Synbiotic Goat Milk Kefir Lowered Peroxisome Proliferator Activated Receptor Gamma (PPAR γ) Gene Expression in Rat Adipose and Liver Tissue

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Research

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Abstract

Background

Kefir is a fermented milk product containing bacteria and yeast, whereas glucomannan from porang (*Amorphophallus oncophyllus*) tuber is known as a prebiotic *in vivo*. Diets with a high fat and high sugar will stimulate metabolic syndrome associated with changes in gene expression including peroxisome proliferator activated receptor gamma (PPAR γ). The purpose of this study was to determine the effect of goat milk kefir enriched with porang glucomannan (synbiotic kefir) and goat milk kefir without glucomannan (probiotic kefir) on blood glucose, hemoglobin A1c (HbA1c), free fatty acid (FFA), tumor necrosis factor alpha (TNF- α), gene expression of peroxisome proliferator activated receptor gamma (PPAR γ), and insulin-producing cells in rats fed a high-fat and high-fructose (HFHF) diet.

Methods

Male Sprague Dawley rats 8–12 weeks old ($n = 30$) treated with HFHF diets for two weeks, and then divided into five dietary groups (each group consisted of 6 rats): 1) normal control (received a standard diet only); 2) rats fed HFHF; 3) rats fed HFHF + probiotic kefir; 4) rats fed HFHF + synbiotic kefir; and 5) rats fed HFHF + simvastatin. The dose of kefir was 3.6 mL/200 g body weight/day and simvastatin was 0.72 mg/day. All of these treatments were carried out for 4 weeks.

Results

There were no significant differences in plasma blood glucose in HFHF rats after and before treatment, but decreased in plasma HbA1c and TNF α ($p < 0.05$) and inhibited the increase of FFA in rats after synbiotic kefir treatment (paired-samples t-test). Probiotic and synbiotic kefir decreased the gene expression of PPAR γ 2 ($p < 0.05$) in both of adipose and liver tissue in HFHF rats but had no effect on the total number of Langerhans islets and insulin-producing cells (one way ANOVA).

Conclusions

Synbiotic kefir could ameliorate the health of rats fed HFHF diet through decreasing HbA1c, TNF α , and PPAR γ 2 gene expression and preventing an increase in FFA. The results indicate that goat milk synbiotic kefir potentially improve metabolic syndrome.

Introduction

Limited physical activity and enhanced exposure to unhealthy foods that are energy-dense (“obesogenic” environment) cause increased obesity. The prevalence of obesity in the last decade is becoming increasingly common and becoming a major nutritional problem throughout the world. The risk factors in

development of obesity are also influenced by genetic factors and physiological problems. Obesity may negatively affect on the development of insulin resistance, type-2 diabetes and metabolic syndrome. Because of the limitations of obesity and metabolic syndrome therapy, prevention strategies are needed [1].

Prevention and treatment of metabolic syndrome can be performed both pharmacologically and nonpharmacologically. Functional food affecting health benefits can be derived from animal or plant sources. Metabolic syndrome can be treated with various approaches, including targeting lipoproteins, blood pressure or anthropometric index. Peroxisome proliferator-activated receptors (PPARs) are play a role in the metabolic control of lipid and lipoprotein levels, i.e. triglycerides (TGs), blood glucose, and abdominal adiposity [2]. PPAR γ is abundantly expressed in adipose tissue and, to a lesser extent, in macrophages and other cell types, and regulates adipogenesis, lipid storage, and glucose homeostasis [3]. PPAR γ 2 is specific for adipose tissue, where it plays a pivotal role in adipogenesis and is an important mediator of insulin sensitivity [4] and a more potent transcription activator [5].

In reducing obesity in mice induced by a high fat diet, a probiotic kefir plays an important role in weight loss and reduce the epididymal fat layer and the diameter of adipocytes. The reduction in gene expression associated with adipogenesis and lipogenesis and also a lowered the levels of proinflammatory markers in epididymal fat has confirmed the role of kefir [6]. Recent studies show that properties of kefir and isolated microorganisms from it have the potential to be anti-atherosclerotic through an enhance in anti-inflammatory cytokines and reduce pro-inflammatory responses [7].

