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## THE EFFECT OF PROCESSED TEMPEH GEMBUS TO HIGH SENSITIVITY C-REACTIVE PROTEIN (hsCRP) AND HIGH-DENSITY LIPOPROTEIN (HDL) LEVELS IN WOMEN WITH OBESITY

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### ABSTRACT

Obesity causes chronic inflammatory reaction is characterized by elevated levels of high sensitivity c-reactive protein (hsCRP). HsCRP and HDL could be used as an early marker of cardiovascular disease risk. Tempeh gembus contain fiber, unsaturated fatty acids and antioxidants, which can reduce the inflammatory reaction. This study determines the effect of processed Tempeh gembus on hsCRP and HDL in obese women. This study included in experimental studies with randomized post-test only control group design involving 40 obese women aged 20 – 50 years. Subjects were randomized into two groups: a control group was given a standard diet low in calories 30 calories/kg body weight, and the treatment group was given a standard diet low in calories 30 calories/kg body weight and Tempeh gembus for 28 days. hsCRP and HDL levels were measured before and after the intervention, food intake was measured by using a 3 x 24-hour recall and physical activity (IPAQ form). HsCRP levels were measured using the ELISA method, whereas HDL levels were measured using the CHOD-PAP method. Wilcoxon test (hsCRP levels) and paired *t*-test (HDL levels) used to test differences before and after intervention each group. Mann Whitney test (hsCRP levels) and independent sample test (HDL levels) used to test differences before and after intervention between groups. There are differences in hsCRP levels before and after the intervention in the control group ( $p = 0.00$ ) and the treatment group ( $p = 0.00$ ). There are differences in HDL levels before and after the intervention in the control group ( $p = 0.00$ ) and the treatment group ( $p = 0.00$ ). There are differences in the decrease hsCRP levels between the two groups ( $p = 0.00$ ). There are differences in the increase in HDL levels between the two groups ( $p = 0.03$ ). Tempeh gembus 150 grams/day can decrease hsCRP levels and increase HDL levels in women with obesity.

**Keywords:** Tempeh gembus; hsCRP; HDL; women; obesity

### INTRODUCTION

Obesity is excessive fat accumulation due to an imbalance between energy intake and energy released by the body so that it can interfere with health (WHO, 2016). Obesity is the cause of half the cases of hypertension that increases the risk of cardiovascular disease (Sizer and Whitney, 2017). Cardiovascular disease is caused by narrowing, blockage of coronary arteries and reduced elasticity of blood vessels due to atherosclerosis (Herrington et al., 2016). Atherosclerosis is a progressive disease, even estimated to have occurred since the age of 10 – 20 years with the formation of fat streaks walking slowly and continues to grow by 3% per year since past the age of 20 years. Atherosclerosis occurs due to the interaction of various risk factors, including obesity, hypertension, diabetes mellitus, smoking habits, the aging process, atherogenic dyslipidemia, and pro-inflammatory conditions (Badimon, Robert and Gemma, 2011).

Obesity can also lead to the occurrence of the reaction inflammation due to their secretion of cytokines and pro-inflammatory (Harford et al., 2011). Inflammatory reactions cause damage to endothelial function resulting in an increase in stroke volume and cardiac output. Excessive adipose tissue causing chronic inflammatory reactions due to cytokine secretion and proinflammation by adipocyte cells are characterized by increased levels of high sensitivity C-reactive protein (hsCRP), Tumor Necrosis Factor (TNF- $\alpha$ ), interferon-gamma (IFN  $\gamma$ ) and interleukin-6 (IL-6) (Guillen et al., 2008; Libby, Ridker and Maseri, 2002). hsCRP is a biomarker that is sensitive to the occurrence of inflammation in the body and is a strong predictor of the incidence of cardiovascular system disease (Tully et al., 2015). Increased levels of hsCRP in the long term indicates a process of chronic inflammation (Pravin and Devang, 2011).

Increased adipose tissue in obesity is closely related to the consumption habits of foods that are high in fat and

low in fiber. Excessive fat intake will affect adipose tissue especially visceral fat to express responses to various stimuli, one of which is an increase in the release of free fatty acids by adipose tissue which can stimulate increased secretion of very low density lipoprotein (VLDL) in the liver which in turn results in increased triglycerides, low density lipoprotein (LDL), and decreased high density lipoprotein (HDL) (Wang and Peng, 2011). Low HDL levels are not able to prevent the activation of pro-inflammatory mediators in the form of cytokines such as TNF- $\alpha$ , IL-6 and CRP. The results showed that subjects with HDL levels greater than 60 mg/dL had a lower risk of developing coronary heart disease because an increase of 1 mg/dL HDL levels could reduce the risk of coronary heart disease by 2% in men and 3% in women (Rajagopal, Suresh and Alok, 2012).

