

## The Effect of Duration of Ramadan Fasting on Food Intake, Lipid Profiles, and Pro-inflammatory Cytokines (TNF- $\alpha$ and IL-6) in Overweight Male Subjects in Malang, Indonesia

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**Abstract :** This study aims to investigate the duration of Ramadan fasting on the daily intake of total foods, body mass index(BMI), plasma lipid profiles (TG, TC, HDL and LDL) and inflammatory markers (TNF  $\alpha$  and IL-6) of overweight male subjects. The research method used in this study is an experimental research method with one group pre-test-post-test design. The respondent for this research is Indonesian male between 20 to 30 years old, do not have a history of diabetes and of hypertension, not an athlete and having body mass index of 25-30 kg/m<sup>2</sup>. The number of Respondents is 23 men. Observations of the respondents were done on 7 day before fasting, 14 days of and 21 days of Ramadan fasting. Parameters measured were food intake, anthropometric, lipid profiles (TG, TC, HDL and LDL), and inflammatory markers (IL6 and TNF- $\alpha$ ). Data of food intake, anthropometric and lipid profiles was analyzed statistically using repeated Anova. Data of TNF- $\alpha$  and IL-6 was analyzed using paired t-test. The correlation between the parameters is done using Spearman Rank method. The results showed that the fasting of Ramadan is able to decrease the food intake and BMI significantly. Total Cholesterol and LDL were significant different after 14 days of Ramadan fasting. The TG, HDL, TNF- $\alpha$  and IL-6 were not significant different.

**Keywords:** Ramadan fasting, Food Intake, BMI, lipid profile, pro-inflammatory cytokines.

### Introduction

Obesity remains a global health problem in both developed and developing countries. According to WHO (World Health Organization), until the 20th century, overweight is common, and become a global epidemic problem. Currently, 1.6 billion adults in worldwide has a problem of overweight, and at least 400 million of them are obese<sup>1</sup>. By 2015, an estimated 2.3 billion adults will be overweight and 700 million are obese<sup>1</sup>. Incidence of obesity in developed countries such as in the countries of Europe, America, and Australia has reached epidemic levels<sup>1</sup>.

In Indonesia, the prevalence of obesity also shows a figure quite alarming<sup>2</sup>. Based on SUSENAS 2004, the prevalence of obesity in children has reached 11%. Based on data from the Health Research<sup>2</sup> in 2007, the national prevalence of obesity is common in people aged  $\geq 15$  years were 10.3% consisted of 13.9% males and 23.8% of women, while the prevalence of overweight in children 6-14 years of age in males 9.5% and 6.4% in

women<sup>2</sup>. This figure is almost the same as the WHO estimates by 10% in children aged 15-17 years. According to the Indonesian Ministry of Health<sup>3</sup>, 9.8 million people from 210 million (4.7%) of people in Indonesia are obese, where 23.0% in men and 43.0% in women in the age group 40-49 years in 12 major cities in Indonesia with increasing age, changes occur in the body, decreasing metabolism, and increased fat in the body that cause an increase in the prevalence of cardiovascular diseases such as coronary heart disease, hypertension and stroke, causing fat to accumulate in the blood vessel walls<sup>3</sup>. Overweight or obesity is a medical condition marked by excess body which causes health adverse effect<sup>4</sup>. Excessive food intake, physical inactivity, and genetic susceptibility simultaneously trigger obesity. Obese people have a slow metabolism, so they are only able to eat a little food<sup>5</sup>. Obesity is indicated by the body mass index (BMI) over 30 kg / m<sup>2</sup>. Obesity triggers various diseases, particularly heart disease, type 2 diabetes, obstructive sleep apnea, cancer, osteoarthritis and asthma<sup>5</sup>. However, not only the diet and physical activity is a major factor of obesity, but also influenced by genes, endocrine disorders<sup>5</sup>, obesity is a cause of death was preventable, although the prevalence of adults and children increases<sup>6</sup>. Obesity is generally a major disease in the modern world (especially in the western world). an unhealthy lifestyle which showed that reduces the high fat diet, regular exercise for 30 minutes every day to maintain a healthy weight and not smoking be a long-term effort of the whole community of physicians, patients, and government policies relating to reducing the risk of atherosclerosis<sup>7</sup>.

