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The Effects of Soyferment-Tempeh on Lipid Profile and Expression of Retinol binding protein 4 (RBP4) and Phosphoenolpyruvate Carboxykinase (PEPCK) Genes in Type 2 Diabetic Mice

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ABSTRACT

Type 2 diabetes can cause oxidative stress leading to the accumulation of reactive oxygen species. Soyferment-Tempeh, a fermented soybean product with aerobic and anaerobic *R. oligosporus* incubation has a high content of isoflavones, which are antioxidants that can regulate oxidative stress in diabetes. In this study, we evaluated the effects of Soyferment-Tempeh on lipid profile and the expression of Retinol binding protein 4 (RBP4) and Phosphoenolpyruvate Carboxykinase (PEPCK) genes in type 2 diabetic mice model. A total of 30 eight-week-old mice were divided into the following six groups: Nondiabetic, diabetic mice, diabetic mice with metformin, and diabetic mice with Soyferment-Tempeh doses of 10, 20, or 40 mg/100 g BW/day, respectively. Treatments were administered orally by gavages. Before and after 3 weeks of treatment, blood was drawn to measure blood glucose levels and lipid profiles. After sacrificing the mice, livers were used for the assessment of RBP4 and PEPCK gene expression. In streptozotocin-induced diabetic mice, supplementation with three separate doses of Soyferment-Tempeh for 21 days decreased blood glucose, total cholesterol, triglycerides, low-density lipoprotein (LDL) level, atherogenic index, and increased high-density lipoprotein (HDL) level significantly ($p<0.001$). RBP4 gene expression was significantly lower in the Soyferment-Tempeh of dose 10mg/100g BW treatment groups ($p<0.05$), but PEPCK gene expression was not significantly different ($p>0.05$). These results demonstrated that supplementation with Soyferment-Tempeh decreases blood glucose level, atherogenic index, improves lipid profile, and decreases RBP4.

Keywords: Soyferment-Tempeh, lipid profile, atherogenic index, retinol binding protein 4, phosphoenol pyruvate carboxykinase

INTRODUCTION

In type 2 diabetes, hyperglycemia and hyperlipidemia can lead to oxidative stress and the accumulation of reactive oxygen species (ROS) (Wang and Wang, 2017). Oxidative stress induces dysregulation of Retinol binding protein 4 (RBP4) (Liu *et al*., 2014) and Phosphoenolpyruvate Carboxykinase (PEPCK) gene expression by an independent insulin mechanism (Yoshiaki *et al.,* 2006). Retinol binding protein 4 is a 21-kDa adipokine that acts to transport vitamin A to target tissues. It is synthesized in the liver and adipose tissue and belongs to the lipocalin family (Tsutsumi *et al*., 1992; Yang *et al*., 2005). Type 2 diabetes mellitus and insulin resistance are linked to RBP4 levels in the blood (Sun et al., 2014). An elevated level of RBP4 induces insulin resistance (Berry *et al*., 2017) and upregulated expression of PEPCK (Tamori *et al*., 2006). RBP4, triglycerides, and insulin resistance all had a close relationship (Graham *et al*., 2006; Vergès *et al*., 2012; Rocha *et al*., 2013). PEPCK is an enzyme involved in gluconeogenesis in the liver and kidney (Hanson and Patel, 1994).

Tempeh is a famous Indonesian traditional fermented soybean that has many beneficial

health effects. One study found that tempeh has a higher isoflavone (genistein) content than other soy products (Haroon *et al*., 2009). Genistein is an antioxidant that can regulate oxidative stress and inflammation in diabetes (El-Kordy and Alshahrani, 2015). Co-incubation with *L. plantarum* and *R. oligosporus* in tempeh fermentation can improve hyperglycemia, hyperlipidemia, and hyperinsulinemia (Huang *et al*., 2018). It has been reported that GABA-Tempeh (prepared by aerobic and anaerobic fermentation of soybean) significantly decreased plasma triacylglycerol level, prevented hyperlipidemia (Watanabe *et al*., 2005), and had the strongest antioxidative activity (Watanabe *et al*., 2007). This study aimed to see how Soyferment-Tempeh affected the lipid profile and the expression of the RBP-4 and PEPCK genes in type 2 diabetic mice. As far as the authors' knowledge, there has been no research on the effect of fermented soybean product prepared with aerobic and anaerobic *R. oligosporus* incubation on the prevention of hyperlipidemia and hyperglycemia, particularly on RBP4 and PEPCK gene expression.