Prevention and management of cardiovascular disease can be done by optimizing the consumption of functional foods with high protein fermented foods that are suspected to be able to prevent cardiovascular disease (Bowen et al., 2018; Anand et al., 2016). *Tempeh gembus* is one of Indonesia's original food products made from fermented tofu-based fermentation that functions as a substrate then the tempeh mushroom (*Rhizopus oligosporus*) is added as a microorganism. The main content of *Tempeh gembus* is fiber. The fiber content in 100 grams of *Tempeh gembus* is 3.93 grams, three times more than the fiber content in soybean *Tempeh* (Sulchan and Endang, 2007; Li, Qiao and Lu, 2012). High-fiber diet ( $\geq 25$  grams of soluble fiber and  $\geq 47$  grams of insoluble fiber) per day can reduce the risk by up to 50% of stroke in the population (Casiglia et al., 2013). Other research shows that the *Tempeh gembus* which is processed into snacks, namely *Kerupuk Gembus* contains quite high fiber as much as 54.4 – 67.4% (Afifah et al., 2019a). The fiber content in *tempeh gembus* has anti-inflammatory, anti-carcinogenic effects, can reduce gastrointestinal transit time which is good for treating diarrhea and constipation (Gropper, Smith, and Groff, 2012). Fresh *Tempeh gembus* contains 1.87% fat, 11.09% fiber, 4.90% protein, 89.67% protein digestibility, 14.03% amino acids, 48.07% antioxidant activity, 0.05% genistein, and 0.07% daidzein (Afifah et al., 2019b). In addition *Tempeh gembus* also contains unsaturated fatty acids linoleic acid (21.51%), linolenic acid (1.82%) and oleic (16.72%) (Sulchan and Endang 2007; Sulchan and Rukmi 2007). Damanik et al. (2018) showed that the saturated fatty acid content in *Tempeh gembus* (12.55%) was higher than the saturated fatty acid in soybean (12.01%) and tofu residue (12.41%). The content of oleic acid in *Tempeh gembus* can suppress the production of pro-inflammatory cytokines. Giving *Tempeh gembus* in rats fed atherogenic diet can reduce hsCRP levels (Dewi et al., 2018). Research conducted by Noviana et al. (2018) on *Tempeh gembus* hydrolyzate which was given 5000 ppm and 8000 ppm bromelain enzymes were able to prevent the microbial activity of *S. aureus*, *B. subtilis*, and *S. mutans*. *Tempeh gembus* also contains fibrinolytic protease-producing microbes, namely *Bacillus pumilus* 2 g (AB968524). Pure fibrinolytic enzymes from *Bacillus pumilus* 2 g are included in the serine protease group of subtilin which can degrade the  $\alpha$  and  $\beta$  chains of fibrinogen quickly so that it has the potential to prevent cardiovascular (Afifah et al., 2014). *Tempeh gembus* has

also been shown to reduce oxidative stress. Giving *Tempeh* as much as 25 g.kg<sup>-1</sup> of Sprague Dawley rats for 28 days on an atherogenic diet, can reduce levels of malonaldehyde and homocysteine. A significant decrease occurred in the administration of fresh *Tempeh gembus* and *gembus gembus* which were given bromelain enzyme 25 ppm (Kurniasari et al., 2017). Giving *Tempeh gembus* with a dose of 8% and 12% for 5 weeks in experimental animals showed a decrease in levels of total cholesterol, LDL cholesterol and increased HDL (Afifah et al., 2014).

### Scientific hypothesis

We investigate several hypotheses in our study:

- Provision of processed *Tempeh gembus* affects the decrease in levels of high sensitivity c-reactive protein (hsCRP),
- Provision of processed *Tempeh gembus* has an effect on increasing levels of high density lipoprotein (HDL).

