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Soy Germ Protein With or Without-Zn Improve Plasma Lipid Profile in Metabolic Syndrome Women

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The aim of this research was to determine the effect of soy germ protein on lipid profile of metabolic syndrome (MetS) patients. Respondents were 30 women with criteria, i.e. blood glucose level > normal, body mass index > 25 kg/m^2, hypertriglyceridemia, low cholesterol-HDL level, 40-65 years old, living in Purwokerto, and signed the informed consent. The project was approved by the ethics committee of the Medical Faculty from Gadjah Mada University-Yogyakarta. Respondents were divided into three randomly chosen groups consisting of ten women each. The first, second, and third groups were treated, respectively, with milk enriched soy germ protein plus Zn, milk enriched soy germ protein (without Zn), and placebo for two months. Blood samples were taken at baseline, one and two months after observation. Two months after observation the groups consuming milk enriched with soy germ protein, both with or without Zn, had their level of cholesterol-total decrease from 215.8 to 180.2 mg/dl (P = 0.03), triglyceride from 240.2 to 162.5 mg/dl (P = 0.02), and LDL from 154.01 to 93.85 mg/dl (P = 0.03). In contrast, HDL increased from 38.91 to 49.49 mg/dl (P = 0.0008). In conclusion, soy germ protein can improve lipid profile, thus it can inhibit atherosclerosis incident.

Key words: soy germ protein, cholesterol total, triglyceride, HDL, LDL, metabolic syndrome

INTRODUCTION

Metabolic syndrome (MetS) is a group of disease symptoms that arise as the effects of metabolic disorders. Hill *et al*. (2003) reported that MetS patients were dominated by women. MetS is charracterized by obesity that is mainly accompanied with two or more of the following four risk factors, i.e. type-2 diabetes mellitus (T-2DM), hypertriglyceridemic, low level HDL-cholesterol, and high blood pressure. While obesity is known to be one of the trigger factors for hypercholesterolemic, hypertriglyceridemic, low level HDL-Cholesterol, and high LDL-Cholesterol level, it is believed that in obesity condition, the adipose tissue and macrophage are infiltrated by inflammatory cytokine, and, as a result, fat is accumulated in the adipose while cholesterol is accumulated in the macrophage. The obesity condition causes excessive endoplasmic reticulum damage due to oxidative stress (Furukawa *et al.* 2004). This is due to the structural changes and the increase of fat and cholesterol synthesis of the adipose.

High level of LDL-Cholesterol might disturb the metabolism and, if it is not recovered immediately, this lipoprotein compound will stay on the wall of blood vein and might generate cholesterol plaque. Sooner or later, the plaque will become thicker, tear the arterial wall layer, and, finally, thrombus will be produce which will cause a disturbance in the arterial blood stream. Continuous thrombus formation will totally plug the coronary blood vein and, in turn, stop the oxygen stream into the heart muscle. This can result in infarction myocardial and eventually a heart attack (Arsana *et al.* 2007). In addition, the high level of HDL-Cholesterol has the possibility to infiltrate the intima tunica, oxidize the first level and form LDL-oxidized (LDL-ox). The LDL-ox that is formed induces the formation of adhesive compounds and patches that attract the monocytes to penetrate the endhotelial layer into the tunica intima. Consequently, the high level of LDL-Cholesterol leads to the constriction and the clog of the blood stream, also called atherosclerosis. Finally, the heart will havedifficulty in pumping blood which is followed by the appearance of sudden heart attack symptoms.

The function of HDL-Cholesterol is to extract excess cholesterol deposites in the vein walls and return it to the liver which will eliminate it through the digestive tract (Staels & Fonseca 2009). Consequently, a high cholesterol-HDL level is very useful in lowering the atherosclerosis risk. Asztalos *et al*. (2000) said that for every increase of 1 unit of cholesterol-HDL the risk of coronary heart disease decreases by 2-3%. Therefore, the HDL level needs to be increased.

