Synbiotic kefir lowered peroxisome proliferator activated receptor gamma (PPARγ) gene expression in rat fed high-fat and high-fructose diet

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Research Article

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Abstract

Kefir is fermented milk product containing bacteria and yeast, whereas glucomannan from porang (*Amorphophallus oncophyllus*) tuber has known as prebiotic *in vivo*. Diets with a high fat and high sugar will stimulate metabolic syndrome. The objective of this study were to determine the effect of synbiotic kefir (goat milk kefir enriched with porang glucomannan) on blood glucose, hemoglobin A1c (HbA1c), free fatty acid (FFA), tumor necrosis factor alpha (TNF-α), gene expression of peroxisome proliferator activated receptor gamma (PPARγ), and insulin producing cells in rat fed high- fat and high- fructose (HFHF) diet. Rats were divided into 5 groups: normal; high fat high fructose (HFHF); HFHF + probiotic kefir; HFHF + synbiotic kefir; and HFHF + simvastatin. There was no significantly differences in plasma blood glucose in HFHF rat after treated with synbiotic kefir. However, synbiotic kefir could decrease HbA1c and plasma TNFα, and inhibit the increasing FFA in HFHF rats. Probiotic and synbiotic kefir could decrease gene expression of PPARγ2 in both of adipose and liver tissue in HFHF rats, but had no effect on total number of Langerhans islet and insulin producing cell. In conclusion, synbiotic kefir could ameliorate the health of rats in condition of high-fat and high-fructose diet, through decreasing in HbA1c, TNFα, and gene expression of PPARγ2 and also prevent the increasing of FFA. Therefore, synbiotic kefir containing porang glucomannan is expected to be a suggestion for the food industry to develop synbiotic-based functional foods which has the potential to improve metabolic syndrome

Introduction

Limited physical activity and enhanched exposure to unhealthy foods that are energy-dense ("obesogenic" environment) causing increased obesity. The prevalence of obesity in the last decade is becoming increasingly common and becoming a major nutritional problem throughout the world. Physiological problems and genetic factors are also additional risks for the development of obesity. The development of insulin resistance, type-2 diabetes and metabolic syndrome are negative health consequences of obesity. Because of the limitations of obesity and metabolic syndrome therapy, prevention strategies are needed (Gregory, 2019).

Prevention and treatment of metabolic syndrome can be done both pharmacological and non-pharmacologically. Functional food affects health benefits can be derived from the animal or plant sources. Therapeutic approaches to metabolic syndrome (MetS) are numerous and may target lipoproteins, blood pressure or anthropometric indices. Peroxisome proliferator-activated receptors (PPARs) are involved in the metabolic regulation of lipid and lipoprotein levels, i.e. triglycerides (TGs), blood glucose, and abdominal adiposity (Botta et al., 2018). PPARγ is abundantly expressed in the adipose tissue and to a lesser extent in macrophages and other cell types, and regulates adipogenesis, lipid storage, and glucose homeostasis (Janani and Kumari, 2015). PPARγ2 is specific for adipose tissue, where it plays a pivotal role in adipogenesis and is an important mediator of insulin sensitivity (Rosen et al., 1999) and a more potent transcription activator (Feige et al., 2006)

Kefir, a probiotic, has recently shown possible health benefits in fighting obesity. Kefir reduced body weight and epididymal fat pad weight and decreased adipocyte diameters in high- fat diets (HFD)-induced obese mice. This was supported by decreased expression of genes related to adipogenesis and lipogenesis as well as reduced proinflammatory marker levels in epididymal fat (Choi et al., 2017). Recent studies show that kefir and derived isolated microorganisms can increase anti-inflammatory cytokines and decrease pro-inflammatory responses, justifying its anti-atherosclerotic potential (Pimenta et al., 2018).

Porang (*Amorphophallus oncophyllus*) is a tuber locally that are often found in the Indonesian forest, and it is being cultivated. Similar to *Amorphophallus konjac*, porang tuber contains glucomannan and has been shown to be a prebiotic *in vivo* (Harmayani et al., 2014). Glucomannan is a water-soluble dietary fiber can improve blood sugar, blood fat concentration, and weight management, and has other health benefits. Staple food from glucomannan noodles can contribute to metabolic syndrome in adults predisposed to type-2 diabetes (Cheang et al., 2017), and has a prebiotic function that selectively enhances the growth of probiotic bacteria such as lactobacilli and bifidobacteria (Al-Ghazzewi and Tester, 2012). Glucomannan from konjac which grows in Japan known to effectively prevent and improve metabolic syndrome in individuals without changing eating habits (Hasegawa et al., 2013).

*T*he purpose of this study was to evaluate synbiotic kefir (goat milk kefir with additional of glucomannan from porang) on gene expression associated with metabolic regulation of lipids and blood glucose i.e. PPARγ in adipose and liver tissue in rat fed a high-fat and high-fructose diet during the experiment.

Materials And Methods

2.1. Kefir preparation

Synbiotic kefir made from a mixture of goat milk, porang glucomannan (as prebiotic), whey protein concentrate (WPC) and kefir grain. Glucomannan from porang tuber was obtained from the Faculty of Agrigultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia. Goat milk was obtained from Ettawah Crossbred goat in Yogyakarta, Indonesia. Whey protein concentrate (WPC) was obtained from Sari Husada Milk industry in Yogyakarta Indonesia. Kefir grain was purchased from local supplier in Yogyakarta.