### MATERIAL AND METHODOLOGY

This study is a true experimental study using a pre-post randomized control group design (Sastroasmoro and Ismael, 2011). Subjects were divided into two groups, namely the control group and the treatment group. The independent variable of this study was the administration of 150 grams of processed *Tempeh gembus* for 28 days while the dependent variable was the levels of hsCRP and HDL. Researchers have obtained Ethical Clearance from the Ethics Commission of the Faculty of Medicine at Sultan Agung University Semarang, Indonesia with number 33/I/2019/Bioethics Commission.

The study was conducted in March 2019 on 40 female prisoners of Class II Penitentiary in the City of Semarang. Subject retrieval is done based on inclusion criteria, namely women aged 20-50 years, body mass index  $\geq 23$  kg.m<sup>-2</sup>, do not have a history or are undergoing liver disease, kidney, cancer, coronary heart disease, stroke, do not smoke, are willing to participate in this study by signing an informed consent.

Physical activity level data were obtained through direct interviews using the IPAQ form and then calculated using the Physical Activity Level (PAL) formula (WHO, 2011). Categorizing the level of physical activity is light (1.40 – 1.69 units), moderate (1.70 – 1.99 units), and heavy (2.00 – 2.40 units). Data on Body Mass Index were obtained based on measurements of body weight and height. Data on nutrient intake was obtained through direct interviews using a food recall form and then analyzed using Nutrisurvey software.

Determination of the subject of this study using consecutive sampling method, and found as many as 73 people were willing to have blood drawn for the initial screening process. There were 40 subjects who met the inclusion criteria which were then divided into 2 groups: one control group and one treatment group with each group consisting of 20 subjects. The control group was given a diet limiting the intake of 30 calories/kg body weight/day while the treatment group as many as 20 people were given a dietary intake limiting the intake of 30 calories/kg body weight/day + processed 150 grams of *Tempeh gembus*/day.





Figure 1 Tofu waste fresh.



Figure 2 Preparation of *Tempeh Gembus*.



Figure 3 Addition *Tempeh* yeast.



Figure 4 *Tempeh Gembus* has been fermented for 36 hours.



Figure 5 *Tempeh Gembus* Balado.



Figure 6 *Tempeh Gembus* Oseng.



Figure 7 *Tempeh Gembus* Bacem.



Figure 8 *Tempeh Gembus* Pepes.



Figure 9 *Tempeh Gembus* Satay.

to the nutritional value (Redman and Ravussin, 2011). Limitation of food intake is given in stages as much as 30 kcal.kg<sup>-1</sup> body weight/day through food menus provided by the correctional institution which refers to a low calorie diet for obesity by considering the gender and physical activity of the subject (Wahyuningsih, 2013). The Japan Atherosclerosis Society (JAS) recommends limiting food intake for obese subjects with mild physical activity levels of 25 – 30 kcal.kg<sup>-1</sup> body weight day, 30 – 35 kcal.kg<sup>-1</sup> body weight/day for subjects with moderate physical activity levels and >35 kcal.kg<sup>-1</sup> body weight/day for subjects with heavy levels of physical activity (Kinoshita et al., 2017). Interventions in the two groups were carried out for 28 days.

The independent variable of this study was the administration of 150 grams of processed *Tempeh gembus*. *Tempeh gembus* which will be given as treatment material is made by researchers and the team, in the Laboratory of Food Technology laboratory polytechnic health of Semarang using tofu waste obtained from tofu craftsmen

food portions while still paying attention in the Cinde-Lamper region of Semarang City, *Tempeh* yeast used is Raprima yeast. *Tempeh gembus* is processed into 5 types of cuisine, namely: bacem, satay, oseng, pepes, and balado.

The dependent variable of this study is the levels of hsCRP and HDL. HsCRP levels were measured using the Enzyme Linked Immunosorbent Assay (ELISA) method (Crowther, 2009). Whereas HDL levels were measured by laboratory workers using the Cholesterol Oxidase-Peroxidase Aminoantipyrine Phenol (CHOD-PAP) method (McPherson and Pincus, 2016). Blood samples were taken by Semarang CITO Laboratory officials twice, namely on the first day before being given an intervention and 1 day after the intervention (the 29<sup>th</sup> day).

## Statistic analysis

Data were analyzed using the version 16.0 of Statistical Package for the Social Sciences (SPSS). Differences in hsCRP levels before and after treatment were analyzed using the Wilcoxon test because the data were not

normally distributed. Differences in treatment effect between the two groups were analyzed using the Mann Whitney test. Differences in HDL levels before and after treatment were analyzed using paired *t*-test because the data were normally distributed. Differences in treatment effect between the two groups were analyzed using the independent sample test.