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Researchers reported that soy protein is able to improve the lipid profile (Anderson *et al.* 1995; Winarsi 2007). It was also reported that soy germs contain higher protein levels than soy seeds (Winarsi *et al*. 2010). However, data about the potential of soy germ protein in improving the profile of metabolic syndrome in women is not available yet.

The aim of thise research was to explore the potential of soy germ protein in decreasing total cholesterol, triglycerides, LDL, and increasing HDL on metabolic syndrome in women patients.

MATERIALS AND METHODS

This research involved 30 metabolic syndrome women fitting the following criterias: BMI > 25 kg/m^2, high blood glucose level in time (> normal), high blood pressure, elevated triglycerides, low levels of high density lipoprotein, age range of 40-65 years, living in Purwokerto, and willing to sign the informed consent. The research was approved by the ethics committee from the Medical Faculty of Gadjah Mada University-Yogyakarta. They were divided into 3 groups of 10 women (Table 1). Group I was treated with milk enriched soy germ protein plus Zn, group II was treated with milk enriched soy germ protein (without Zn), and group III was the placebo (Table 2) for the period of two months with 25 g/d. Throughout the research, recalls of food consumption was performed for 8 times. Their blood samples were taken 3 times, i.e. baseline, and continued with 1 and 2 months after observation. Two ml of blood was collected in venoject-tubes containing EDTA, intravenously, and finally the total cholesterol level, triglycerides, HDL, and LDL was determined.

Determination of the Total Cholesterol Level (CHOD-PAP Method). Blood sample was centrifuged at 2000 rpm for 20 minutes to obtain the plasma. As much as 1000 µl of reagent was used as the blank. Plasma of 10 µl was added in 1000 µl of cholesterol reagent Dyasis®, and then incubated for 10 minutes. The sample was read after the blank was read using photometer 5010 in 546 nm wavelength.

Determination of the Triglycerides Level (glycerol-3-phosphateoxidase (GPO) method). An amount of 10 µl blood sample was added to 1000 µl of reagent. 1000 µl of reagent was used as the blank. Each solution was homogenized and incubated for 20 minutes at 20-25 ^{o}C, or 10 minutes at 37 ^{o}C. The absorption of each sample was read in 500 nm wavelength. Triglycerides level was then calculated with the following formula:

$$C = \frac{A_{sample}}{A_{standard}} \times C_{st}$$

C = Triglycerides level, A = Absorbances, C_{st} = Triglycerides standard level (200 mg/dl).

Determination of the Cholesterol-HDL Level (Burstein Method). 0.1 ml of plasma was added to a reaction tube containing 0.25 ml of cholesterol-HDL reagent, and homogenized. A sample of the solution was incubated for 10 minutes at room temperature, and centrifuged in 2000 rpm for 10 minutes. 100 µl of the supernatant was reacted with 1000 µl of reagent. The sample was read in 546 nm wavelength.

Determination of the LDL-Cholesterol Level (Freidewald Method) (Astawan *et al*. 2005). Cholesterol-LDL level measurement was carried out by using the following formula:

Cholesterol-LDL level = Total cholesterol –
(Triglyceride / 5) – cholesterol-HDL

Statistical Analysis. The data obtained was presented as an average ± standard error. All of the data was analyzed by using an analysis of variance (Anova). Pearson's correlation coefficient was used to determine the correlation between variables, if there was significantly difference ($P < 0.05$).

Table1. Profile of metabolic syndrome women

Profile	A	B	C	P=
Age (Year)	50.2 ± 2.0	51.5 ± 2.3	49.5 ± 2.3	0.81
BMI (kg/m^2)	31.0 ± 1.1	31.2 ± 0.7	33.3 ± 0.9	0.21
Blood glucose level (mg/dl)	221.2 ± 15.36	213.7 ± 6.8	223.3 ± 8.83	0.81
Triglyceride level (mg/dl)	236.4 ± 37.9	204 ± 22.8	212 ± 30.4	0.74
HDL level (mg/dl)	45.19 ± 3	48.73 ± 2	45.87 ± 2.3	0.61
Blood pressure				
Sistole (mmHg)	156 ± 9	149 ± 8	154 ± 6	0.81
Diastole (mmHg)	106 ± 2	108 ± 7	102 ± 4	0.71

A: milk enriched soy germ protein plus Zn, B: milk enriched soy germ protein without Zn, C: placebo, n: 10, data presented in average ± SEM.