MATERIALS AND METHODS

Soyferment-Tempeh

About 1000g of Anjasmara soybeans from Gunung Kidul, Indonesia were soaked in room temperature tap water for 24h. Then, the soaked soybeans were boiled for 15min in the water previously used for soaking. In a hot state, the boiled soybeans were drained and cooled to room temperature. The next step is the peeling of the soybeans. Then, soaked again in fresh tap water followed by further boiling. After cooling to room temperature, the boiled soybeans were fermented by inoculating the spore suspension (25mL of spore suspension *R. oligosporus* FNCC 6010) into the soybeans. About 120g of inoculated beans were packed into perforated polyethylene plastic bags and aerobically incubated for 20h at 37°C. (conventional Tempeh). For anaerobic incubation, all of the conventional Tempeh was transferring into Oxoid AnaeroJar 2.5L containing AnaeroGen 2.5L (Thermo) and then re-incubated for 23h at 37°C. The methods followed the steps previously reported by Aoki *et al*. (2003), Yusof *et al*. (2013), and Hwang *et al.* (2019) with different *Rhizopus* and small modifications.

Animals

Thirty male BALB/C mice, (weight range 20-25g, 8 weeks of age), were collected from The Center for Food & Nutrition Studies, UGM, Yogyakarta, Indonesia. They were housed in cages in an animal room (22-25°C room temperature on a 12h daylight cycle); food and water were provided ad libitum during the experiment using normal diets (AIN 93M, D10012M-Research Diet, USA). The Medical and Health Research Ethics Committee of the Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia, gave their approval to this study, which was given the number KE/FK/0335/EC.

Induction of type 2 diabetes mellitus

Following the procedure outlined by Lee *et al*. (2010), type 2 diabetes mellitus was induced by intraperitoneal injection of 50mg/kg BW streptozotocin (Sigma, St. Louis, MO, USA) 15min after injection of 120mg/kg BW nicotinamide (NA) (Sigma-Aldrich, USA). Five days after induction, fasting blood glucose levels were assessed, and mice were classified as diabetic if they had a fasting blood glucose was ≥196 mg/dL (Lee, *et al*., 2018).

Experimental design

The treatment was divided into six groups. Nondiabetic, diabetic mice, diabetic mice with metformin, diabetic mice with Soyferment-Tempeh doses 10, 20, or 40mg/100g BW/daily, respectively. The Soyferment-Tempeh was administered orally by gavages for 21 days. At the end of treatment, the animals were sacrificed.

Blood biochemical examination

Plasma glucose levels were determined by the GOD-PAP method (Diasys®, Germany). Serum lipid profiles were enzymatically analyzed using a commercial kit (DyaSis®, Germany).

Gene expression analysis using quantitative polymerase chain reaction (qPCR)

According to the producer's protocol, RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, USA) was used for the synthesis of the first-strand cDNA from RNA templates. The real-time PCR assay was performed by using ExcelTaq™ 2X Fast Q-PCR Master Mix (SMOBIO, China). Primers for the genes and the internal control (GAPDH) (Table I). The samples were run on Bio-Rad Real-Time PCR detection system CFX-96 according to the following program: 95°C for the 20s; 95°C for 3s x40; 59.8°C for 30s. The ΔΔCT method of relative quantification was used to evaluate the fold change in expression during data processing.

