

Soy Germed Protein Plus Zn as an Inducer Insulin Secretion on Type-2 Diabetes Mellitus

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Hyperglycemic induces pancreatic cells to produce inadequate insulin. However, previous studies revealed that soy protein induce pancreatic cells to secrete insulin. Hence, this study was aimed to investigate effect of soy germed protein on the insulin and blood glucose level of type-2 diabetes mellitus with Zn enrichment. The research involved twenty four women that characterized with having more blood glucose level than normal, body mass index more than twenty three kg/m², and age more than forty years old. They were divided into three groups randomly, eight woman for each group. The first, second and third group were treated respectively with milk containing soy germed protein plus Zn, this product without Zn, and placebo, all for two months. Blood samples were taken at baseline, one and two months after observation. Results showed that two months after observation the insulin level increased from 194.79 to 519.82 pmol/ml (P = 0.033) in group consuming milk containing soy germed protein with or without Zn, supported by significantly reduced blood glucose level. This result might be correlated with the potency of isoflavones in soy germ protein to protect pancreatic beta cells membrane from free radicals attack. Therefore, this maintain the cells integrity and to secrete optimal insulin.

Key words: insulin, soy germed protein, type-2 diabetes mellitus, Zn

INTRODUCTION

Hyperglycemic condition induces beta cells to produce interleukine-1 beta and C-reactive protein (Larsen *et al.* 2007; Winarsi & Purwanto 2009), followed by lower cell function (Maedler *et al.* 2004; Welsh *et al.* 2005). It is proved that inflammatory cytokine islet plays a role in type-2 diabetes pathogenesis, with resulting in pancreatic incapability to produce adequate insulin to compensate insulin resistance condition.

Researchers reported that soy protein could control blood glucose level (Hermansen *et al.* 2001; del Carmen Crespillo *et al.* 2003) by inducing the pancreatic cells to hypertrophy, and then influenced insulin secretion. Winarsi *et al.* (2009) added that the protein content of soy germed was higher (42%) than soy non-germed (36.5%). The soy germed protein may be more potency on pancreatic cell than soy non-germed. Daidzein and glycitein were important isoflavone compounds in soy germed protein (Song *et al.* 2003; Winarsi *et al.* 2009). In general soy isoflavone had been proved as antioxidant and immunostimulator (Winarsi *et al.* 2005a,b). According to that finding, the isoflavone of soy germed play a role to protect beta cells integrity and induces their action.

Winarsi *et al.* (2005a) also stated that woman above 40 years old had lower of Zn status. The Zn status correlate with beta cells capabillity to secrete insulin. Chausmer (1998) postulated that supplementation of Zn

could improve insulin sensitivity and suppress complication diseases. Therefore, Zn is needed by type-2 diabetes patient. In this study, we investigated the effect of soy germed protein milk enrich with Zn on the level of plasma insulin and blood glucose of type-2 diabetes patient.

MATERIALS AND METHODS

Subjects were 24 women from Diabetic Clinic of the Margono Soekarjo General Hospital, Purwokerto in 2009, characterized by blood glucose in time level above normal (> 200 mg/dl), the Body Mass Index more than 23 kg/m², and the age of more than 40 years old, and live in Purwokerto. All participants had to sign the informed consent. All subjects were divided into three randomly groups, each group consisted of eight women (Table 1). First group was treated with milk containing soy germed protein plus Zn, second group was given milk containing soy germed protein without Zn, and third group was given milk without soy germed protein or Zn (placebo) (Table 2), for the period of two months with 25 g/d. Blood samples were taken three times, at baseline, and then continued by 1 and 2 months after observation. As much as 3 ml blood samples were collected in tubes with ethylene diaminetetraacetic acids (EDTA), intravenously.

Part of the whole collected blood was analysed for glucose level using Glucocard Tes Strip II. Blood was centrifuged at 3000 rpm for 10 minutes. Subsequently two different layers were formed; the above layer was the plasma separated from the second layer (erythrocyte).

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Table 1. Characteristics (mean and SE) of type-2 diabetic women at baseline

	A (n = 8)	B (n = 8)	C (n = 8)
Age (years)	57.88 ± 1.97	59 ± 2.42	61.18 ± 3.04
BMI (kg/m ²)	25.57 ± 0.97	25.62 ± 1.03	25.88 ± 0.93
Blood glucose (mg/dl)	313 ± 36.97	324 ± 50.14	320 ± 40.4

A = group consuming soy germed protein+Zn; B = group consuming soy germed protein without Zn; C = group consuming placebo; n = 8; mean ± SEM.