## RESULTS AND DISCUSSION

Subject characteristics consisting of age, level of physical activity, Body Mass Index before and after treatment are presented in Table 1. All subjects in the study were in the age group of 21–50 years. The mean age in the control group ( $35.05 \pm 8.54$  years) was lower than in the treatment group ( $36.50 \pm 9.37$  years). The Mann Whitney test showed that there was no significant difference in age between groups ( $p = 0.64$ ), so age was not a confounding variable in the study. The mean level of physical activity during the study in the control group ( $1.52 \pm 0.17$  units) was higher than the treatment group ( $1.48 \pm 0.10$  units). The Mann Whitney test showed that there was no difference in the average level of physical activity between groups ( $p = 0.84$ ), so the level of physical activity was not a confounding variable in the study. Based on WHO (2011), the level of subject activity in this study was included in the mild category (1.40 – 1.69 unit). Table 1 also shows that the average Body Mass Index (BMI) before and after the study in the control group ( $30.00 \pm 5.61$  kg.m<sup>-2</sup>;  $29.63 \pm 5.42$  kg.m<sup>-2</sup>) than the treatment group ( $28.22 \pm 2.49$  kg.m<sup>-2</sup>;  $27.32 \pm 2.50$  kg.m<sup>-2</sup>). The Mann Whitney test showed that there was no difference in mean Body Mass Index before ( $p = 0.51$ ) and after research ( $p = 0.16$ ), confounding in the study.

Table 2 shows data on energy, protein, fat and carbohydrate intake patterns before the study. The mean energy intake before the study in the control group ( $2306.96 \pm 545.24$  kcal) was higher than in the treatment group ( $2057.70 \pm 241.06$  kcal), the Mann Whitney test showed that there were significant differences in energy intake between groups ( $p = 0.01$ ) so that energy intake before the study becomes confounding variable. The mean protein intake before the study in the control group ( $84.86 \pm 19.08$  grams) was higher than the treatment group ( $72.59 \pm 19.16$  grams), the independent sample test showed that there were significant differences in energy intake between groups ( $p = 0.04$ ) so that protein intake before it can become confounding variables. The mean fat intake before the study in the control group ( $86.8 \pm 31.84$  grams) was higher than the treatment group ( $60.57 \pm 18.75$  grams), the independent sample test showed that there were significant differences in energy intake between groups ( $p = 0.00$ ) so that fat intake before the study can be a confounding variable. The mean carbohydrate intake in the control group ( $296.79 \pm 77.45$  gram) was higher than the treatment group ( $276.42 \pm 35.50$  gram), but the independent sample test showed no difference in carbohydrate intake before the inter-group study ( $p = 0.29$ ) so that carbohydrate intake before the study did not become a confounding variable.

Nutrient intake data subject control and treatment groups during the study are presented in Table 3. Table 3 Based on average energy intake in the control group ( $1924.35 \pm 218.62$  kcal) is higher than that of the treatment

group ( $1883.81 \pm 187.89$  kcal), independent sample test showed that there were no significant differences in energy intake between groups ( $p = 0.53$ ) so that energy intake was not a confounding variable in the study. The mean protein intake in the control group ( $p = 0.53$ ) was higher than the treatment group ( $96.21 \pm 10.93$  gram), the independent sample test showed no significant differences in protein intake between groups ( $p = 0.53$ ) so that protein intake is not a confounding variable in the study. The mean fat intake in the control group ( $42.76 \pm 4.85$  grams) was higher than the treatment group ( $41.86 \pm 4.17$  grams), the independent sample test showed no significant difference in fat intake between groups ( $p = 0.53$ ) so that fat intake does not become a confounding variable in the study. The mean carbohydrate intake in the control group ( $288.611 \pm 32.75$  grams) was higher than the treatment group ( $282.54 \pm 28.16$  grams), the independent sample test showed no significant difference in carbohydrate intake between groups ( $p = 0.53$ ) so that carbohydrate intake is not a confounding variable in the study.