Synbiotic kefir was prepared traditionally according to Otles and Cagindi (2003) with slight modification. Goat milk, 0.1% whey protein concentrate (WPC), 0.3% porang glucomannan were mixed, pasteurized at 75^{o}C for 15 min, and cooled at room temperature. Kefir grains (2%) were inoculated into pasteurized milk and incubated at room temperature for 18 h. After incubation, the kefir was filtered to separate kefir grain. Probiotic kefir prepared with goat milk, WPC and kefir grain without glucomannan.

2.2. Animal experimental

Male Sprague Dawley rats of 8-12 weeks old were divided into 5 groups (each group consist 6 rats): 1) Normal control (negative control rats) that received standard diet only, 2) Rat fed high-fat/high-fructose (HFHF) (positive control), 3) Rat fed HFHF + probiotic kefir, 4) Rat fed HFHF + synbiotic kefir, 5) Rat fed HFHF + simvastatin. The dose of kefir was 3.6 mL/200 g body weight/ day, for 4 weeks. The dose of simvastatin was 0.72 mg/day.

Before treatment, the rats were acclimated with standard diet AIN-93 for 1 week, and then fed high fat and high fructose for 2 weeks. The rats were then divided into 5 groups as above. High fat and high fructose diet were administered until the end of experiment (4 weeks). The composition of standard diet and high-fat and high-fructose diet were formulated according to Reeves et al.(1993) and de Castro et al. (2013) with slight modification. All procedures related to animal experiment in this study were approved by Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine Universitas Gadjah Mada, Indonesia (Approval number: KE/FK/95/EC/2015).

Table 1

Composition of standard (AIN-93) and high fat/high fructose diets

No.	Ingredient (g/kg)	AIN-93M*	High fat + fructose**
1	Fructose	-	321,6
2	Casein	140,00	190,25
3	Condensed milk	-	158
4	Soy bean oil	40,00	20
5	Tallow (beef fat)	-	185
6	Fiber/ alpha cel	50,00	25
7	Wheat bran	-	54,15
8	Mineral Mix	35,00	35
9	Vitamin Mix	10,00	10
10	DL-Methionine	1,80	1,8
11	Cholin Chloride	2,50	2,5
12	Corn starch	620,70	-
13	Sucrose	100,00	-

*Reeves (1993) , ** de Castro et al. (2013)

2.3. Blood analysis

Fasting plasma blood glucose was done by enzymatic photometric test using Glucose Oxidase Phenol 4-Aminoantipyrine Peroxidase (GPO-PAP) method according to instructions in Kit (Dia Sys, Holzheim-Germany). Glycosylated hemoglobin (HbA1c) analysis according to the instructions in Rat HbA1c ELISA Kit (ELabScience, Wuhan, China). Analysis of plasma FFA was done according to

instruction in Rat FFA ELISA Kit (Qayee-Bio, Shanghai, China). Plasma TNFα was analyzed according to the instructions in Rat specific ELISA kits for measurement of TNF α (eBioscience, Bender MedSystem, Vienna, Austria).

2. *4. Gene expression analysis*

PPARγ2 gene expression was analyzed through 4 stages: 1) isolation of RNA from white adipose tissue and liver tissue, 2) reverse transcription from RNA to cDNA using reverse transcriptase enzyme, 3) cDNA amplification by PCR and 4) quantification and detection of cDNA products with real-time PCR.

Total RNA was extracted from adipose tissue using the TRIzol reagent, and mRNA levels were analyzed by real-time polymerase chain reaction (PCR). Reverse transcription of total RNA by using the First Strand cDNA Synthesis Kit (Roche) Transcriptor. Transcription Reagents to produce cDNA. Real-time PCR is carried out in a mixture (final volume 20 µL) containing 2µL cDNA (DNA template), 10 µL Evagreen, Forword GAPDH 1 µL, Reverse GAPDH 1 µL, RNAse free water 6 µL. Likewise for Forword and Reverse PPARγ2 with additional reagents totaling 20 µL as well. The amount of mRNA was calculated as the ratio to the value of glyceraldehydes-3-phosphate dehydrogenase (GADPH) in each cDNA sample. The primary nucleotide sequences used to detect each mRNA are designed using the Primary Express Software according to the sequences available in the GenBank database. The primary nucleotide sequences are as in the following Table (Takahashi and Ide, 2008).

[Please see the supplementary files section to view Table 2.]

Optimization of cDNA amplification products that have been done using conventional PCR with a program at a temperature of 95^{o}C for 5 minutes; 95^{o}C 1 minute; 58^{o}C 1 minute; 72^{o}C 1 minute with a 34x cycles. Continued temperature of 72^{o}C 5 minutes and 12^{o}C 5 minutes. The optimization program for Real Time (RT) –PCR was at a temperature of 95^{o}C for 5 minutes; 95^{o}C 1 minute; 60^{o}C 30 seconds and 72^{o}C 1 minute with 39x cycles. Continued melt curve 65-95^{o}C for 5 seconds, and then reading the plate. The average change in the level of gene expression ($2^{-\Delta\Delta CT}$) PPARgamma-2 was analyzed according to Livak and Schmittgen (2001).