Table 2. Chemical compositions (mean ± SE) of supplement given to subjects

	Milk containing		Placebo
	soy germed protein+Zn	Milk containing soy germed protein	
Water (%)*	4.79 ± 0.08	4.6 ± 0.008	4.5 ± 0.003
Ash (%)*	22.68 ± 0.07	22.57 ± 0.09	22.4 ± 0.006
Fat(%)*	1.11 ± 0.01	1.02 ± 0.003	1.04 ± 0.003
Protein (%)*	42 ± 0.05	39.47 ± 0.09	36.72 ± 0.037
Carbohydrate (%)*	29.39 ± 0.04	32.27 ± 0.02	35.03 ± 0.32
Total (%)	100 ± 0.005	100 ± 0.03	100 ± 0.03

*3 replications.

Determining the Plasma Insulin Level. The insulin level was determined using an Elisa Diagnostic Automation, Inc. INSULIN Microplate ELISA Cat. No. 1606. Briefly, 25 µl plasma sample, controls and reference were loaded into assigned wells. One hundred µl enzyme was conjugated into each well and mixed for 5 seconds, incubated for 30 minutes at 25 °C. Incubation mixture was removed and the wells were rinsed five times with washing buffer. One hundred µl solution buffer containing hydrogen peroxide and the 100 µl tetramethylbenzidine were dispensed into each well, then was incubated for 15 minutes at room temperature. The reaction was stopped by 50 µl stop solution and the optical density was determined at 450 nm with a microwell reader.

Determining Blood Glucose Level. Blood glucose level was determined using an Glucocard Tes Strip II, GT-1620, Arkray, Inc. 57 Nishi Aketa-CHO, Higashi-Kujo, Japan. A drop of blood was collected by using a lancing device, and was touch the blood to the tip of a test strip. The test strip draw blood into the reaction chamber automatically, and start the test measure.

Statistical Analysis. Data collected were analyzed statistically using Analysis of Variance (ANOVA).

RESULTS

Plasma Insulin Level of Type-2 Diabetes Mellitus.

At baseline time, the insulin range level was 249.68-307.02 pmol/ml, that were not significantly different between groups ($P = 0.79$). After 1 month of observation, the insulin level was also not different ($P = 0.34$), but at 2 months after observation, the insulin level increased from 194.79 to 519.82 pmol/ml ($P = 0.033$) in group consuming milk containing soy germed protein plus Zn (Figure 1), however it was not different with the consuming milk containing soy germed protein without Zn ($P = 0.08$).

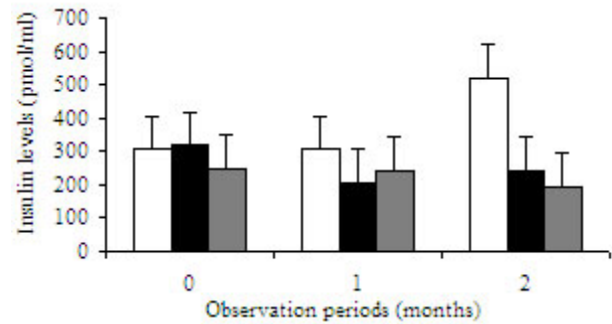


Figure 1. Insulin levels of type-2 diabetes. □ soy germ protein+Zn, ■ soy germ protein, ▒ placebo.

Blood Glucose Level of Type-2 Diabetes Mellitus. At baseline time, the blood glucose range level was very high i.e. 313-324.5 mg/dl, but was not significantly different between groups ($P = 0.98$) as well as after 1 month observation ($P = 0.40$). At two months after observation, the blood glucose level decreased from 315.38 to 185.38 mg/dl ($P = 0.04$) in group consuming milk containing soy germed protein plus Zn, but not different with the milk containing soy germed protein without Zn ($P = 0.39$).

DISCUSSION

Type 2 diabetes is characterized by impaired insulin secretion and chronic insulin resistance. Soy germed protein contains amino acids that can induce insulin secretion more than soy non-germed protein, since soy germed contain protein higher than the seed (Winarsi *et al.* 2009). Four amino acids that are important to induce insulin secretion, i.e. leucine, isoleucine, alanine and arginine (Newsholme *et al.* 2006). Arginine dominate in soy protein, followed by leucine, alanine, and isoleucine.