Table 4 shows the levels of hsCRP before and after the intervention. In the control group, the mean hsCRP level before the intervention was  $7.31 \pm 0.75$  mg.L<sup>-1</sup> whereas after the intervention the mean hsCRP level became  $5.65 \pm 0.88$  mg.L<sup>-1</sup>. In the treatment group, the mean hsCRP level before intervention was  $5.63 \pm 1.23$  mg.L<sup>-1</sup>, whereas after the intervention was  $3.69 \pm 1.35$  mg.L<sup>-1</sup>. There was a significant difference between the mean levels of hsCRP before and after the intervention in the two groups ( $p = 0.00$ ;  $p = 0.00$ ). The mean hsCRP level before the intervention in the control group ( $7.31 \pm 0.75$  mg.L<sup>-1</sup>) was higher than the treatment group ( $5.63 \pm 1.23$  mg.L<sup>-1</sup>), there was a significant difference to the average hsCRP level before the intervention between the two group ( $p = 0.00$ ). The mean hsCRP level after intervention in the control group ( $5.65 \pm 0.88$  mg.L<sup>-1</sup>) was higher than the treatment group ( $3.69 \pm 1.35$  mg.L<sup>-1</sup>), there was a significant difference to the average hsCRP level after the intervention between the two group ( $p = 0.00$ ). There was a significant difference in the decrease in hsCRP levels after the intervention in both groups ( $p = 0.03$ ).

In this study, the mean hsCRP level in the treatment group decreased by  $1.94 \pm 0.29$  mg.L<sup>-1</sup>. This shows that giving as much as 150 grams of *Tempeh gembus* per day for 28 days is effective in reducing levels of hsCRP. The decrease in hsCRP levels in the treatment group can be caused by the presence of fiber in *Tempeh gembus*. Low fiber intake can increase proinflammatory cytokines IL-6, TNF- $\alpha$ , and IL-18. Increasing IL-6 can consistently increase CRP levels. High fiber intake can reduce fat oxidation resulting in decreased inflammation. Fiber is a protective factor to counter increasing CRP levels (Ma et al., 2006). Other studies have shown that fiber in fermented soybeans can reduce cholesterol levels so that it contributes positively to the anti-inflammatory effect. The fiber in fermented soybean consists of several monosaccharides including glucose, arabinose, galactose, and uronic acid which are components of cellulose and non-cellulose polysaccharides. The main non-cellulose polysaccharide from soybean fiber is arabinogalactan. The positive effect on the anti-inflammatory effect can be seen significantly in decreasing the levels of C-Reactive Protein (Kim et al., 2014).



Besides fiber, *Tempeh gembus* also contains antioxidants in the form of isoflavones (daidzein and genistein) and unsaturated fatty acids (oleic, linoleic and linolenic fatty acids) which include essential fatty acids (Sulchan and Endang 2007; Sulchan and Rukmi 2007). The anti-inflammatory mechanism by isoflavones is carried out by inhibiting the NF- $\kappa$ B transcription system and modulating arachidonic acid (AA) metabolism and Nitric Oxide (NO) production by inhibiting protein levels and the activity of proinflammatory enzymes (phospholipase A2 (PLA2), lipoxygenase (LOX), COX-2, and iNOS) (Jie et al., 2016). This study is in line with other studies that show that interventions with soy-based foods can reduce high sensitivity levels of C-Reactive Protein by 25% (Kone, 2014). Apart from isoflavones, antioxidant activity *Tempeh gembus* probably derived from amino acids/peptides bioactive. *Tempeh gembus* containing amino acids such as tyrosine, methionine, histidine, lysine, cysteine and tryptophan. Activity of antioxidants in soybean gembus with ABTS method was  $63.14 \pm 1.16\%$  (Agustina et al., 2018).

