice bran due to oryzanol content. The objective of this study was to analyze the effect of intervention of rice bran oil (RBO) emulsion beverage on inflammatory biomarkers of High Sensitivity C-Reactive Protein (HsCRP) and Interleukin-6 (IL-6) among metabolic syndrome (MS) adult subjects. This study used a randomized single blind controlled trial design. Thirty five adult subjects (age 34-57 years) with MS are divided into 2 groups. Treatment group (n=18) was given RBO emulsion beverage for 4 weeks. The control group (n=17) was given placebo emulsion beverage (without RBO). Nutrition intake was assessed by 24 hours recalls method every week. HsCRP and IL-6 levels were analyzed at pre and post intervention. After 4 weeks intervention, there was a significant change of the HsCRP level (p<0.05) in both groups, while for IL-6 serum levels a significant change only in control groups. However, the level of HsCRP and IL-6 serum in the treatment group no significant difference (p>0.05). The intervention of rice bran oil seems give benefit for inflammation status on MS subject.

1. Introduction
Dietary and human activity are currently changes e.g. excessive energy intake and increased sedentary behavior (lack of physical activity) [1]. These will lead to obesity and metabolic syndrome (MS). Metabolic syndrome incident mostly was caused by insulin resistance and the presence of flux due to excess fatty acids. Inflammation was suspected to play a role in the syndrome [2]. International Diabetes Federation (IDF) recommends that central obesity criterion as the main criterion plus 2 of 4 other criteria i.e. low HDL levels, high triglycerides levels, hypertension, and high fasting blood glucose level [3].

Many studies reported that there were high prevalence of MS in several regions in Indonesia. Soewondo et al. [4] reported that prevalence MS in Jakarta using ATP criteria III of Asia at the age of 25–64 years was 28.4%. Other research in Jakarta reported that the prevalence of MS in elderly men (55–85 years) was 6.6% [5]. While in the Bogor region the prevalence of MS in adults reached 36.2%, where the proportion of men (44%) was greater than women (28.6%) [6].

People with MS conditions, their inflammation showed by the high level of the cytokine (e.g. tumor necrosis factor and interleukin-6) and also by the increasing level of acute phase reactants, e.g. C-reactive protein (CRP). High concentration of CRP is an indicator for inflammatory state and related...
with higher risk of heart disease and diabetes [7]. The presence of inflammation will result in an increase in CRP concentration up to 1000 times [8]. Otsuka et al.[9] reported that in adult men (>35 years) CRP levels are related with enhancement of metabolic syndrome risk. In addition, other studies had shown that there was an increasing of IL-6 levels in MS men[10].

The inhibition of the oxidation reaction through dietary therapy is expected to prevent from the emergence of metabolic disorder e.g. obesity-related complications, insulin resistance, and dyslipidemia[11],[12]. One of the food commodities that can be utilized is rice bran. The major bioactive components in rice bran are γ-oryzanol and ferulic acid which is an antioxidant and anti-inflammatory[13],[14]. Xu et al.[15] showed that the antioxidant activity of γ -oryzanol was higher than that of vitamin E.

The γ-oryzanol compound presented in the rice bran oil fraction has been developed in the form of a RBO emulsion beverage. It has a better acceptance compared to rice bran powder beverage. Damayanthi et al.[16] reported that rice bran oil emulsion beverage intervention lowered total cholesterol and LDL cholesterol levels in obese adult subjects significantly. The study about the effect of γ-oryzanol in inflammatory status in human subjects with metabolic syndrome is still very limited. The purpose of this study was to study the effect of RBO emulsion beverage intervention for the inflammatory biomarkers of High sensitive C-reactive protein (HsCRP) and interleukin-6 (IL-6) on metabolic syndrome subjects.

2. Method

2.1. Design, location, and time

This study used a randomized single blind controlled trial design. The productions of the RBO emulsion beverage were conducted at the Laboratory of the National Institute of Pharmaceutical Navy, Jakarta. This study was conducted in Mintohardjo Navy Hospital and Headquarters of Navy Jakarta. The analysis of inflammatory biomarkers (HsCRP and IL-6) was conducted at the Integrated Laboratory of Faculty of Medicine University Indonesia. This study was conducted from June 2015–June 2016. These research passed ethical review conducted by Ethics Committee of Faculty of Medicine, University of Indonesia Number: 870/UN2.F1/ETIK/2014.

2.2. Material and equipment

The materials used in the production of RBO emulsions beverage were RBO commercial, emulsifiers (sugas ester), flavors, low-calorie sweetener, salt, and water. For analysis of HsCRP levels used the HsCRP Human ELISA brand DRG® kit and for IL-6 levels used the Quantikine® ELISA Human IL-6 Immunoassay R & D™ brand kit.