2. *5. Immunohistochemical of insulin-producing cells*

Mouse monoclonal insulin primary antibody (abcam, [K36aC10] ab6995, Cambridge, USA) used in this analysis. Pancreatic tissue slides were counterstained with haematoxylin and mounted with coverslips and observed under the light microscope. The number of Langerhans islet and insulin-producing cells were calculated using colony counter, and then documented by Opti Lab (SOP No A-007) microscope.

2.6. Statistical analysis

Data from this analysis were presented as mean±standard deviation. Data of blood plasma analysis before and after treatments including fasting blood glucose, HbA1c, FFA, and TNFα. The difference between the mean of blood plasma analysis in before and after treatments was analyzed by Paired-Samples *t*-Test. One way ANOVA followed by Duncan's Multiple Range Test (DMRT) were used for statistical analyses in gene expression of PPAR, total number of Langerhans islet and insulin-producing cells (p-values of less than 0.05 indicated significant differences). Statistical analyses were performed by using the SPSS version 17 Software.

Results And Discussion

3.1. Blood glucose

Table 3

The average of blood glucose in rat before and after treatments

Treatments	Fasting blood glucose (mg/dL)	
	Before treatment	After treatment
Normal control	94,11 ±19,02[a]	95,51 ±21,10[a]
HFHF	104.05 ±12,93[a]	118.90 ± 11,33[a]
HFHF+kefir	112.81 ±9,54 [a]	114.34 ±18,81 [a]
HFHF+kefir +Glucomannan	116.98 ±6,76 [a]	105.08 ±11,93 [a]
HFHF+simvastatin	221,37 ±6,76 [a]	82,35 ±11,93 [b]

Different letter in the same row indicate significantly different (P<0,05)

The average of fasting blood glucose levels in rats fed a high fructose and high fat diet, before and after being treated with goat's milk kefir, goat's milk kefir + porang glucomannan and simvastatin shown on Table 3.

Based on Table 3, showed that in negative controls (normal rats, only receiving standard diet) showed blood glucose levels were still within the normal glucose range and there was no difference before and after treatment. All rats fed high fed and high fructose demonstrated risk factor for metabolic syndrome with fasting blood glucose > 100 mg/dL. Rats that only received high-fructose and high-fat feed (positive control), showed higher glucose levels after being given the diet for 5 weeks (post-test) compared to pre-test, although not significantly. Goat milk kefir enriched with porang glucomannan could reduce blood glucose levels, but the decrease was not significant, which is only 11.9 mg / dL. However, a konjac-derived glucomannan supplement (3.6 g/day) administered for 28 days reduced blood lipid and glucose levels by enhancing fecal excretion of neutral sterol and bile acid and alleviated the elevated glucose levels in hyperglycemic diabetic subjects (Chen et al., 2003). Different from a previous study by Hadisaputro et al. (2012), skim milk kefir given at a dose of 3.6 ml / day for 4 weeks could significantly reduce blood glucose levels in the amount of 111.00 mg / dL. In the present study, the decrease in low

blood glucose was possible that synbiotic kefir was still not enough to play a role in reducing blood glucose in rats that consume HFHF diets during the experiment. The study by Hadisaputro et al. (2012), diabetic rats did not fed a HFHF diet. The low dose of glucomannan in kefir and the difference in the conditions of the subjects may cause not significant reduction in blood glucose levels.

In the present study, simvastatin could significantly reduce blood glucose levels in rats fed HFHF diet, which was around 139.02 mg / dL. This results in accordance with a previous study by Salunkhe et al. (2016), that mice fed high-fat diet and treated with rosuvastatin showed lower blood glucose might be due to improved glucose uptake, but beta cells activity is inhibited through lowered insulin level and inhibited Ca^{2+} signaling in beta cells resulting in lowered insulin secretion. Therefore, duoble effects on glucose homeostasis by rosuvastatin due to increased insulin sensitivity, meanwhile there is an inhibition of beta cells activity. In other study by Tagaki et al. (2008), also showed that glucose uptake in adipose tissue upregulated in pravastatin-treated mice fed high fat/high sucrose diet and db/db mice. In contrast to study by Wang et al. (2013) and Van Stee et al. (2018), simvastatins can increases the risk on T2DM particularly in prediabetic subjects due to hyperglycemia by impairing the function of islet β cells and have a negative effect on glucose homeostasis, especially on fasting blood glucose level. Individual types of statin may have a different effect on glucose metabolism (Kim et al., 2018). Atorvastatin in high dose causes worsening of the glycemic control in patients with DM (Angelidi et al., 2018). Based on the results of these studies, the possible effect of statins on blood glucose levels depends on the dose and type of statin and the condition of the subject used for the study.