Table 2. The chemistry composition of milk rich soy germ protein*

Component	Milk enriched soy germ protein + Zn	Milk enriched soy germ protein	Placebo
Water (%)	4.79 ± 0.08	4.6 ± 0.008	4.5 ± 0.003
Ash (%)	22.68 ± 0.07	22.6 ± 0.09	22.4 ± 0.006
Fat (%)	1.11 ± 0.01	1.02 ± 0.003	1.04 ± 0.003
Protein (%)	42 ± 0.05	39.5 ± 0.09	36.7 ± 0.037
Carb (%) by difference	29.39 ± 0.04	32.27 ± 0.02	35.03 ± 0.32
Total (%)	100 ± 0.005	100 ± 0.03	100 ± 0.03

*data presented in 3 repeated, average ± SEM, Winarsi and Purwanto (2010).

RESULTS

The baseline time of each treatment group showed no difference in data, i.e. ages (P = 0.55), BMI (P = 0.1), level of triglycerides (P = 0.83), HDL (P = 0.4), and blood pressure (P = 0.64). It means that at the initial time, the subjects' conditions were homogenous. It could be concluded that the change that exist after observation was the effect of giving the supplement to the subjects.

The Total Cholesterol Level. In baseline time, the total cholesterol level was 216.7 ± 68 mg/dl, but it did not show the difference among treatment groups (P = 0.69). One month after observation, their cholesterol level did not change (P = 0.9), but two months later the level had decreased from 215.8 to180.2 mg/dl (P = 0.03). In fact, in the group consuming milk enriched with soy germ protein without Zn, the level had also significantly decreased from 215.8 to 182.6 mg/dl (P = 0.04). Therefore, the cholesterol level in a group consuming milk enriched with soy germ protein plus Zn was not different from the group consuming it without Zn (P = 0.89) (Figure 1).

The Triglyceride Level. At baseline time the average level of triglyceride was 208.05 ± 26.64 mg/dl, and the levels were not different among treatment groups (P = 0.74). Triglyceride is the lipid found in blood circulation, so it is also called lipid. One month after observation, the level of triglyceride did not change (P = 0.73), but two months later, their level had decreased from 240.2 to 162.5 mg/dl (P = 0.02) in the group consuming milk enriched with soy germ protein plus Zn, while in the group consuming milk enriched with soy germ protein without Zn, the level decreased from 244.7 to 176.2 mg/dl (P = 0.03) (Figure 2). Both of the groups did not have different triglyceride levels (P = 0.35).

The HDL Level. Initially, the HDL levels of MetS patients was 47.3 ± 2.4 mg/dl, and was not different among the treatment groups (P = 0.61). This was also true for one month after observation. However, two months after observation started, the level increased from 38.91 to 49.49 mg/dl (P = 0.0008) in the group consuming milk enriched with soy germ protein plus Zn, while in the group consuming it but without Zn, the level increased from 38.91 to 43.41 mg/dl (P = 0.03) (Figure 3). Thus, the Zn enrichment did not influence the HDL level.

The LDL-Cholesterol Level. The average LDL-Cholesterol level in the baseline time was 159.6 ± 8.06 mg/dl, and was not different among the treatment groups (P = 0.64). One month later, the LDL level did not change (P = 0.74). However, two month after, the level decreased from 154.01 to 93.85 mg/dl (P = 0.03) in the group consuming milk enriched with soy germ protein plus Zn, while, in the group consuming milk enriched with soy germ protein without Zn, the level decreased from 154.01 to 115.44 mg/dl (P = 0.04). Both of the groups did not show any difference in terms of LDL-Cholesterol level (P = 0.64) (Figure 4).

In this study, a recall of food consumption was also conducted. However, the results yield no difference between the groups for both dietary protein intake (P = 0.14) and the calories (P = 0.17). Thus, their food intake had no effect on their lipid profile.