Fasting gives the digestive chance to rest, improve the regeneration of cells of the gastrointestinal tract instruments, as well as reducing the workload of digestion. Those are occurred due to a diet change from three meals a day to two times and reducing habits of snacks consumption during fasting<sup>7</sup>. Furthermore, fasting can reduce blood sugar levels, triglyceride and blood pressure and accidental change of low density lipoprotein (LDL), without the addition of HDL and increasing human growth hormone response (HGH) which protects muscle and metabolic balance<sup>8,9,10</sup>. When fasting, a change in diet of three meals a day to two times. When not fasting, some people often eat snacks that contain lots of triglyceride. So that by fasting, we can reduce the habit. But of course, these benefits will be achieved when we apply a good diet when breaking and dawn<sup>7</sup>.

## Methods

### Subject and Treatments

Subjects for this study were male aged 19-29 years, BMI 25-30 kg/m<sup>2</sup>, do not have a history of diabetes, do not have hypertension and not an athlete. Male is used as subjects because they can do full time of Ramadan fasting.

The protocol of the research have already approved by Ethical Committee Faculty of Medicine of Universitas Brawijaya (311 / EC / KEPK / 05 / 2015).

At a day before and 14<sup>th</sup> day of Ramadan fasting, blood sampling was taken to be analysed of total cholesterol (TC), high-density lipoprotein (HDL), low density lipoprotein (LDL), Triglyceride (TG), and inflammatory indicators (TNF- $\alpha$  and IL-6). During the fasting, subjects are asked to record their food intake of breakfast and dinner. End Measurement was performed at 21<sup>th</sup> day of Ramadan fasting. At this measurement, blood was taken to be analysed of TC, HDL, LDL, TG and pro-inflammatory cytokines (TNF- $\alpha$  and IL-6).

### Food Intake

Subjects are asked to record their food intake of breakfast, lunch, dinner and other foods for a week before Ramadan fasting. During the fasting, the subjects are asked to record their food intake of breakfast and dinner.

Food intake has been assessed by trained research staff from Nutrition Study Programme Medical Faculty Brawijaya University using the 24 h repeated food recall combined with a 2-days food record. Dietary assessment was performed using questionnaire and visual aids (models of food and food photos) directly to the participant. The 24 h repeated food recall combined with a 2-days food record were collected from all participants before fasting, at 14<sup>th</sup> and at 21<sup>st</sup> Ramadan fasting. Data is processed using Nutrisurvey<sup>11</sup>.

## Physical and metabolic data

Participants completed an anthropometric measurement (body weight, height and BMI) by trained staff from Nutrition Program Medical Faculty. General physical exam and medical history were collected by care provider in Medical Faculty of Brawijaya University.

## Lipid Profile Analysis

Lipid profiles of blood were analyzed using CHOD-PAP method for Total Cholesterol (TC) and HDL Cholesterol; and GPO-PAP method for triglycerides (TG)<sup>12</sup>.

## Pro-inflammatory cytokines

Pro-inflammatory cytokines (IL-6 and TNF- $\alpha$ ) were measured using commercial available Enzyme-linked Immunosorbent Assay (ELISA)<sup>13</sup>.

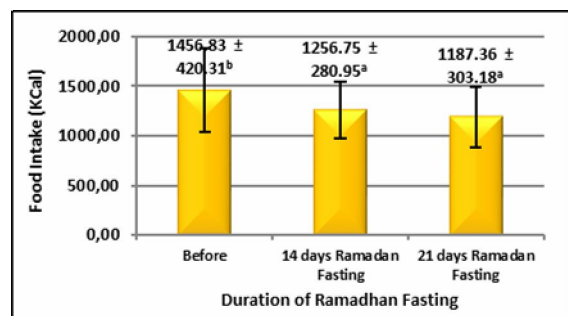
## Statistical Analysis

The data of food intake, body mass index, and plasma lipids (TC, TG, HDL and LDL) was analyzed using repeated ANOVA followed by LSD test. The data of pro-inflammatory cytokines (IL-6 and TNF- $\alpha$ ) was analyzed using paired t-test. The correlation between pro-inflammatory cytokines and other parameter observed was analyzed using Rank Spearman Correlation.