Table I. Primer sequences for the quantitative PCR

Gene	Forward primer	Reverse primer
RBP-4	CTGCTGGCGGCTCTGG	GCAAAAAGAGACCCTCGGGA
PEPCK	GTGTTTGTAGGAGCAGCCATGAGA	GCCAGGTATTTGCCGAAGTTGTAG
GAPDH	TGTTTGTAGGAGCAGCCATGAGA	TTGCTGTTGAAGTCGCAGGAG

Statistical methods

The results were expressed as the mean (SD). One-way ANOVA was used to examine the effects of Soyferment-Tempeh on fasting blood glucose, cholesterol, HDL, RBP4, and PEPCK gene expression, followed by a post hoc Dunnett's multiple comparisons test. The effects of Soyferment-Tempeh on triglycerides, LDL, and atherogenic index were analyzed by a Kruskal-Wallis followed by post hoc Dunn-Bonferroni tests. If the p-value was less than 0.05, the data was considered statistically significant.

RESULTS AND DISCUSSION

Soyferment-Tempeh

Soyferment-Tempeh is a fermented soybean product with aerobic and anaerobic *R. oligosporus* incubation. Studies showed that, compared with the conventional tempeh (only aerobic condition), anaerobically fermentation increases the free amino acid levels, particularly gamma-aminobutyric acids (GABA) (Aoki *et al.,* 2006). Similar to GABA, antioxidant activity increased during aerobic and anaerobic fermentation (Watanabe *et al*., 2007). Compared to Aoki *et al*. (2003), Yusof *et al*. (2013), and Hwang *et al*. (2019), the GABA level of our Soyferment-Tempeh was lower (manuscript in preparation).

Effect of Soyferment-Tempeh on blood glucose and lipid profile level

The mice induced with streptozotocin and nicotinamide had blood glucose levels that were higher than normal, suggesting type 2 diabetes mellitus. Soyferment-Tempeh supplementation at doses of 10, 20, or 40 mg/100g BW for 3 weeks significantly reduced blood glucose ($p<0.001$) (Table II), cholesterol ($p<0.001$) (Table III), triglycerides ($p<0.05$) (Table IV), and LDL ($p<0.05$) (Table V) in mice induced with streptozotocin and nicotinamide. The Atherogenic index also significantly decreased ($p<0.05$) (Table VI). After treatment with Soyferment-Tempeh at three different doses (Table VII), HDL increased significantly ($p<0.001$).

Our results on the diabetic mice suggested that Soyferment-Tempeh at doses of 10, 20, or 40 mg/100g BW can lower blood glucose levels, even exceed metformin. According to Shim *et al*. (2007), glycemic control can be improved by supplementing soybean isoflavones. Treatment with Soyferment-Tempeh at a dosage of 10 mg/100g BW resulted in the greatest reductions in triglycerides, LDL, and the atherogenic index, as well as an improvement in HDL, which implies that the treatment of Soyferment-Tempeh may improve lipid metabolism. Watanabe *et al.* (2006) reported that GABA-Tempeh affected HDL and lowered LDL cholesterol levels compared with other groups. Tempeh has a higher content of genistein, a major component of soy isoflavones, than other soy products (Haron *et al*., 2009). Research by Shim *et al*. (2007) found that the plasma cholesterol level was lowered significantly after supplementation with soybean isoflavone extract. However, there was no change in plasma triglyceride levels. Another research showed that supplementation with genistein for three weeks significantly reduced serum triglyceride and cholesterol levels in diabetic groups (Lee, 2006).

Effect of Soyferment-Tempeh on RBP4 and PEPCK gene expressions

Hepatic RBP4 gene expression was significantly different ($p<0.05$) after treatment with Soyferment-Tempeh at a dose of 10 mg/100g BW compared to control DM groups (Figure 1). The RBP4 gene's melting temperature began to be detected at 70.50°C, while the control gene's melting temperature (GAPDH) began to be detected at 67.50°C. The melting curve of the RBP4 gene product was detected at 84.50°C. The melting curve of GAPDH was detected at 82°C. The RBP4 gene's CT value was first detected at 25.88 cycles, while the GAPDH cycle's CT value was first detected after 15 cycles.