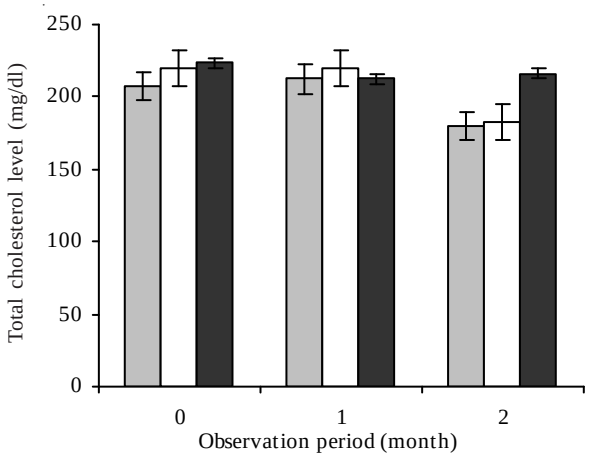

Figure 1. Total cholesterol level in metabolic syndrome women. ▫: milk enriched soy germ protein +Zn, □: milk enriched soy germ protein, ■: placebo.

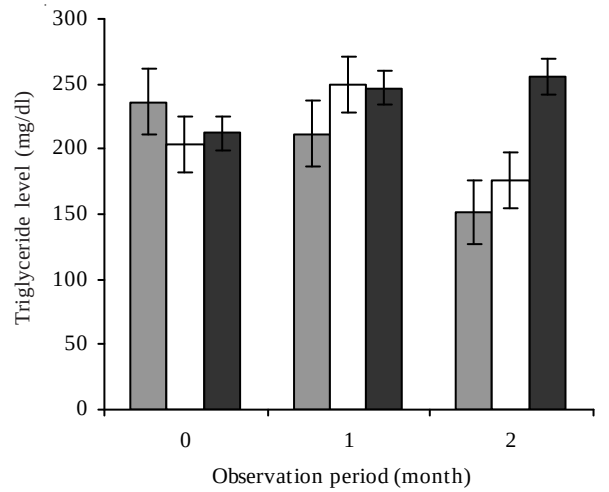

Figure 2. Triglyceride level in metabolic syndrome women. ▫: milk enriched soy germ protein +Zn, □: milk enriched soy germ protein, ■: placebo.

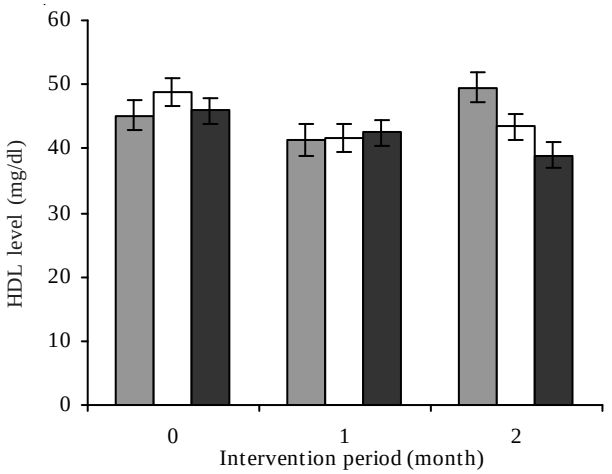

Figure 3. HDL level in metabolic syndrome women. ▫: milk enriched soy germ protein +Zn, □: milk enriched soy germ protein, ■: placebo.

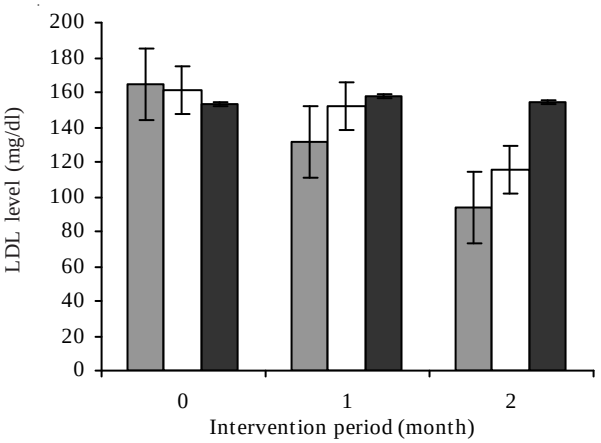

Figure 4. LDL level in metabolic syndrome women. ▣: milk enriched soy germ protein +Zn, □: milk enriched soy germ protein, ■: placebo.