The arginine induces insulin secretion. The process involves cation of amino acids transporter enter into beta cells that leads membrane depolarization (Sener *et al.* 2000), resulting an increase of Ca^{++} intracellular level. Depolarization of membrane plasma induces activation of voltage dependent calcium channels, as a result, increases of Ca^{++} sitosolic and stimulates insulin secretion.

Furthermore, metabolism of L-arginine in beta cells increases production of urea through the activity of arginase or Nitric Oxide (NO) by Nitric oxide synthase (NOS). In this case, inducible nitric oxide synthase (iNOS) regulates cytokine proinflammatory existency (Ortis *et al.* 2006). Therefore, in that condition, consumption of L-arginine and its metabolism have a negative effect on cell function. This causes increase level of NO which continuously suppresses insulin secretion, and may correlates to mitochondrial role as coupling stimulation of secretor.

Brosnan (2003) argues that arginine does not have clinical effect. This amino acid quickly disappears in epithelial intestine cells, because it is conversed to ornithine and citrulline, and then removed to kidney or liver, and changed in to proline when it is secreted. Broca

et al. (2003) assures that L-arginine is changed into L-glutamate, and then stimulates insulin secretion. As an insulin secretion stimulator, the role of L-glutamate is debated. The molecular mechanism may be to promote insulin secretion. The intracellular role of L-glutamate on insulin secretion is induced by nutrition. Causing of glutamate role by insulin secretion amplified pathway was stimulated glucose (Maechler & Wollheim 1999).

During hyperglycemic period the level of cell glutamate increases in islet of human, mouse and mice (Rubi *et al.* 2001; Bertrand *et al.* 2002). The activated mitochondria beta cells indirectly stimulates insulin exocytosis. It is proven that glutamate could be transported into secretory granules, and promote Ca^{++} -dependent exocytosis (Hoy *et al.* 2002). Glutamate role is initiated from the action of beta cells expressing L-glutamate decarboxylase (GAD). GAD overexpression reduces L-glutamate content in beta cells islet (Rubi *et al.* 2001), and then suppresses the secretory response. The role of glutamate on insulin secretion remains debatable. Increase of intracellular glutamate level occurred with addition 16.7 mmol/L glucose in mouse islet, but the insulin secretion was not observed (MacDonald & Fahien 2000). Incubation in 10 mmol/L L-glutamine increased L-glutamate level 10 fold, but did not stimulate the insulin secretion. Firstly, these findings confused Newsholmes *et al.* (2006) about glutamate role. However, the other study assured that incubation in glucose significantly improved L-glutamate level in islet mouse and mice (Bertrand *et al.* 2002). Broca *et al.* (2003) against this glutamate hypothesis. In their study, L-glutamine increased L-glutamate content, but did not affect insulin secretion. However, the other findings showed that activation of glutamate dehydrogenase (GDH) lowers L-glutamate level, and in this case insulin secretion increased. The debate explains our finding that arginine which is more abundant in soy germed protein stimulates beta cells to secrete insulin. Kanetro *et al.* (2008) added that arginine and glycine amino acids in soy germed protein regulates insulin hormone. This soy compound has potency to lower the risk of obesity and diabetes by improving insulin resistance condition, and then regulates secretion of insulin from beta cells pancreatic (Bhathena & Velasquez 2002).

Beside arginine, amino acid leucine was also reported able to stimulate release of insulin by two different mechanisms. First, leucine performs transamination into alpha-ketoglutarate, subsequently mitochondrial oxidation. Second, by activation of allosteric glutamate dehydrogenase (GDH) that causes oxidation of glutamate to the intermediate Krebs cycle, i.e. alpha-ketoglutarate + ammonia. This GDH influence explains that secretion of insulin which is stimulated by leucine was inhibited by higher glucose. Gao *et al.* (1999) added that glucose inhibited leucine stimulation from glutaminolytic and secretion of insulin in islet of isolated mice, by increasing ATP and GTP intracellularly, while ADP reduced and inhibited GDH activities. The important role of GDH in secretion of insulin was mediated by glucose and reversed action to catalyze production of glutamate as a cofactor lead to exocytosis of insulin granules (Maechler & Wollheim 1999).