Table II. Plasma glucose levels after 3 weeks of treatment

Group Treatment	n	Blood glucose (mg/dL)		
		Baseline	End	Change from baseline
Nondiabetic	5	55.95±7.436	69.19±1.497	13.24±8.506
DM	5	227.48±17.156	245.64±3.810	18.17±19.214
DM-Metformin	5	220.94±15.925	155.65±2.532	-65.29±16.414***
DM-SoyT 10	5	257.89±3.175	138.44±2.427	-119.45±4.325***
DM-SoyT 20	5	224.05±13.526	117.50±2.956	-106.55±11.918***
DM-SoyT 40	5	258.10±4.676	103.00±0.980	-155.54±4.335***

The data were presented as the mean (SD), and statistical analysis was performed using a one-way ANOVA with Dunnett's multiple comparisons post-hoc analysis where ***p<0.001 significant difference from control (DM).

Table III. Cholesterol levels after 3 weeks of treatment

Group Treatment	n	Cholesterol (mg/dL)		
		Baseline	End	Change from baseline
Nondiabetic	5	83.15 ± 1.788	84.28 ± 2.414	1.13 ± 0.723
DM	5	174.93 ± 3.923	171.22 ± 4.489	-3.72 ± 0.814
DM-Metformin	5	172.60 ± 3.176	142.14 ± 4.193	-30.46 ± 5.565***
DM-SoyT 10	5	178.90 ± 2.241	106.86 ± 4.442	-72.04 ± 6.007***
DM-SoyT 20	5	178.10 ± 5.239	123.65 ± 1.642	-54.45 ± 5.904***
DM-SoyT 40	5	175.62 ± 3.369	99.40 ± 1.057	-76.21 ± 4.136***

The data were presented as the mean (SD), and statistical analysis was performed using a one-way ANOVA with Dunnett's multiple comparisons post-hoc analysis where ***p<0.001 significant difference from control (DM).

Table IV. Triglyceride levels after 3 weeks of treatment

Group Treatment	n	Triglycerides (mg/dL)		
		Baseline	End	Change from baseline
Nondiabetic	5	63.60 ± 3.080	65.89 ± 3.637	2.28 ± 0.835
DM	5	129.19 ± 2.974	131.09 ± 3.271	1.90 ± 0.750
DM-Metformin	5	128.06 ± 1.759	104.11 ± 4.020	-23.95 ± 4.223
DM-SoyT 10	5	129.33 ± 1.803	84.38 ± 3.040	-44.95 ± 2.338*
DM-SoyT 20	5	125.38 ± 3.817	92.77 ± 1.449	-32.61 ± 4.141
DM-SoyT 40	5	130.91 ± 6.290	86.75 ± 2.050	-44.16 ± 6.396*

The data were presented as the mean (SD), and statistical analysis was performed using a Kruskal-Wallis with the Dunn-Bonferroni tests post-hoc analysis where *p<0.05 significant difference between DM *vs* DM-Soy T10 or DM *vs* DM-Soy T40.

Table V. LDL levels after 3 weeks of treatment

Group Treatment	n	LDL (mg/dL)		
		Baseline	End	Change from baseline
Nondiabetic	5	24.50 ± 2.219	26.19 ± 2.175	1.69 ± 0.771
DM	5	81.24 ± 3.412	83.97 ± 1.524	2.724 ± 2.937
DM-Metformin	5	84.01 ± 1.594	63.81 ± 1.646	-20.204 ± 2.090
DM-SoyT 10	5	83.74 ± 2.448	38.73 ± 2.772	-45.006 ± 3.469*
DM-SoyT 20	5	82.49 ± 2.211	51.81 ± 1.069	-30.684 ± 2.567
DM-SoyT 40	5	83.32 ± 1.593	46.69 ± 1.285	-36.628 ± 2.128*

The data were presented as the mean (SD), and statistical analysis was performed using a Kruskal-Wallis with the Dunn-Bonferroni tests post-hoc analysis where *p<0.05 significant difference between DM *vs* DM-Soy T10 or DM *vs* DM-Soy T40.