Table 5 shows data on HDL levels before and after the intervention. In the control group, the mean HDL level before the intervention was  $29.25 \pm 5.05$  mg.dL<sup>-1</sup> whereas after the intervention the mean HDL level was  $35.45 \pm 3.79$  mg.dL<sup>-1</sup>. In the treatment group, the mean HDL level before the intervention was  $32.50 \pm 5.62$  mg.dL<sup>-1</sup> whereas after the intervention the average HDL level was  $41.90 \pm 2.73$  mg.dL<sup>-1</sup>. There was a significant difference between the mean HDL levels before and after the intervention in the two groups ( $p = 0.00$ ;  $p = 0.00$ ). The mean HDL levels before the intervention in the control group ( $29.25 \pm 5.05$  mg.dL<sup>-1</sup>) were lower than the treatment group ( $32.50 \pm 5.62$  mg.dL<sup>-1</sup>), there were no significant differences in the mean HDL levels before the intervention between both groups ( $p = 0.06$ ). The mean HDL levels after the intervention in the control group ( $35.45 \pm 3.79$  mg.dL<sup>-1</sup>) were lower than the treatment group ( $41.90 \pm 2.73$  mg.dL<sup>-1</sup>), there were significant differences in the mean HDL after the intervention between the two groups ( $p = 0.00$ ). There was a significant difference in the increase in HDL levels after the intervention in both groups ( $p = 0.00$ ). In this study, the mean HDL levels in the treatment group increased by  $9.40 \pm 4.48$  mg.dL<sup>-1</sup>. The provision of 150 grams of processed *Tempeh gembus* for 28 days is effective in increasing HDL levels in the treatment group.

The main content of *Tempeh gembus* is fiber. High fiber intake can increase the excretion of bile acids and cholesterol through feces thereby reducing bile acids to get back into the liver. The reduction of bile acids to the liver causes an increase in the use of cholesterol to bile acids so that it has an effect on increasing HDL (Buse, Kenneth and Harles, 2017). Besides fiber, the increase in HDL levels in the treatment group was due to the presence of flavonoids in *Tempeh gembus*. Flavonoids can increase the amount of Apolipoprotein A-1. Apolipoprotein A-1 acts as an enzyme cofactor for LCAT and as a ligand of interaction with lipoprotein receptors in tissues in HDL. An increase in Apolipoprotein A-1 is expected to increase HDL levels (Gropper, Smith, and Groff, 2012). According to the American Association of Clinical Endocrinologists (AACE, 2012) HDL levels of

60 mg.dL<sup>-1</sup> can reduce the risk of coronary heart disease (Jellinger et al., 2012). However, in this study there were no respondents with HDL levels reaching 60 mg.dL<sup>-1</sup> after the intervention. Increased levels of HDL that do not reach optimal values are caused by several factors, one of which is exercise (Whitney and Sharon, 2015). Other studies have shown that adult women who exercise regularly, such as aerobic exercise three times a week, can experience increased levels of HDL (Wang and Peng, 2011). WHO states that aerobic exercise performed by adults aged 18-64 years with moderate intensity for 150 minutes/week or high intensity for 75 minutes/week can increase HDL levels and be beneficial for heart health (WHO, 2011). Regular exercise can improve the work function of Apolipoprotein A-1 as an HDL receptor in reducing cholesterol from blood vessel walls (Kingwell and Michael, 2013). In this study, respondents only did aerobics once a week so this might be the cause of HDL cholesterol levels not reaching optimal values. In addition to lack of exercise, respondents' physical activity is also included in the mild category. The types of activities most frequently carried out by respondents were sleeping, watching TV, sweeping, washing clothes, cooking and making batik.

Changes in HDL and hsCRP levels also occurred in the control group. This is presumably due to the provision of restriction of food intake through a low calories diet of 30 calories per kg body weight/day in the control group. Based on the results of the study, it is known that the control group's diet before the intervention was 2306.96 kcal while during the intervention it decreased to 1924.35 kcal.

Changes in calorie intake resulted in an increase in HDL levels of  $6.20 \pm 2.35$  mg.dL<sup>-1</sup>, from  $29.25 \pm 5.05$  mg.dL<sup>-1</sup> to  $35.45 \pm 3.79$  mg.dL<sup>-1</sup>. Foods that have been consumed will undergo metabolic processes and produce energy in the form of adenosine triphosphate (ATP) to carry out physical activity (Rodwell et al., 2018). When doing physical activity, energy needs will increase, so if glucose as the main energy source is insufficient there will be an increase in fat metabolism. This causes a decrease in body fat percentage and an increase in HDL cholesterol (Whitney and Sharon, 2015). Research on obese subjects shows that food intake restrictions affect changes in lipid profile, including increased levels of HDL (Fothergill et al., 2016). Food intake restrictions are also able to protect against age-related diseases including inflammation by reducing oxidative stress (Omodei and Luigi, 2011; Rebrin, Michael and Rajindal, 2011).