The equipment used in the production of RBO emulsions beverage were bottle plastic, large pan, homogenizer, and scales. Equipments for sampling of the subject's blood were a syringe, blood tube, cotton, gauze and plaster. While for analysis of HsCRP and IL-6 levels were used Micro plate, micro pipette, and ELISA reader.

2.3. Research subjects

Target population was men with waist circumference ≥90 cm who worked at Mintohardjo Hospital Navy and Navy Headquarters Jakarta. Subjects were selected based on inclusion criteria: 1) aged 34–60 years old; 2) waist circumference ≥90 cm; 3) had two of the four criteria of MS, i.e. triglyceride levels≥150 mg/dL, HDL levels <40 mg/dL, blood pressure ≥130/85 mm/Hg and fasting blood glucose ≥100 mg/dL and <126 mg/dL. While the exclusion criteria were: 1) suffering from diabetes mellitus; 2) regularly taking cholesterol-lowering drugs or hypertension medications; 3) suffering from chronic illness; and 4) smoking. Subjects that met the inclusion criteria were then randomized to determine the type of treatment to be accepted for each subject.
2.4. Intervention
Subjects entered a run-in period of 1 week, and during this time the subjects were asked to limited consumption of high antioxidant food and supplements. After the run in period, subjects were gathered for blood collection, weight measurement and other data collection. Blood collection was conducted at 07.30 until 10.00 a.m. by medical personnel. Thirty five adult subjects (age 34–57 years) with MS were divided into 2 groups i.e. 18 people in the treatment group who were given RBO emulsion beverage, two bottles (± 200 ml) a day equivalent to 57.6 mg γ-oryzanol and 17 people in the control group were given placebo (without RBO) beverage in the same volume for 4 weeks. To monitor compliance, subjects are reminded to consume the product via short message service (sms) on a regular basis. After the intervention ended, subjects were asked for fasting for 8 -10 hours and then the bloods were taken for post-intervention.

2.5. Data analysis
Data analysis was conducted by using microsoft excel software. Homogeneity test was conducted on both groups to know the variance of data. The normality test used Kolmogorov-Smirnov's one-simple test. The Mann-whitney test was used to analyzed the level of education subjects. An independent pair t test was performed to test whether there are significant differences before and after the intervention. An independent t test was conducted to find out how much effect of rice bran oil emulsion beverage intervention on metabolic syndrome subject.

3. Results and discussion
3.1. Characteristics subject
Characteristics subjects are presented in table 1. The age of the subject in this study ranged from 34-57 years old. Based on independent t-test there is no difference in age between the control group and the treatment group (p>0.05). Most subjects have high school education level. There is no difference in the level of education between the control group and the treatment group (p>0.05).

Table 1. Characteristics subject.

<table>
<thead>
<tr>
<th>Variabel</th>
<th>Control group (n=17)</th>
<th>Treatment Group (n=18)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year), mean±SD</td>
<td>44.6±5.9</td>
<td>43.4±7.0</td>
<td>0.572</td>
</tr>
<tr>
<td>Education Levela</td>
<td></td>
<td></td>
<td>0.212</td>
</tr>
<tr>
<td>Elementary, n(%)</td>
<td>1(5.9)</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>High School Junior, n(%)</td>
<td>0(0%)</td>
<td>0(0%)</td>
<td></td>
</tr>
<tr>
<td>High School Senior, n(%)</td>
<td>13(76.4)</td>
<td>12(66.7)</td>
<td></td>
</tr>
<tr>
<td>Diploma, n(%)</td>
<td>2(11.8)</td>
<td>4(22.2)</td>
<td></td>
</tr>
<tr>
<td>Undergraduate, n(%)</td>
<td>1(5.9)</td>
<td>2(11.1)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²), Mean±SD</td>
<td>29.8±4.3</td>
<td>31.7±3.5</td>
<td>0.183</td>
</tr>
<tr>
<td>Waist circumference (cm), Mean±SD</td>
<td>100.9±8.4</td>
<td>105.5±7.9</td>
<td>0.105</td>
</tr>
<tr>
<td>HsCRP (mg/L),Mean±SD</td>
<td>4.26±3.09</td>
<td>6.90±4.52</td>
<td>0.053</td>
</tr>
</tbody>
</table>

Independent t-test; aMann-Whitney

The nutritional status of the subjects was determined using the body mass index (BMI) indicator. The mean BMI subjects in the control group were 29.8±4.3 kg/m² and the treatment group 31.7±3.5 kg/m², and according to WHO [17] it was included in the category of obese. There was no difference in nutritional status between the two groups (p>0.05). Emanuela et al. [18] stated that obesity is a risk factor for metabolic and cardiovascular diseases at the population level. Central obesity became a main concern which is a high risk factor for cardiovascular disease [19]. Measurement of waist circumference is one indicator in determining central obesity. All subjects in this study had waist circumference above 90 cm. There was no difference of waist circumference between the two groups.
HsCRP and IL-6 levels before the intervention were not significantly different between control and treatment groups. It means before intervention both of groups had the same characteristic.