Table VI. The atherogenic index after 3 weeks of treatment

Group Treatment	n	Atherogenic index		
		Baseline	End	Change from baseline
Nondiabetic	5	-0.10 ± 0.024	-0.09 ± 0.026	0.01 ± 0.004
DM	5	0.60 ± 0.018	0.70 ± 0.026	0.10 ± 0.030
DM-Metformin	5	0.55 ± 0.013	0.43 ± 0.030	-0.12 ± 0.052
DM-SoyT 10	5	0.59 ± 0.053	-0.02 ± 0.013	-0.61 ± 0.023*
DM-SoyT 20	5	0.66 ± 0.015	0.23 ± 0.011	-0.42 ± 0.026
DM-SoyT 40	5	0.68 ± 0.037	0.10 ± 0.014	-0.58 ± 0.026*

The data were presented as the mean (SD), and statistical analysis was performed using a Kruskal-Wallis with the Dunn-Bonferroni tests post-hoc analysis where *p<0.05 significant difference between DM *vs* DM-Soy T10 or DM *vs* DM-Soy T40.

Table VII. HDL levels after 3 weeks of treatment

Group Treatment	n	HDL (mg/dL)		
		Baseline	End	Change from baseline
Nondiabetic	5	80.14 ± 1.332	81.84 ± 1.817	1.70 ± 0.700***
DM	5	32.25 ± 1.436	25.94 ± 1.325	- 6.31 ± 0.789
DM-Metformin	5	35.85 ± 0.905	38.33 ± 2.737	2.48 ± 3.086***
DM-SoyT 10	5	33.49 ± 3.748	88.03 ± 2.980	54.54 ± 6.029***
DM-SoyT 20	5	28.03 ± 1.474	54.25 ± 1.280	26.22 ± 2.663***
DM-SoyT 40	5	28.16 ± 2.987	68.79 ± 0.499	40.63 ± 2.556***

The data were presented as the mean (SD), and statistical analysis was performed using a one-way ANOVA with Dunnett's multiple comparisons post-hoc analysis where ***p<0.001 significant difference from control (DM).

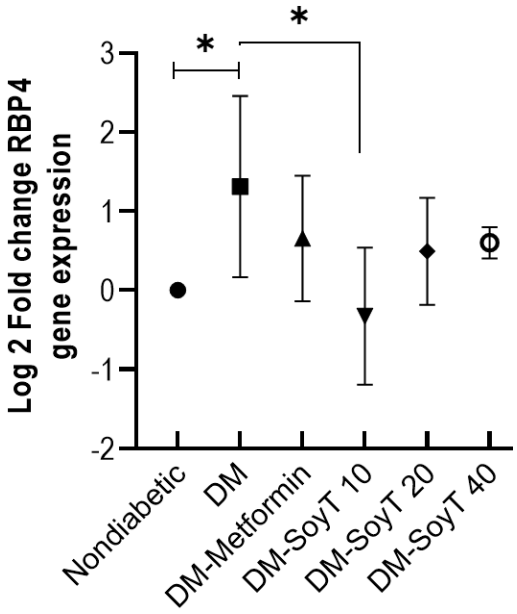

Figure 1. Log 2 fold change in liver RBP4 gene expression. A one-way ANOVA with Dunnett's multiple comparisons post-hoc analysis was used for statistical analysis, with *p<0.05 indicating a significant difference from control (DM).

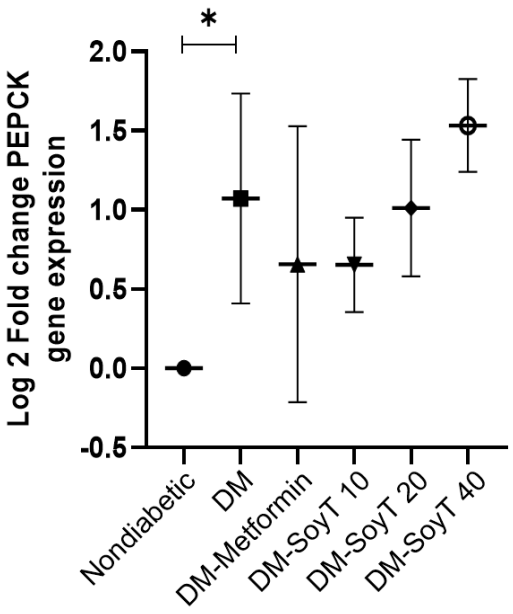

Figure 2. Log 2 fold change in liver PEPCK gene expression. A one-way ANOVA with Dunnett's multiple comparisons post-hoc analysis was used for statistical analysis, with *p<0.05 indicating a significant difference from control (DM).