3.2. Hemoglobin (Hb)A1c

Table 4

The average of HbA1c in rat before and after treatments

Treatments	HbA1c (ng/mL)	
	Before treatment	After treatment
Normal control	21,47±5,18 [a]	24,33±3,35 [a]
HFHF	24,98±2,92 [a]	26,45±4,60 [a]
HFHF+kefir	26,02±4,79 [a]	35,44±18,99 [a]
HFHF+kefir +Glucomannan	28,89±4,12[a]	23,56±3,47[b]
HFHF+simvastatin	22,72±4,64 [a]	33,61±16,45 [a]

Different letter in the same row indicate significant difference (P<0,05)

Based on Table 4, HbA1c levels in rats after treatment with synbiotic kefir showed lower ($p<0.05$) compared to before treatment. However, other groups of rats including those who received probiotic kefir treatment did not show any significant difference before and after treatment. This indicates that porang glucomannan added to kefir could improve glucose metabolism so that reduce glycosylated hemoglobin. According to Schrezenmeir and de Vrese (2001), a synergistic effects of these two components probiotics and prebiotics that makes it a more effective supplement compared with probiotic or prebiotic separately. Other study by Patel et al. (2011), the fructose diet will be rapidly metabolized by the liver, causing changes in carbohydrate and lipid metabolism as well as hepatic inflammation which will lead to the development of hyperglycemia, insulin resistance, hyperinsulinemia, and hypertriglyceridemia as major risk factors for diabetes complications). It was further stated by Sangwan and Singh (2014) that the administration of a high fructose diet (68.35%) over a long period of time can induce complications related to type 2 diabetes, namely high blood glucose, glycosylated HbA1c, cholesterol, triglycerides and oxidative stress. However, the results study indicate that the administration of fermented milk containing probiotics *Lactobacillus rhamnosus* GG (150 g/kg standars diet) can reduce the increase in glycosylated hemoglobin (HbA1c) in rats induced by diabetes by feeding high fructose feeds (Sangwan and Singh, 2014). Additionally, the 24 individuals with T2DM had significantly decreased HbA1c by 7.7% after glucomannan noodles intervention (Cheang et al., 2017).

3.3. Free fatty acid (FFA)

Table 5

The average of plasma FFA in rat before and after various treatments

Treatments	FFA (ng/mL)	
	Before treatment	After treatment
Normal control	54.20±5.47a	61.41±2.19b
HFHF	60.15±4.66a	63.25±3.74b
HFHF+kefir	59.92±2.74a	62.24±2.84a
HFHF+kefir +Glucomannan	59.59±4.49a	62.85±4.13a
HFHF+simvastatin	54.04±8.32a	61.21±6.21b

Different letter in the same row indicate significant difference ($P<0,05$)

Based on the Table 5 showed that the average of plasma FFA levels in rats after various treatment were higher ($p<0.05$) in all groups of rats than before treatment, although the increasing FFA after kefir treatment showed not significant. This indicate that probiotic and synbiotics kefir could maintain plasma FFA levels in HFHF rats. In a previous study, konjac-glucomannan supplementation (5%) in baboons resulted in lower than baseline values for triglycerides and circulating free fatty acids after 9 weeks Venter et al.(1990). The lower dose of glucomannan from porang tuber in the present study compared to the previous study by Venter et al. (1990) resulted in no decrease in plasma FFA. According to Martin (1985) and Venter et al. (1990), increased levels of circulating FFA can stimulates fibrinogen synthesis in the liver. Elevated plasma fibrinogen is characteristic of insulin resistance in the liver (insulin may regulate synthesis of fibribnogen). Glucomannan from konjac which is fermented in the colon can decrease FFA production including propionate leading a decrease in fibrinogen synthesis. Therefore, that colonie production and absorption of SCFA (propionate) from soluble fiber may contribute to this fiber's metabolic effects (Venter et al., 1990). Sobczak et al. (2019) reported that each type of FFA has different effects on physiological processes, including the regulation of lipolysis and lipogenesis in adipose tissue, inflammation, endocrine signalling and the composition and properties of cellular membranes. Alterations in such processes due to altered plasma FFA concentrations/profiles can potentially result in the development of insulin resistance and coagulatory defects.

3.4. Tumor necrosis factor alpha (TNFα)

Table 6

The average of plasma TNFα in rat before and after treatments

Treatments	TNFα (pg/mL)	
	Before treatment	After treatment
Normal control	157.66± 15.71a	166.66± 20.84a
HFHF	170.33±23.54a	271.33±167.86a
HFHF+kefir	159.33±14.06a	208.00±44.68b
HFHF+kefir +Glucomannan	176.50±13.79a	155.00±6.63b
HFHF+simvastatin	169.33±11.07a	192.33±50.49a

Different letter in the same row indicate significant difference (P<0,05)

Based on Table 4, there were no decrease in TNFα levels in rat after various treatments, except in kefir added glucomannan treatment. This indicates that porang glucomannan added to kefir can play a role in reducing the occurrence of inflammation through decreased production of pro-inflammatory cytokines in rats fed high-fat high fructose..

The effect of soluble fiber in porang glucomannan on the improvement of metabolic syndrome in the present study is in accordance with a previous study using chitosan fiber by Chang et al. (2012), which is given to rats with metabolic disorders (induced by diabetes), can improve insulin resistance and chronic inflammation, through decreased lipid absorption and slowed absorption of glucose in the small intestine after eating, resulting in a decrease in hepatic lipids and weight of adipose tissue, and reduced plasma adipocytokine levels including leptin, TNF alpha and plasminogen activator inhibitor-1 (PAI-1).