## Result and Discussion

### Food Intake

The food intake before Ramadan fasting there is difference significant with. The food intake during 14 days and 21 days of Ramadan fasting. The result of testing difference with LSD showed the average of food intake 21 days of Ramadan fasting is lower and significantly different than food intake before Ramadan fasting. But not significantly different with food intake 14 days of Ramadan fasting.

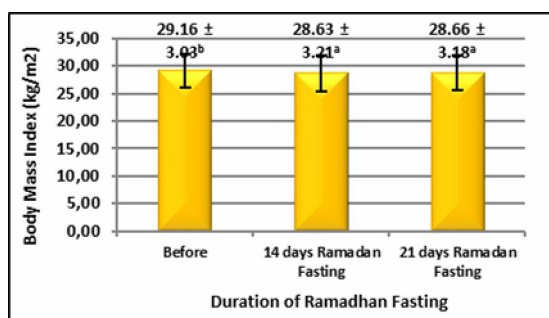


**Figure 1. The Average of Food Intake Before, 14 days and 21 days of Ramadan Fasting**

In this study, decreasing food intake could be attributed to the changing of eating patterns from 3 times to 2 times a day. An overweight subject who has fasting eat fewer calories after 14 days and 21 days of Ramadan fasting<sup>14</sup>.

### Body Mass Index

Figure 2 shows that the BMI before fasting Ramadan is higher than and significantly different with body mass index at 14 days and at 21 days of Ramadan fasting.

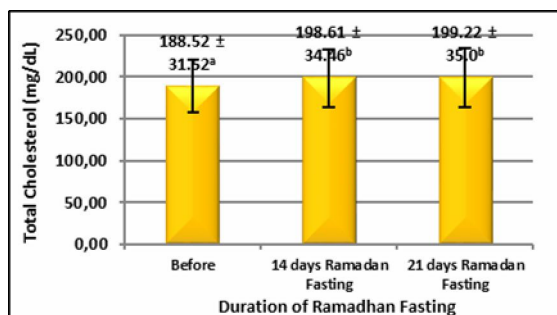


**Figure 2. The Average of Body Mass Index Before, 14 days, and 21 days of Ramadan Fasting**

A decrease in the amount of intake of food or drink between dawn and break their time; patients also control or limit the amount or type of food intake of the night after breaking; also due to the restriction activity of the fasting causes weight loss<sup>15</sup>.

### **Total Cholesterol (TC)**

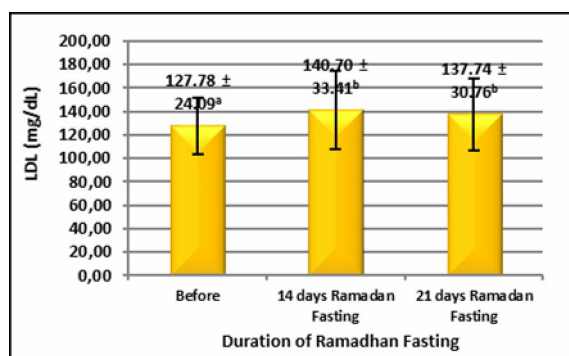
The total cholesterol before, 14 days of Ramadan fasting, and 21 days of Ramadan fasting was significant difference. The average of total cholesterol before fasting Ramadan is lower than and significantly different with total cholesterol 14 days and 21 days of Ramadan fasting. Total cholesterol after fasting Ramadan is the highest and significantly different with before fasting. Than total cholesterol before fasting Ramadan significantly different with total cholesterol 21 days of Ramadan fasting. This result is consistent with research conducted by Barkia, et al<sup>16</sup>. His study informed that Ramadan fasting could significant increasing total cholesterol. Total cholesterol of overweight subjects who have Ramadan fasting increase significantly. Then Nematy, et al.<sup>17</sup>, in their research show Ramadan fasting significantly improves total cholesterol, and Khaled et al.<sup>14</sup>, also showed that there was increase total cholesterol significantly in overweight.



**Figure 3. The Average of Total cholesterol Before, 14 days of Ramadan fasting, and 21 days of Ramadan fasting**

### **Low Density Lipoprotein (LDL)**

The LDL before, 14 days of Ramadan fasting and 21 days of Ramadan fasting was significant difference. There are significant different between before, 14 days of Ramadan fasting and 21 days of Ramadan fasting. The LDL of 21 days of Ramadan fasting is higher and significantly different with LDL before Ramadan fasting, but not significantly different with LDL 14 days of Ramadan fasting.