Interventions in the form of limiting food intake in the control group also resulted in a decrease in hsCRP levels of  $1.65 \pm 0.57$  mg.L<sup>-1</sup>, from  $7.31 \pm 0.75$  mg.L<sup>-1</sup> to  $5.65 \pm 0.88$  mg.L<sup>-1</sup>. A decrease in subject's hsCRP levels can be caused by a decrease in fat intake. It is known that the mean fat intake in the control group before the intervention is  $86.58 \pm 31.84$  grams to  $42.76 \pm 4.85$  grams after the intervention. Fat intake is excessive will affect visceral fat to express a response to various stimuli one of them is an increase in spending of free fatty acids by adipose tissue that can stimulate increased secretion of VLDL in the liver which in turn will result in an increase in triglycerides, LDL and decrease HDL (Gropper, Smith and Groff, 2012; Wang and Peng, 2011). This increase

will trigger the release of HDL from the liver to carry cholesterol in the circulation (reverse cholesterol by hepatic lipase, thus reducing circulating HDL levels. As the result the reverse cholesterol transport process is

**Table 1** Respondent Characteristics.

Respondent Characteristics		Control Group			Treatment Group			p
		Mean $\pm$ SD	Median	Min-Max	Mean $\pm$ SD	Median	Min – Max	
Age (years)		35.05 $\pm$ 8.54	34.00	21 – 50	36.50 $\pm$ 9.37	39.00	21 – 48	0.64*
Body Mass Index (kg.m <sup>-2</sup> )	Before	30.00 $\pm$ 5.61	28.40	25.10 – 47.67	28.22 $\pm$ 2.49	27.73	25.08 – 33.23	0.51*
Body Mass Index (kg.m <sup>-2</sup> )	After	29.63 $\pm$ 5.42	27.99	25.00 – 47.03	27.32 $\pm$ 2.50	27.14	24.15 – 32.47	0.16*
Level of physical activity (unit)	During	1.52 $\pm$ 0.17	1.42	1.40 – 1.79	1.48 $\pm$ 0.10	1.43	1.40 – 1.73	0.84*

Note: \* Mann Whitney test.

**Table 2** Diet Before Research.

Respondent Characteristics		Control Group			Treatment Group			p
		Mean $\pm$ SD	Median	Min – Max	Mean $\pm$ SD	Median	Min-Max	
Energy (kcal)		2306.96 $\pm$ 545.24	2284.65	916.40 – 3328.10	2057.70 $\pm$ 241.06	1954.68	1823.6 – 2714.50	0.01*
Protein(grams)		84.86 $\pm$ 19.08	87.90	45.90 – 118.40	72.59 $\pm$ 19.16	71.65	39.50 – 111.30	0.04**
Fat (grams)		86.58 $\pm$ 31.84	82.55	26.90 – 131.30	60.57 $\pm$ 18.75	59.41	30.80 – 109.00	0.00**
Carbohydrate (grams)		296.79 $\pm$ 77.45	289.90	122.00 – 451.10	276.42 $\pm$ 35.50	274.33	216.80 – 364.70	0.29**

Note: \* Mann Whitney test; \*\* independent sample test.

**Table 3** Nutrition Intake During Research.

Respondent Characteristics		Control Group			Treatment Group			p
		Mean $\pm$ SD	Median	Min – Max	Mean $\pm$ SD	Median	Min – Max	
Energy (kcal)		1924.35 $\pm$ 218.62	1955.07	1456.65 – 2457.00	1883.81 $\pm$ 187.89	1895.40	1512.81 – 2299.05	0.53**
Protein(grams)		96.21 $\pm$ 10.93	97.75	72.83 – 122.85	94.19 $\pm$ 9.39	94.77	75.64 – 114.95	0.53**
Fat (grams)		42.76 $\pm$ 4.85	43.45	32.37 – 54.60	41.86 $\pm$ 4.17	42.12	33.62 – 51.09	0.53**
Carbohydrate (grams)		288.61 $\pm$ 32.75	293.26	218.50 – 368.55	282.54 $\pm$ 28.16	284.31	226.92 – 344.86	0.53**
Tempeh		0.00 $\pm$ 0.00	0.00	0.00 – 0.00	61.37 $\pm$ 19.05	56.88	35.88 – 94.90	0.00**
Gembus (%)								

Note: \*\* independent sample test.