Supplementation with combination of fiber (konjac glucomannan) and bacterial cellulose in high-fat - diet-induced obesity in mice had a more positive effect on obesity-associated hepatic inflammation by reducing levels of TNFα and IL6 and suppressing the protein expression of nuclear factor erythroid 2–related factor 2 (Nrf-2) in comparison with supplementation with bacterial cellulose or konjac glucomannan alone(Zhai et al., 2018). In addition, glucomannan and spirulina combination blocks detrimental effects promoted by hypercholesterolemic diets in Zucker rats, one of which could decrease plasma TNF-α as one of an inflammation biomarkers (Vázquez-Velasco et al., 2015).

3.5. PPARγ-2 gene expression

The average change in the level of gene expression ($2^{-\Delta\Delta CT}$) PPARγ2 of white adipose tissue in HFHF rats treated kefir with or without glucomannan was not significantly different from simvastatin treatment. The rats treated with kefir had a lower change of level PPAR γ2 gene expression than HFHF rats without kefir ($p<0.05$) (Table 7).

Table 7

The average of the relative expression of genes PPARγ2 of white adipose tissue in rat with various treatments

Perlakuan	PPARγ2 gene expression		
	Δ CT	Δ Δ CT	$2^{-\Delta\Delta CT}$
HFHF	-3,74 ±1,08a	-0,67±1,08a	1,96 ±1,24a
HFHF+ kefir	-2,41 ±1,12ab	0,65 ±1,12ab	0,80 ±0,55b
HFHF+ kefir+Glucomannan	-1,00 ±0,94bc	2,06 ±0,94bc	0,29 ±0,23b
HFHF+ simvastatin	-2,22 ±1,12c	0,66 ±1,12c	0,70 ±0,52b

Different letter in the same column indicate significant diffefrence ($p<0,05$)

Normal control rat had an average of Δ CT of – 3.07; average of Δ Δ CT of 0.00 and average of changes in gene expression of PPAR γ2($2^{-\Delta\Delta CT}$) of 1.00.

Based on Table 8, the pattern of the changes in the expression of PPARγ2 genes in liver tissue was similar to that of white adipose tissue, in which rat treated with kefir added or without glucomannan showed lower changes in gene expression ($p < 0.05$) compared to the rats without kefir treatment. Likewise, the simvastatin treatment rat also had lower changes in PPARγ2 gene expression ($p < 0.05$) than in HFHF rat.

Table 8

The average of the relative expression of genes PPARγ2 of liver tissue in rat with various treatments

Treatments	PPARγ2 gene expression		
	Δ CT	Δ Δ CT	$2^{-\Delta\Delta CT}$
HFHF	0,00 ±1,59a	-6,28±1,59a	123,46 ±120,56a
HFHF+ kefir	1,88 ±1,12b	-4,41 ±1,12b	27,25 ±21,01b
HFHF+ kefir+Glucomannan	3,84 ±0,89c	-2,44 ±0,89c	6,33 ±3,78b
HFHF+ simvastatin	2,7 7 ±1,97bc	-3,51 ±1,99bc	19,79 ±17,89b

Different letter in the same column indicate significant diffefrence ($p<0,05$)

Normal control rat had an average of Δ CT of 6.29 ; Δ Δ CT of 0.00 and average of changes in gene expression of PPAR γ2 ($2^{-\Delta\Delta CT}$) of 1.00.

Based on Table 7 and Table 8 showed that the change in PPARγ2 gene expression in liver tissue was higher than adipose tissue. Normally, PPARγ2 is most abundantly expressed in adipocytes and plays major adipogenic and lipogenic roles in the tissue (Lee et al., 2018). Because the rats in the present study received high fat and high fructose diet, it was possible to cause fatty liver. According to Souza-Mello (2015), in non-alcoholic fatty liver disease (NAFLD) showed a high abnormality of PPARγ expression in the liver (Lee et al., 2018).

In the present study, the change in gene expression was the lowest in rats tissue that treated synbiotic kefir, although this difference was not significant compared to probiotic kefir treatment. It was possible that kefir containing probiotics synergize with prebiotics glucomannan play a role in down-regulation of PPARγ2 expression in white adipose and hepatic tissue. The result in the present study was similar to a previous study by Choi *et al*. (2017), that mice fed high fat diet supplemented by 0.2% kefir powder for 8 weeks lowered PPARγ gene expression in the epididymal fat. In other study, shown that mice fed a high-fat diet and 1×10^7 or 1×10^9 CFU /mice probiotic *L. plantarum* LG42 supplementation daily for 12 weeks can reduce PPARγ expression in adipose tissue (Park *et al.,* 2014). Decreased levels of

PPAR-γ and GLUT4 mRNA after high fructose treatment also enhanced by Lr263 administration (Hsieh et al., 2013). It was further emphasized by Zhai et al. (2018), that besides reducing PPARγ expression, the mixed bacterial cellulose/konjac glucomannan it also lowered protein expression of PPARγ by reducing the size of cells in the adipose tissue of high-fat diet fed mice.