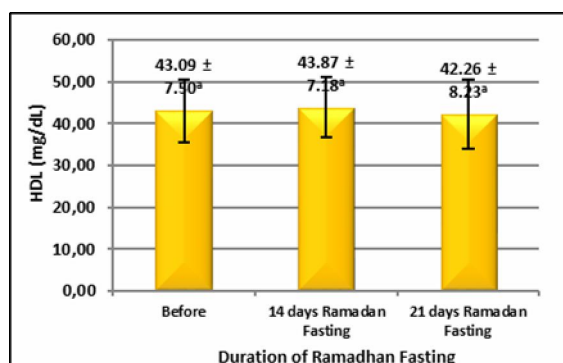


**Figure 4. The Average of LDL Before, 14 days of Ramadan fasting, and 14 days of Ramadan fasting**

Fasting decreases body weight, thus adipose tissue become shrink. This makes the fat and cholesterol stored in fatty tissues have no place to go into the bloodstream, thereby causing an increase in cholesterol. Because of decreasing Body weight, adipose tissue become shrink thus the fat and cholesterol that normally stored in fatty tissue have no-where to go but the bloodstream, then IT causing a rise in cholesterol. This effect is not permanent and cholesterol levels will drop as the weight stabilizes. Medications used to treat high cholesterol, such as Z-hydroxy-Z-Coareductase inhibitors, are not effective in controlling cholesterol when it comes from fatty tissue stores<sup>7,18</sup>.

### High Density Lipoprotein(LDL)

Statistical difference test with repeated ANOVA showed there was no significant difference HDL before Ramadan fasting, 14 days of Ramadan fasting, and 21 days of Ramadan fasting.



**Figure 5. The Average of HDL Before, 14 days, and 21 days of Ramadan fasting**

The absence of differences in levels of HDL cholesterol can be explained by the mechanism of Reverse Cholesterol Transport, HDL cholesterol is released as tiny particles of poor cholesterol, containing Apo A, C, and E, and so-called nascent HDL. Nascent HDL comes from the small intestine and liver, flattened shape and containing Apo A-1. Nascent HDL will approach the macrophages to take up cholesterol from macrophages, nascent HDL turn into mature HDL cholesterol, which is round<sup>19</sup>.

In addition, no significant decrease in HDL is probably caused by the respondents who do not do much activity. The food consumed by respondents are foods with unsaturated fats, such as vegetable oil.

### Triglyceride (TC)

Figure 6 shows that triglyceride before Ramadan fasting, 14 days of Ramadan fasting, and 21 days of Ramadan fasting are not significantly different.

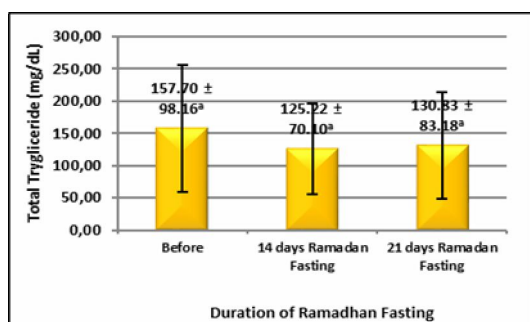


Figure 6. The Average of Triglyceride Before, 14 days, and 21 days of Ramadan fasting

The effect of Ramadan fasting on lipid profile, vary in many studies, possibly due to changes in diet and reduced activity. Ziaee et al.<sup>4</sup> found that there is no difference in levels of triglycerides (TG) were significant before and after Ramadan despite TG levels decreased of Ramadan. This condition is thought to result from the consumption of a diet that is high in carbohydrates, especially sugars. Another cause is a change in the pattern of consumption of complex carbohydrates, such as cereals, fruit and vegetables, into simple carbohydrates such as sugary drinks or with artificial sweeteners of Ramadan<sup>7</sup>.