**Table 4** hsCRP levels before and after the intervention.

hsCRP levels (mg.L <sup>-1</sup> )	Control Group (n = 20)			Treatment Group (n = 20)			p
	Mean $\pm$ SD	Median	Min – Max	Mean $\pm$ SD	Median	Min – Max	
Pre Intervention	7.31 $\pm$ 0.75	7.15	6.20 – 8.90	5.63 $\pm$ 1.23	5.35	4.40 – 9.30	0.00*
Post Intervention	5.65 $\pm$ 0.88	5.60	4.40 – 7.30	3.69 $\pm$ 1.35	3.40	2.40 – 7.70	0.00*
$\Delta$	1.65 $\pm$ 0.57	1.55	2.90 – 0.20	1.94 $\pm$ 0.29	2.00	2.40 – 1.00	0.03*
p		0.00***			0.00***		

Note : p-value <0.05 = significant; \* Mann Whitney test; \*\*\* Wilcoxon.

**Table 5** HDL levels before and after the intervention.

HDL levels (mg/dL)	Control Group (n = 20)			Treatment Group (n = 20)			p
	Mean $\pm$ SD	Median	Min – Max	Mean $\pm$ SD	Median	Min – Max	
Pre Intervention	29.25 $\pm$ 5.05	29.00	20.00 – 38.00	32.50 $\pm$ 5.62	34.00	21.00 – 39.00	0.06**
Post Intervention	35.45 $\pm$ 3.79	36.00	27.00 – 42.00	41.90 $\pm$ 2.73	42.00	36.00 – 47.00	0.00**
$\Delta$	6.20 $\pm$ 2.35	6.00	2.00 – 10.00	9.40 $\pm$ 4.48	8.00	2.00 – 20.00	0.00**
P		0.00****			0.00****		

Note p-value <0.05 = significant; \*\* independent sample test; \*\*\*\* paired t test.

transport).

HDL is esterified into cholesterol esters which can be directly carried to the liver to be directly excreted or exchanged with triglycerides from VLDL and chylomicrons. When cholesterol esters are excessive, triglyceride-rich HDL (low density HDL) is broken down

reduced and cholesterol levels in the circulation and tissue increase (Murray, Granner and Rodwell, 2017). In one condition, an increase in excess cholesterol levels in the circulation causes an abnormal reaction that causes the activation of the scavenger macrophage. This macrophage is responsible for cleaning cholesterol and low-density

HDL from circulation by phagocytosis. Macrophages that are full of cholesterol will then become foam cells that cause activation of pro-inflammatory cytokines (IL-1, IL-6, and TNF  $\alpha$ ). Activation of pro-inflammatory cytokines is an early sign of inflammation. Continued inflammation will cause CRP expenditure from the liver (Pitsavos et al., 2006). Other studies have shown that low fat intake can inhibit cytokine release and reduce levels of hsCRP in the blood (Camhi et al., 2010). This is due to adipose tissue reducing the expenditure of free fatty acids causing a decrease in total cholesterol, LDL, triglyceride levels and an increase in HDL that affect macrophages, thereby impacting on decreasing hsCRP levels (Murray, Granner and Rodwell, 2017).

Intervention in the form of limiting long-term food intake has a very strong protective effect on the risk of atherosclerotic cardiovascular disease (CVD) as evidenced by a decrease in blood pressure, LDL cholesterol, hsCRP, IL-6, TNF- $\alpha$ , and an increase in HDL cholesterol levels (Dolinsky and Dyck, 2011). Restricted food intake has been shown to improve mitochondrial function, reduce oxidative stress and increase nitric oxide production involved in the prevention of atherosclerosis, reduction in blood pressure, weakening of left ventricular hypertrophy, resistance to myocardial ischemic injury and prevention of heart failure (Weiss and Luigi, 2011).

## CONCLUSION

The administration of processed *Tempeh gembus* for 28 days can reduce high sensitivity c-reactive protein (hsCRP) by 1.93 mg.L<sup>-1</sup> and increase HDL levels by 9.40 mg.dL<sup>-1</sup> in obese women in penitentiary class II Semarang city, Indonesia. Changes in hsCRP and HDL levels also occurred in the control group. This is thought to be due to a standard diet of 30 calories/kg body weight/day for 28 days.

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# THE EFFECT OF PROCESSED TEMPEH GEMBUS TO HIGH SENSITIVITY C-REACTIVE PROTEIN (hsCRP) AND HIGH-DENSITY LIPOPROTEIN (HDL) LEVELS IN WOMEN WITH OBESITY

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