Regulation of insulin in type-2 diabetes after consumption of milk containing soy germed protein might be correlated with the potency of isoflavone as reported by Winarsi *et al.* (2005b) that soy isoflavone improved the cellular antioxidant protection of cell membrane. This includes pancreatic beta cells membrane from free radical attack, hence the cell integrity can be maintained and secrete optimal insulin.

The soy germed protein contains many isoflavone antioxidant compounds. These compounds are different between soy non-germed protein and soy germed protein. In soy non-germed protein, the concentration of isoflavones glycitein is very low, but high in the soy germed protein. However, currently lack of research explores glycitein potency, while the isoflavone was reported as the strongest radical scavenger (Hubert *et al.* 2008), hence is potential as beta cells membrane protector.

Zn metabolism disorder was also occurred in type-2 diabetes and obesity subject. This condition resulted in lowering Zn and antioxidant status (Winarsi *et al.* 2005b). This condition disturbs cells and stimulates dysfunction of endothelial cells that could cause other vascular diseases. Zn is a cell-structured compound (Winarsi *et al.* 2005b), so that for the integrity of cell membrane Zn supplementation is needed. However, our study showed that in group of women patients consuming soy germed protein plus Zn was not different with the one consuming soy germed protein without Zn. It may be correlated with adequate Zn status initially so that was synergistically improved integrity of beta cell membrane, and then the function of cell is better especially in insulin secretion. This potency was supported by significantly reduced blood glucose level from 315.38 into 185.38 mg/dl ($P=0.04$) (Figure 2) in groups consuming milk containing soy germed protein plus Zn after 2 months of observation, although it was not different in the without Zn group ($P=0.39$). Simon and Taylor (2001) reported that Zn supplementation attenuated hyperglycemia in *db/db* mice. Thus, suggesting that Zn plays a role in pancreatic function and peripheral tissue glucose uptake. Adequate Zn supplementation may improve glycemic control and delay progression of diabetes, showed less weight in group receiving soy germed protein plus Zn compared with control groups. In this research we use 280 ppm Zn for two months attenuated

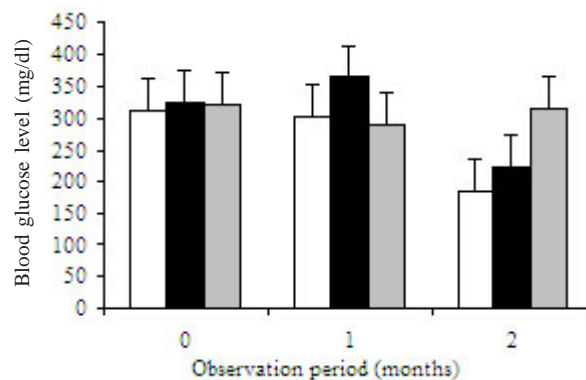


Figure 2. Blood glucose levels of type-2 diabetes. □ soy germ protein+Zn, ■ soy germ protein, ▒ placebo.

blood glucose, thus elevated insulin content of pancreatic islets. This finding supported Bégin-Heick *et al.* (1985) that Zn modulated high insulin secretory response of pancreatic islets to glucose.

Based on this study result, the blood glucose level was decreased for patient consumed soy milk enrich with or without Zn, but not up to normal range. This might be due to the impaired of beta cells function. Researchers also reported that by increasing the age, show decreasing affect of beta cell function as shown by the lowering glucose tolerance in T2DM (Scheen *et al.* 1996; Samos & Roos 1998). Glucose intolerance due to ages was more due to defect beta cells function compare to low cells mass (Clark *et al.* 2001; Guiot *et al.* 2001).

Chronical hyperglycemic as the characteristic of T2DM disturb beta cell pancreatic action, and cause impaired secretion of insulin. These conditions affected regulation of beta cells turnover (Donath & Halban 2004). Normal blood glucose level was very important to be maintained. Based on this study, the level of blood glucose was significantly lower after two months of observation having soy milk enrich with or without Zn. We found from this study that consuming milk contain soy germed protein for two months induces beta cell action to secrete insulin. The level of insulin subject increased from 194.79 to 519.82 pmol/ml, supported by decrease of blood glucose level from 315.38 to 185.38 mg/dl. For further research one can study the time length consumption of soy milk enrich with Zn to ensure the T2DM blood glucose patient able to reach up to the normal level.

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