3.2. Energy and nutrients intake
The energy and nutrients intake of the subject was shown in table 2. There was no significant difference (p>0.05) in energy and nutrients intake before and after intervention. There is no difference energy and nutrients intake during intervention period for treatment group. While for control group there was significant higher protein intake during intervention (p<0.05).

Table 2. Energy and nutrients intake before and during intervention.

<table>
<thead>
<tr>
<th></th>
<th>Control group (n=17)</th>
<th>Treatment group (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before intervention</td>
<td>During intervention</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>1462±513</td>
<td>1561±333.6</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>41.4±19.5 a</td>
<td>48.9±13.4 b</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>40.5±22.6</td>
<td>45.6±11.2</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>226.8±69.6</td>
<td>229.6±52.0</td>
</tr>
</tbody>
</table>

Figures followed by the different letters in the same row showed a significant difference for each groups (p<0.05)

3.3. The effect of RBO emulsion beverage intervention on HsCRP levels
The effect of RBO emulsion beverage intervention on HsCRP levels was presented on table 3. Average of HsCRP level on both groups before and after intervention were more than 3 mg/L, these showed a high risk to cardiovascular disease [7]. Level of HsCRP can be used as a clinical criteria for the metabolic syndrome and part from assessment risk disease cardiovascular. The presence of a high level CRP showed a persistent infection [8].

Table 3. Effect of rice bran oil emulsion beverage intervention on HsCRP levels.

<table>
<thead>
<tr>
<th>HsCRP level (mg/L)</th>
<th>Control group (n=17)</th>
<th>Treatment group (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>4.26±3.09 a</td>
<td>6.90±4.52 a</td>
</tr>
<tr>
<td>After</td>
<td>3.12±2.32 b</td>
<td>4.87±3.36 b</td>
</tr>
<tr>
<td>Delta</td>
<td>-1.14±2.00</td>
<td>-2.03±3.75</td>
</tr>
</tbody>
</table>

Values followed by the different letters in the same column showed a significant difference for each groups (p<0.05)

There was no significant difference (p>0.05) of HsCRP level between control and treatment groups at before intervention. After intervention HsCRP level on both group showed a decreased significantly (p<0.05). When inflammation occurs, there was increasing of oxidative stress caused immune cells produce free radicals [2]. The rice bran oil contains the bioactive component γ-oryzanol which has high antioxidant activity. Damayanthi's study showed that the administration of 31.45 mg γ-oryzanol significantly decreased oxidized-LDL in vitro [20].

3.4. The effect of RBO emulsion beverage intervention on Interleukin-6 levels
Interleukin-6 levels ranged from 0.971-9.952 pg/mL. After intervention, levels of IL-6 were decreased in both groups. IL-6 is a mediator produced by macrophage cells, dendritic cells, endothelium or Th2 cells in an inflammatory reaction that serves as a signal between cells to regulate systemic responses [21]. Tanaka and Kishimoto mentioned that IL-6 production related with inflammation on disease chronic [22]. MS is a chronic disease, with decreased IL-6 due to interventions indicating a tendency for improvement in the state of inflammation. But there was no significant difference of IL-6 between control and treatment group after intervention. Other studies reported that in 4 months intervention of
γ-oryzanol combined with niacin significantly decreases TNFα levels [23]. TNFα is one of cytokines that induce the release of IL-6 from several cell types [24].

| Table 4. Effect of rice bran oil emulsion beverage intervention on Interleukin-6 level. |
|----------------------------------|----------------------------------|
| Interleukin-6 level (pg/mL)      | Control group (n=17)             | Treatment group (n=18)          |
| Before                           | 3.287±1.457                     | 3.437±1.914                     |
| After                            | 2.617±1.386                     | 2.503±0.778                     |
| Delta                            | -0.670±1.174 *                  | -0.934±1.999 *                  |

Figures followed by the same letters showed no significant difference (p>0.05)

4. Conclusion
There was no significant differences characteristics subject for age, education, nutritional status, circumference waist, energy and nutrients intake except for protein between treatment and control groups. These intervention has not been showed improvement on HsCRP and IL-6 state due to the length of time of intervention.

References
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