The carbohydrates are a source of energy for the first time in the use of energy by the body, but the amount of carbohydrate reserves stored by the body which is usually only a few hundred grams, especially in the form of glycogen in the liver and muscles<sup>20</sup>. This reserve can provide the energy needed for body functions perhaps only for a half day. Therefore, there will be a progressive shrinkage of adipose tissue, resulting in free fatty acid levels in plasma increases during fasting and heavy exercise that shows fatty acid needs enormous as an energy source<sup>20</sup>. This state is achieved by hydrolyzing TG back into fatty acids and glycerol, then both of these compounds are transported to active tissues where both can be oxidized to produce energy<sup>20</sup>.

### Inflammatory Marker

Figure 7 and Figure 8 show that there was no significant difference in TNF- $\alpha$  and IL-6 before and 21 days of Ramadan fasting.

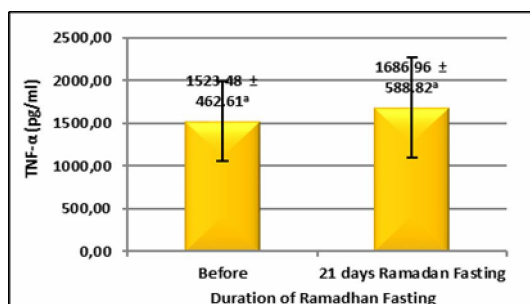


Figure 7. The Average of TNF- $\alpha$  Before and 21 days of Ramadan fasting

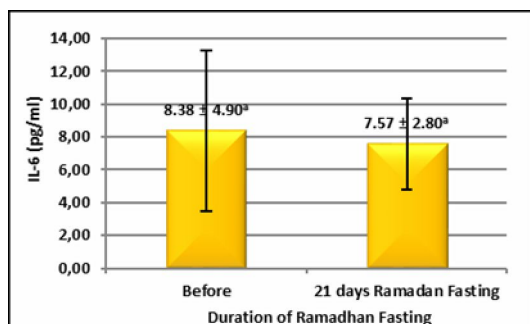


Figure 8. The Average of IL-6 Before and 21 Days Of Ramadan Fasting

Adipose tissue is fatty tissue that reserves the fat ready for use in the body as energy and as an endocrine organ<sup>21</sup>. Adipose cells actively produce several hormones and cytokines, such as Adiponectin, Leptin, Angiotensin, Resitin, PAI-1, TNF- $\alpha$ , and IL-6<sup>21</sup>. Thus adipose cells play a role in the development of insulin resistance and obesity. Cytokine, IL-6 and TNF- $\alpha$  than as an inflammatory reaction in the body's defence mechanisms also have an important role as a hormone in the metabolism of glucose and lipids. In this study, Ramadan fasting did not change IL-6 and TNF- $\alpha$  significantly. This is because BMI is quite low (less than 30), so that the body cannot decrease the adipose tissue is not much, causing IL-6 and TNF- $\alpha$  produced also becomes insignificant<sup>21</sup>.

### Correlation between Food Intake and BMI and Pro-inflammatory Cytokines (TNF- $\alpha$ and IL-6)

The correlation between food intake and BMI and the Pro-inflammatory Cytokines is presented in Table 1. Although the cytokines are most affected by BMI and food intake (Faris *et al.*, 2012), this study showed no correlation between food intake and TNF- $\alpha$  and IL-6 and BMI and TNF- $\alpha$  and IL-6.

**Table 1. Correlation between Food Intake and BMI and TNF- $\alpha$  and IL-6**

|               | Food Intake       |       | BMI               |       |
|---------------|-------------------|-------|-------------------|-------|
|               | Spearman<br>$r_s$ | P     | Spearman<br>$r_s$ | P     |
| TNF- $\alpha$ | 0.020             | 0.896 | -0.148            | 0.327 |
| IL-6          | 0.532             | 0.176 | -0.067            | 0.657 |

No significant correlation between food intake with inflammatory markers, inflammatory markers and BMI with the BMI because most of the subjects is quite low, below 30 kg / m<sup>2</sup>, precisely between 23-24.9 kg / m<sup>2</sup>, so that duration of fasting for 21 days cannot decrease the adipose tissue<sup>21</sup>.

In conclusion, this study showed food intake and BMI of the overweight subjects significantly decreased after 21 days of Ramadan fasting. Total cholesterol and LDL increase significantly. The TG, HDL, TNF- $\alpha$  and IL-6 of the subjects were not significant difference after 21 days of fasting.

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