After three weeks of treatment with Soyferment-Tempeh at doses of 10, 20, or 40mg/100g BW, hepatic PEPCK gene expression in diabetic mice groups did not vary significantly ($p>0.05$) from control DM groups (Figure 2). The PEPCK gene's melting temperature began to be detected at 70°C, while the GAPDH gene's melting temperature began to be detected at 67.50°C. The melting curves of both PEPCK and control gene products were detected at 82°C. The PEPCK gene's CT value began to be detected at 19.40 cycles. while the GAPDH gene's CT value began to be detected after 15 cycles.

According to Tamori *et al*. (2006), in adipose-specific GLUT4 knockout mice, RBP4 secretion into the circulation is increased due to reduced glucose influx in adipocytes. Increasing RBP4 in circulation: i) inhibits insulin signals in the skeletal muscles, causing the reduction of glucose uptake, and ii) upregulates the expression of PEPCK in the liver, causing an increase in glucose output. Both of these conditions together can cause increased blood glucose levels. In our study, diabetic animal models exhibited a lower fold change of RBP4 expression ($p<0.05$) and PEPCK expression ($p>0.05$) after treatment with Soyferment-Tempeh at a dose of 10mg/100g BW compared with diabetic mice groups. A previous study found that isoflavones decrease the expression of the RBP4 gene in white adipose tissue (Duru *et al*., 2018). On the contrary, in a study by Velpen *et al.* (2014), supplementation with isoflavones did not affect RBP4 gene expression.

In the diabetic state, hyperglycemia is a typical feature that can cause ROS regeneration, resulting in insulin resistance (Valverde *et al.,* 2003). In this condition, insulin fails to suppress hepatic gluconeogenesis (Hatting *et al*., 2018) which causes an increase in PEPCK gene expression. Increased PEPCK can be caused by increased RBP4 expression which leads to increased glucose output from the liver, thus raising blood glucose (*Yang et al.,* 2005*; Graham et al.,* 2006). In this study, we found that Soyferment-Tempeh which was prepared with anaerobic incubation of the conventional tempeh, at certain doses can improve hyperglycemia and lipids profile, by reducing liver RBP4 gene expression.

CONCLUSION

This study showed the effects of Soyferment-Tempeh on decreasing triglyceride, LDL, and the atherogenic index, and increasing HDL level. Although not accompanied by a significant decrease of PEPCK gene expression, Soyferment-Tempeh at certain doses can reduce the expression of the liver RBP4 gene in type 2 diabetic mice. These results suggest that the protective effect of Soyferment-Tempeh may be through the reduction of the liver RBP4 gene expression. Thus, Soyferment-Tempeh can be used as a diabetic protection supplement.

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REFERENCES

Aoki, H., Uda, I., Tagami, K., Furuya, Y., Endo, Y., & Fujimoto, K. (2003). The production of a new tempeh-like fermented soybean containing a high level of gamma-aminobutyric acid by anaerobic incubation with Rhizopus. *Bioscience, biotechnology, and biochemistry*, 67(5), 1018–1023. https://doi.org/10.1271/bbb.67.1018

Berry, D., Noy, N. (2017). Retinol Binding Protein 4: Role in Diabetes and Cancer. Adipocytokines, Energy Balance, and Cancer. Energy Balance and Cancer. 12. Switzerland: Springer International Publishing Services. p. 89-107.

Duru, K. C., Kovaleva, E. G., Danilova, I. G., van der Bijl, P., & Belousova, A. V. (2018). The potential beneficial role of isoflavones in type 2 diabetes mellitus. *Nutrition research (New York, N.Y.)*, *59*, 1–15. https://doi.org/10.1016/j.nutres.2018.06.005.