Consumption of these dietary fibers, especially mixed BC/KGM, resulted in an improved antioxidant defense system and reduced lipid peroxidation in the liver by increasing the activity of antioxidant enzymes and reducing the formation of MDA in the liver. Moreover, supplementation with these fibers regulated the levels of leptin and adiponectin and inhibited the protein expression of PPARγ by reducing the size of cells in the adipose tissue of high-fat diet fed mice (Zhai et al., 2018).

In the rats fed high-fructose high-fat (HFHF) diet without kefir supplementation showed the highest changes in PPAR gene expression ($p < 0.05$) in both of adipose and liver tissue. These results was in accordance with the results by May et al. (2015), that PPARγ expression level was significantly higher in high fat diet than normal diet rats which is mainly related to fat formation. PPARγ2 is also expressed in the liver, specifically in hepatocytes, and its expression level positively correlates with fat accumulation induced by pathological conditions such as obesity and diabetes (Lee et al., 2018).

3.6. Immunohistochemical of ß-cells

The average number of Langerhans islet and insulin-producing beta cells with various treatments shown on Table 9.

Table 9

The average number of Langerhans islet and insulin-positive ß-cells in rat with various treatments

Treatments	Langerhans^{ns}	Insulin-positive ß-cells ^{ns}
Normal control	3.04± 0.78	96.95 ± 94.19
HFHF	2.47± 0.76	46.08 ± 2.,59
HFHF+ kefir	2.90± 0.71	71.74 ± 22.42
HFHF+ kefir+Glucomannan	3.55± 0.65	82.14 ± 45.27
HFHF+ simvastatin	3.05± 0.82	107.35 ± 79.95

ns:non significant

HFHF: high- fat high- fructose

The average number of Langerhans islets and insulin-producing beta cells in rats fed high fat and high fructose without kefir supplementation, showed the lowest number, although statistically not significantly different (Table 9). There was no change in the number of Langerhans islets and insulin-producing beta cells in all treatments, indicating a high-fructose high-fat diet received during the

experiment did not cause beta cell damage. This was also evidenced by unchanged the average of fasting blood glucose levels in HFHF rats before and after being treated with kefir (Table 2).

According to Linnemann et al. (2014), individuals with type 2 diabetes have decreased beta cell mass compared to nondiabetic individuals, and fasting blood glucose will increase if the volume (mass) of cells is less than the 1.1% threshold (Ritzel et al., 2006). If it is below this threshold value, changes in insulin sensitivity and functional damage in insulin secretion will have a major impact on blood glucose. The HFHF diet in this study had not yet led to diabetes, but only causes prediabetes because blood glucose levels range from 100 mg / dL to 125 mg / dL, which is at risk of becoming diabetic ($\geq$ 126 mg/dL), whereas the normal blood glucose was < 100 mg/dL (Gholi et al., 2016; Khan et al., 2019).

Immunohistochemical (IHC) staining of pancreatic showed that insulin-producing beta cells show a brown color on using rat anti-insulin antibodies (Figure 1). Based on Figure 7 showed that HFHF rats were rarely seen on Langerhans islet and very weak intensity of IHC staining on insulin-producing beta cells and there were few insulin-producing beta cells (Figure 1), although the number of Langerhans islets and beta cells in all treatment groups is not statistically different (Table 9). Rat that received kefir treatment showed intense beta cell stining intensity as in negative control rats (normal normal rats). The HFHF rat given simvastatin showed less strong in IHC staining intensity compared to kefir treatment.

Conclusion

The metabolic syndrome caused by the habit of consuming high-fat and high- fructose diets can be improved by consuming synbiotic kefir, through decreasing in HbA1c, TNFα, and gene expression of PPARγ2 and also prevent the inceasing of FFA. Therefore, synbiotic kefir containing porang glucomannan is expected to be a suggestion for the food industry to develop synbiotic-based functional foods which has the potential to improve metabolic syndrome.

Declarations

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Conflict of Interest

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

References

Al-Ghazzewi, F.H., Tester, R.F., 2012. Efficacy of cellulase and mannanase hydrolysates of konjac glucomannan to promote the growth of lactic acid bacteria. J Sci Food Agric. 92, 2394-2396.

Angelidi, A.M., Stambolliu, E., Adamopoulou, K.I., Kousoulis, A.A., 2018. Is Atorvastatin Associated with New Onset Diabetes or Deterioration of Glycemic Control? Systematic Review Using Data from 1.9 Million Patients. Int J Endocrinol. 2018, 1-18.

Botta, M., Audano, M., Sahebkar, A., Sirtori, C.R., Mitro, N., Ruscica, M., 2018. PPAR agonists and metabolic syndrome: an established role?. Int J Mol Sci. 19, 1-21.

Chang, H-P., Yao, H-T., Chiang, M-T., 2012. effects of high and low molecular weight chitosan on plasma cholesterol, glucose and adipocytokines in diabetic rats induced by streptozotocin and nicotinamide. J Food Drug Anal. 20, 661-667.

Cheang, K-U., Chen, C-M., Chen, C-Y. O., Liang, F-Y., Shih, C-K., Li, S-C., 2017. Effects of Glucomannan Noodle on Diabetes Risk Factors in Patients with Metabolic Syndrome: A Double-Blinded, Randomized Crossover Controlled Trial. J Food Nutr Res. *5,* 622-628.