DISCUSSION

Facts stated that in subjects that were identified as hypercholesterolemia and obese, with a BMI level of 32.28 kg/m^2, there was a big risk of diabetes. The results showed that their blood glucose level in baseline time was more than the normal level allowed. Consequently, these subjects experienced diabetes, particularly type-2 diabetes mellitus (T2-DM). They were characterized as insulin resistant conditions. Even-though the insulin level was not analyzed, in insulant resistant conditions, the hormones do not have the ability to push the glucose into cells to perform metabolism, and the glucose produced is accumulated in blood. The excess of glucose in the blood is changed into glycerol-3-phosphate and fatty acids at the same time, and used to synthesis triglyceride. Triglyceride, then, transports hepatic very low density lipoprotein (VLDL) into extra hepatics tissues, is lysed by lipoprotein lipase to become the intermediate density lipoproteins (IDL) (high triglycerides and protein is lost), and is changed to LDL. Therefore, the increase of cholesterol tissue is shown in the blood level.

This finding supported a research by Potter *et al.* (1998) in which consuming 50 g/d soy protein had resulted in a decrease of total cholesterol level for subjects with hypercholesterolemia. The same result was also reported in hypercholesterolemia rabbits or monkey (Anthony *et al.* 1996; Anthony *et al.* 1998). Soy protein has been known to suppress cholesterol and bile acids absorption in the small intestine. However, they also induced the excretion of bile acids and fecal steroids giving theliver more opportunity to change the cholesterol in the body into bile salt, This phenomenon affected the decrease of total cholesterol in the body. Winarsi *et al.* (2010) reported that protein content in soy germ was higher than that of in soy non germ. Consequently, the soy germ protein's ability to decrease the total cholesterol in the body was higher (15.27%) than soy non germ protein (11-12%).

Kanetro *et al*. (2008) added that soy germ protein, which is enriched with amino acids such as arginine and leusin, have potential in inducing insulin secretion. Winarsi and Purwanto (2010) also said that insulin level of type T-2DM was significantly increased after consuming soy germ protein, with or without Zn, for two months. As a result, the decrease of cholesterol level was correlated with the increase of insulin level, whose activity was also assumed to increase. The glucose was not accumulated in blood, but it was pushed into cells and transformed into energy in the metabolic process. The same condition was also found in lipid synthesis where glucose was not changed into fat, including cholesterol, which resulted in an inhibition of triglyceride breaking. It can be concluded that soy germ protein, with or without Zn, could decrease the cholesterol level. In other words, Zn did not affect the cholesterol level of metabolic syndrome patients.

Triglyceride. Fat from diet and the body are made from triglycerides. A high level of triglyceride is often correlated with the occurrence of heart disease. Consequently, subjects with metabolic syndrome are considered a risk for high triglycerides levels. Higher levels of triglycerides might cause excess body weight or obesity because cholesterol in the blood originates from the fat in a diet or synthesized from other macro nutrients, like carbohydrate. Unused calories from a diet is directly changed into triglycerides, and saved in adipose tissues.

Krajcovicova-Kudlackova *et al*. (2005) claimed that soy protein contains higher amounts of leusin than other chained branch amino acids. Leusin is a substrate of protein synthesis that has a role in maintaining glucose and insulin homeostasis. Body weight is regulated by the potential change in body composition, including non-fat mass, and the stabilization of blood fasting or postprandial glucose levels. Besides the eight essential amino acids, soy protein also contains non-essential amino acids in higher levels than that of chicken eggs. These amino acids induce the release of glucagon hormone, which is responsible for breaking enzymes that save fat, increasing fat metabolism (fat burning), and decreasing cholesterol synthesis. The amount of non-essential amino acids in soy germ protein is assumed higher than that of soy protein since the amount of protein in soy germ is higher than that of soy. Thus, the potential of suppressing cholesterol is also high.