El-Kordy, E. A., & Alshahrani, A. M. (2015). Effect of genistein, a natural soy isoflavone, on pancreatic β-cells of streptozotocin-induced diabetic rats: Histological and immunohistochemical study. *Journal of microscopy and ultrastructure,*3(3),108–119. https://doi.org/10.1016/j.jmau.2015.03.005.

Graham, T. E., Yang, Q., Blüher, M., Hammarstedt, A., Ciaraldi, T. P., Henry, R. R., Wason, C. J., Oberbach, A., Jansson, P. A., Smith, U., & Kahn, B. B. (2006). Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. *The New England journal of medicine*, *354*(24), 2552–2563.

https://doi.org/10.1056/NEJMoa054862.

Haron, H., Ismail, A., Azlan, A., Shahar, S., Su Peng, L. (2009). Daidzein and genestein contents in tempeh and selected soy products. *Food Chemistry*.115(4),1350-1356. https://doi.org/10.1016/j.foodchem.2009.01.053

Hanson, R. W., & Patel Y. M. (eds) (1994). P-enolpyruvate Carboxykinase: The Gene and the Enzyme, John Wiley & Sons, Inc., New York. doi: 10.1002/9780470123157.ch6.

Hatting, M., Tavares, C., Sharabi, K., Rines, A. K., & Puigserver, P. (2018). Insulin regulation of gluconeogenesis. *Annals of the New York Academy of Sciences*, *1411*(1), 21–35. https://doi.org/10.1111/nyas.13435.

Huang, Y. C., Wu, B. H., Chu, Y. L., Chang, W. C., & Wu, M. C. (2018). Effects of Tempeh Fermentation with *Lactobacillus plantarum* and *Rhizopus oligosporus* on Streptozotocin-Induced Type II Diabetes Mellitus in Rats. *Nutrients*, *10*(9), 1143. https://doi.org/10.3390/nu10091143

Hwang, J. H., Wu, S. J., Wu, P. L., Shih, Y. Y., & Chan, Y. C. (2019). Neuroprotective effect of tempeh against lipopolysaccharide-induced damage in BV-2 microglial cells. Nutritional neuroscience, 22(12), 840–849. https://doi.org/10.1080/1028415X.2018.1456040.

Lee, J., Yee, S. T., Kim, J. J., Choi, M. S., Kwon, E. Y., Seo, K. I., & Lee, M. K. (2010). Ursolic acid ameliorates thymic atrophy and hyperglycemia in streptozotocin-nicotinamide-induced diabetic mice. *Chemico-biological interactions*, *188*(3), 635–642. https://doi.org/10.1016/j.cbi.2010.09.019

Lee J. S. (2006). Effects of soy protein and genistein on blood glucose, antioxidant enzyme activities, and lipid profile in streptozotocin-induced diabetic rats. *Life sciences*, *79*(16), 1578–1584. https://doi.org/10.1016/j.lfs.2006.06.030

Liu, Y., Wang, D., Li, D., Sun, R., & Xia, M. (2014). Associations of retinol-binding protein 4 with oxidative stress, inflammatory markers, and metabolic syndrome in a middle-aged and elderly Chinese population. *Diabetology & metabolic syndrome*, *6*(1), 25. https://doi.org/10.1186/1758-5996-6-25

Rocha, M., Bañuls, C., Bellod, L., Rovira-Llopis, S., Morillas, C., Solá, E., Víctor, V. M., & Hernández-Mijares, A. (2013). Association of serum retinol binding protein 4 with atherogenic dyslipidemia in morbid obese patients. *PloS one*, *8*(11), e78670. https://doi.org/10.1371/journal.pone.0078670

Shim, J. Y., Kim, K. O., Seo, B. H., & Lee, H. S. (2007). Soybean isoflavone extract improves glucose tolerance and raises the survival rate in streptozotocin-induced diabetic rats. *Nutrition research and practice*, *1*(4), 266–272. https://doi.org/10.4162/nrp.2007.1.4.266