Chen, H-L., Sheu, W. H-H., Tai, T-S., Liaw, Y-P., Chen, Y-C., 2003. Konjac supplement alleviated hypercholesterolemia and hyperglycemia in type 2 diabetic subjects—a randomized double-blind trial. J Am Coll Nutr . 22, 36–42.

Choi, J-W., Kang, H.W., Lim, W-C., Kim, M-K., Lee, I-Y., Cho, H-Y., 2017. Kefir prevented excess fat accumulation in diet-induced obese mice. Biosci Biotech Bioch. 81, 958–965.

Crespo, M.J., Quidgley, J., 2015. Simvastatin, atorvastatin, and pravastatin equally improve the hemodynamic status of diabetic rats. World J Diabetes. 6, 1168–1178.

deCastro, U.G.M., dos Santos, R.A.S., Silva, M.E., de Lima, W.G., Campagnole-Santos, M.J., Alzamora, A.C., 2013. Age-dependent effect of high-fructose and high-fat diets on lipid metabolism and lipid accumulation in liver and kidney of rats. Lipids Health Dis. 12, 1-11.

Feige, J.N., Gelman, L., Michalik, L., Desvergne, B., Wahli, W., 2006. From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions. Prog Lipid Res. 45, 120–159.

Gholi, Z., Heidari-Beni, M., Feizi, A., Iraj, B., Askari, G., 2016. The characteristics of pre-diabetic patients associated with body composition and cardiovascular disease risk factors in the Iranian population. J Res Med Sci. 21: 1-6.

Gregory, J.W., 2019. Prevention of obesity and metabolic syndrome in children. Front. Endocrinol. 10, 1-9.

Hadisaputro, S., Djokomoeljanto, R.R.J., Judiono, Soesatyo, M.H.N.E., 2012. The effects of oral plain kefir supplementation on proinflammatory cytokine properties of the hyperglycemia Wistar rats induced by streptozotocin. Acta Medica Indonesiana - The Indonesian Journal of Internal Medicine. 44, 100-104.

Harmayani, E., Aprilia, V. , Marsono, Y., 2014. Characterization of glucomannan from *Amorphophallus oncophyllus* and its prebiotic activity *in vivo*. Carbohyd Polym. 112, 475-479.

Hsieh, F-C., Lee, C-L., Chai, C-Y., Chen, W-T., Lu, Y-C., Wu, C-S. 2013. Oral administration of *Lactobacillus reuteri* GMNL-263 improves insulin resistance and ameliorates hepatic steatosis in high fructose-fed rats. Nutr Metab. 10, 1-14.

Janani, C., Kumari, B.D. R., 2015. PPAR gamma gene – a review. Diabetes Metab Syndr. 9, 46–50.

Khan, R.M.M., Chua, Z.J.Y, Tan, J.C., Yang, Y., Liao, Z., Zhao, Y., 2019. From pre-diabetes to diabetes: diagnosis, treatments and translational research. Medicina. 55, 1-30.

Kim, J., Lee, H.S., Lee, K-Y., 2018. Effect of statins on fasting glucose in non-diabetic individuals: nationwide population-based health examination in Korea. Cardiovasc Diabetol. 17, 1-11.

Lee, Y.K., Park, J.E., Lee, M., Hardwick., J.P., 2018. Hepatic lipid homeostasis by peroxisome proliferator-activated receptor gamma 2. Liver Res. 2, 209–215.

Linnemann, A.K., Baan, M., Davis, D.B., 2014. Pancreatic β-Cell proliferation in obesity. Adv. Nutr. 5, 278–288.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 25, 402–408.

Mei, L., Tang, Y., Li, M., Yang, P., Liu, Z., Yuan, J., Zheng, P., 2015. Co-Administration of cholesterol-lowering probiotics and anthraquinone from *Cassia obtusifolia L*. ameliorate non-alcoholic fatty liver. Plos One. 10, 1-16.

Otles, S., Cagindi, O., 2003. Kefir: A probiotic dairy-composition, nutritional and therapeutic aspects. Pak J Nutr. 2, 54-59.

Park, J-E., S.-H. Oh, S-H., Y.-S. Cha, Y-S., 2014. Lactobacillus plantarum LG42 isolated from gajami sik-hae decreases body and fat pad weights in diet-induced obese mice. J Appl Microbiol. 116, 145—156.

Patel, J., Matnor, N. A., Iyer, A., Brown, L., 2011. A regenerative antioxidant protocol of vitamin e and *α*-lipoic acid ameliorates cardiovascular and metabolic changes in fructose-fed rats. Evid Based Complement Alternat Med. 1-8.

Pimenta F.S., Luaces-Regueira M., Ton A.M.M., Campagnaro B.P. , Campos-Toimil M., Pereira T.M.C., Vasquez E.C., 2018. Mechanisms of action of kefir in chronic cardiovascular and metabolic

diseases. Cell Physiol Biochem. 48, 1901–1914.

Reeves, P.G., Neilsen, F. H., Fahey, G.C. JR., 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr. 123, 1939-1951.

Ritzel, R.A., Butler , A.E., Rizza, R.A., Veldhuis, J.D., Butler, P.C., 2006. Relationship between beta-cell mass and fasting blood glucose concentration in humans. Diabetes Care. 29, 717–718.