Cholesterol is a result of triglyceride breaking. Thus, a decrease in cholesterol level also suppresses the level of triglyceride. This finding has been reported in many previous researches, and it is believed that the decrease of triglyceride plasma level correlates with anti-atherogenicity of the HDL, particularly in the case of cholesterol transport.

Increasing HDL levels with milk enriched with soy germ protein supplements improves the regulation of lipid metabolish which may be correlated with the potential of the isoflavone binding protein found in the milk. Song *et al*. (2003) said that soy isoflavone showed its capability when it is mixed with protein. This finding supported the opinion of Yousef *et al*. (2004) who mentioned that white guinea pigs treated with 5 mg/kg body weight of soy isoflavones for 13 weeks had increased HDL level as much

as 39%. This was the same with results from a study by Anderson *et al.* (1995) whose clinically tested subjects had a 2% increase in HDL levels when treated with soy protein.

Isoflavones, found in milk enriched with soy germ protein, has an estrogen-like characteristic. This phytoestrogen reduces lipase hepatic activity, but induces apolipoprotein A-1 (protein compound in lipoprotein) production. Apolipoprotein A-1 is a protein active with lecithin cholesterol acyltransferase (LCAT), an enzyme that changes free cholesterol to ester cholesterol (Miller 2003). Consequently, a higher level of esterified cholesterol will progressively increase HDL levels.

Similar opinion could be found in Supriyanto (2004), in which estrogen-like soy isoflavones induced apolipoprotein A-1 production, and contributed in increasing HDL levels. Initially, HDL was released as a HDL *nascent* containing apolipoprotein (apo) A, C, and E. Next, the HDL *nascent* blocked the macrophage and let cholesterols save them. Finally, the HDL nascent changed into adult HDL. That finding was also supported by the macrophage level, although it was not significant, recording a monocyte decrease from 1.25 10^3 to 1.01 10^3/ul ($P = 0.04$). The number of monocytes was greater than the normal range, thus the decrease was needed. The cholesterol saving the macrophage was also fewer, which meant more HDL was formed. HDL-cholesterol is good cholesterol. It cleans the blood vessels from the excess of LDL-cholesterol. Higher HDL-cholesterol level supports LDL-cholesterol level lower than 150 mg/dl, which is a good marker to inhibit atherosclerosis.

In high level of LDL, corked and clothed vessels are found in blood deposits. Lower LDL levels achieved using soy germ protein supplements might be correllated with the antioxidative potential of soy isoflavones. Soy and its products function as the source of isoflavone. Winarsi *et al*. (2010) suggested that soy germ protein isolate contained larger amounts of isoflavones than ordinary soy protein isolate.

Jenkins *et al*. (2002) added that consumption of soy isoflavones (168 mg/d) could decrease the risk of cardiovascular occurance through degradation of the LDL-OX. This was also supported by a statement from Yousef *et al*. (2004) that stated that white guinea pigs which were treated with 5 mg/kg body weight of soy isoflavones for 13 weeks had a 22% decrease in LDL-cholesterol levels. Zhan and Ho (2005) conducted 23 clinical tests from 1995 until 2002 and concluded that isoflavones of soy protein decreased LDL levels by up to 5.25%. Consequently, the decreasing LDL level of metabolic syndrome patients may prove the antioxidative potential of isoflavones in soy germ protein. Lower levels of LDL may cause a decrease in VLDL hepatic secretion. VLDL, which has a high risk oxidation and is very atherogenic, can suppress triglycerides levels (fewer triglyceride was hydrolized by lipase hepatic enzyme, resulting in a much lower VLDL level) and prevent the formation of LDL-OX. Decreasing LDL level by soy germ protein enriched with isoflavones may inhibit atherosclerosis development.

It can be concluded that milk enriched soy germ protein, with or without Zn, improved the lipid profile of women with metabolic syndrome. It is suggested that consuming soy germ protein regularly might prevent the development of metabolic syndrome.

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