Sun, L., Qi, Q., Zong, G., Ye, X., Li, H., Liu, X., Zheng, H., Hu, F. B., Liu, Y., & Lin, X. (2014). Elevated plasma retinol-binding protein 4 is associated with increased risk of type 2 diabetes in middle-aged and elderly Chinese adults. The Journal of nutrition, 144(5), 722–728. https://doi.org/10.3945/jn.113.189860

Tamori, Y., Sakaue, H., & Kasuga, M. (2006). RBP4, an unexpected adipokine. *Nature medicine*, *12*(1),30–31. https://doi.org/10.1038/nm0106-30

Tsutsumi, C., Okuno, M., Tannous, L., Piantedosi, R., Allan, M., Goodman, D. S., & Blaner, W. S. (1992). Retinoids and retinoid-binding protein expression in rat adipocytes. *The Journal of biological chemistry*, *267*(3), 1805–1810.

Valverde AM, Burks DJ, Fabregat I, Fisher TL, Carretero J, White MF, Benito M. Molecular mechanisms of insulin resistance in IRS-2-deficient hepatocytes. Diabetes. 2003 Sep;52(9):2239-48. doi: 10.2337/diabetes.52.9.2239. PMID: 12941762.

van der Velpen, V., Geelen, A., Hollman, P. C., Schouten, E. G., van 't Veer, P., & Afman, L. A. (2014). Isoflavone supplement composition and equol producer status affect gene expression in adipose tissue: a double-blind, randomized, placebo-controlled crossover trial in postmenopausal women. *The American journal of clinical nutrition*, *100*(5), 1269–1277. https://doi.org/10.3945/ajcn.114.088484

Vergès, B., Guiu, B., Cercueil, J. P., Duvillard, L., Robin, I., Buffier, P., Bouillet, B., Aho, S., Brindisi, M. C,, Petit, J. M. (2012). Retinol-binding protein 4 is an independent factor associated with triglycerides and a determinant of very low-density

lipoprotein-apolipoprotein b100 catabolism in type 2 diabetes mellitus. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 32(12), 3050–3057. https://doi.org/10.1161/ATVBAHA.112.255190.

Wang, J., & Wang, H. (2017). Oxidative Stress in Pancreatic Beta Cell Regeneration. *Oxidative medicine and cellular longevity*, *2017*, 1930261. https://doi.org/10.1155/2017/1930261

Watanabe, N., Endo, Y., FUJIMOTO, K., AOKI, H. (2006). Tempeh-like Fermented Soybean (GABA-tempeh) Has an Effective Influence on Lipid Metabolism in Rats. *Journal of Oleo Science*, 55(8), 391-396. https://doi.org/10.5650/jos.55.391

Watanabe, N., Fujimoto, K., & Aoki, H. (2007). Antioxidant activities of the water-soluble fraction in tempeh-like fermented soybean (GABA-tempeh). International journal of food sciences and nutrition, 58(8), 577–587. https://doi.org/10.1080/09637480701343846

Yang, Q., Graham, T. E., Mody, N., Preitner, F., Peroni, O. D., Zabolotny, J. M., Kotani, K., Quadro, L., & Kahn, B. B. (2005). Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature*, *436*(7049),356–362. https://doi.org/10.1038/nature03711

Yoshiaki, I., Saori, O., Takashi, N., Naoyuki, N. (2006). Oxidative stress induces phosphoenolpyruvate carboxykinase expression in H4IIE cells. *Bioscience, Biotechnology, and Biochemistry,* 7*0*(9), 2191-2198. https://doi.org/10.1271/bbb.60135

Yusof, M. H., Ali, N. M., Yeap, S. K., Ho, W. Y., Beh, B. K., Koh, S. P., Long, K., Abdul Aziz, S., & Alitheen, N. B. (2013). Hepatoprotective Effect of Fermented Soybean (Nutrient Enriched Soybean Tempeh) against Alcohol-Induced Liver Damage in Mice. *Evidence-based complementary and alternative medicine*, 2013. https://doi.org/10.1155/2013/274274