Rosen, E.D., Sarraf, P., Troy, A.E., Bradwin, G., Moore, K., Milstone, D.S., Spiegelman, B. M., Mortensen, R..M., 1999. PPARγ is required for the differentiation of adipose tissue in vivo and in vitro. Mol Cell. 4, 611–617.

Salunkhe, V.A., Mollet, I.G., Ofori , J.K., Malm, H.A., Esguerra, J.L.S., Reinbothe, T.M., Stenkula, K.G., Wendt, A., Eliasson, L., Vikman, J., 2016. Dual effect of rosuvastatin on glucose homeostasis through improved insulin sensitivity and reduced insulin secretion. EBioMedicine. 10, 185-194.

Sangwan, S., Singh, R., 2014. Therapeutic effects of probiotic fermented milk (LGG and *L. casei* NCDC 19) on progression of type 2 diabetes . J Innov Bio. 1, 078-083.

Schrezenmeir, J., de Vrese, M., 2001. Probiotics, prebiotics, and synbiotics–approaching a definition. Am J Clin Nutr. 73, 361s–364s.

Sobczak, A.I.S., Blindauer, C.A., Stewart, A.J., 2019. Changes in plasma free fatty acids associated with type-2 diabetes. Nutrients. 11, 1- 42.

Takahashi, Y., Ide, T., 2008. Effects of soy protein and isoflavone on hepatic fatty acid synthesis and oxidation and mRNA expression of uncoupling proteins and peroxisome proliferator-activated receptor γ in adipose tissues of rats. J Nutr Biochem. 19, 682–693.

Toshiyuki Takagi, T., Matsuda, M., Abe, M., Kobayashi, H., Fukuhara, A., Komuro, R., Kihara, S., Caslake , M.J., McMahon, A., Shepherd, J., Funahashi, T., Shimomura, I., 2008. Effect of pravastatin on the development of diabetes and adiponectin production. *Atherosclerosis.* 196, 114–121.

van Stee, M.F., de Graaf, A.A., Groen, A.K., 2018. Actions of metformin and statins on lipid and glucose metabolism and possible beneft of combination therapy. Cardiovasc Diabetol. 17, 1-22.

Vázquez-Velasco, M., González-Torres, L., López-Gasco, P., Bastida, S., Benedí, J., González-Muñoz M.J., Sánchez-Muniz, F.J., 2015. Effects of glucomannan/spirulina-surimi on liver oxidation and inflammation in Zucker rats fed atherogenic diets. Journal of Physiology and Biochemistry. 71, 611–622.

Venter, C.S., Vorster, H.H., Van Der Nest, D.G., 1990. Comparison between physiological effects of konjac-glucomannan and propionate in baboons fed"Western" diets. J Nutr. 120, 1046-1053.

Wang, L., Duan, G., Yong Lu, Y., Pang, S., Huang, X., Jiang, Q., Dang ,N., .2013.The effect of simvastatin on glucose homeostasis in streptozotocin induced type 2 diabetic rats. J Diabetes Res. 2013, 1-5.

Zhai, X., Lin, D., Zhao, Y., Li*a,* W., Yang, X., 2018. Enhanced anti-obesity effects of bacterial cellulose combined with konjac glucomannan in high-fat diet-fed C57BL/6J mice. Food Funct. 9, 5260-5272.

Figures

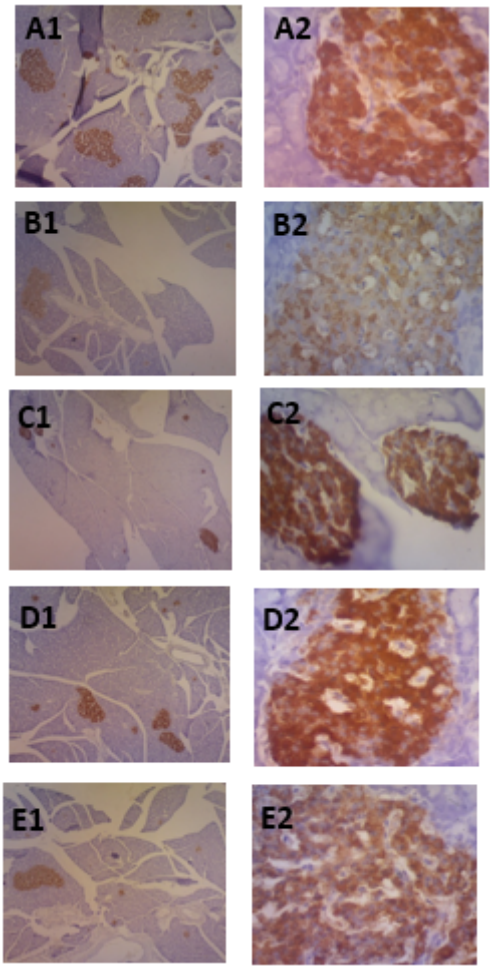

Figure 1

Immunohistochemical of insulin-producing β-cells (showed brown colour). A: normal control; B: HFHF; C: HFHF+kefir; D:HFHF+kefir+glucomannan; E: HFHF+simvastatin. A1-E1 :magnification 40x and A2-E2: magnification 400x.

Supplementary Files

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