Porang (*Amorphophallus oncophyllus*) is a tuber locally that is often found in Indonesian forests, and it is being cultivated. Similar to *Amorphophallus konjac*, porang tuber contains glucomannan and has been shown to be a prebiotic *in vivo* [8], that selectively enhances the growth of probiotic bacteria such as lactobacilli and bifidobacterial [9]. Glucomannan is a water-soluble dietary fiber that can improve blood sugar, blood fat concentration, and weight management and has other health benefits. Subject with metabolic syndrome will be comfortable consuming glucomannan as a substitute for main carbohydrates, in the form of noodles given 4 weeks can reduce the risk of metabolic syndrome and reduce oxidative stress [10].

The purpose of this study was to evaluate the effect of synbiotic kefir (goat milk kefir with additional glucomannan from porang) and probiotic kefir (goat milk kefir without porang glucomannan) on gene expression associated with the metabolic regulation of lipids and blood glucose, i.e. PPAR γ in adipose and liver tissue in rats fed a high-fat and high-fructose diet.

Materials And Methods

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

Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia. Fresh goat milk was originated from Ettawah Crossbred goats in Yogyakarta, Indonesia. Whey protein concentrate (WPC) was obtained from the Sari Husada Milk industry in Yogyakarta, Indonesia. Kefir grain was purchased from a local supplier in Yogyakarta.

The methods of synbiotic kefir preparation was done according to [11] with slight modification. Goat milk, 0.1% whey protein concentrate (WPC), and 0.3% porang glucomannan were mixed, pasteurized at 75°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 grains. Probiotics kefir were prepared with goat milk, WPC and kefir grain without glucomannan. Synbiotics kefir were prepared by addition of glucomannan into probiotics kefir.

Animal experiments

Male Sprague Dawley rats 8-12 weeks old were divided into 5 groups (each group consisted of 6 rats): 1) normal control (negative control rats) that received a standard diet only, 2) rats fed high-fat/high-fructose (HFHF) (positive control), 3) rats fed HFHF + probiotic kefir, 4) rats fed HFHF + synbiotic kefir, and 5) rats 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.

The rats were adapted with standard diet AIN-93 for 1 week and then treated with a high fat and high fructose diet for 2 weeks. The rats were then divided into 5 groups as described above. A high-fat and high-fructose diet was administered until the end of the experiment (4 weeks). The composition of the standard diet and high-fat and high-fructose diet were prepared according to [12,13] with slight modification. All treatments which given to animal experiments in this study were already approved by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine Universitas Gadjah Mada, Indonesia (Approval number: KE/FK/95/EC/2015).

Table 1 Formulation 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)

Fasting plasma blood glucose was measured by an enzymatic photometric test using the glucose oxidase phenol 4-aminoantipyrine peroxidase (GPO-PAP) method according to the instructions in the kit (Dia Sys, Holzheim-Germany). Glycosylated hemoglobin (HbA1c) analysis was performed according to the instructions in the Rat HbA1c ELISA Kit (ELabScience, Wuhan, China). Analysis of plasma FFA was performed according to the instructions of the Rat FFA ELISA Kit (Qayee-Bio, Shanghai, China). Plasma TNF α was analyzed according to the instructions in rat-specific ELISA kits for the measurement of TNF α (eBioscience, Bender MedSystem, Vienna, Austria).

Gene expression analysis

PPAR γ 2 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 TRIzol reagent, and mRNA levels were analyzed by real-time polymerase chain reaction (PCR). Reverse transcription of total RNA was performed using the First Strand cDNA Synthesis Kit (Roche) Transcriptor. Transcription reagents to produce cDNA. Real-time PCR was carried out in a mixture (final volume 20 μ L) containing 2 μ L cDNA (DNA template), 10 μ L Evagreen, 1 μ L GAPDH, 1 μ L GAPDH, and 6 μ L RNase-free water. Likewise for Forward and Reverse PPAR γ 2 with additional reagents totaling 20 μ L as well. The amount of mRNA was calculated as the ratio to the value of glyceraldehyde-3-phosphate dehydrogenase (GADPH) in each cDNA sample. The primary nucleotide sequences used to detect each mRNA were designed using Primary Express Software according to the sequences available in the GenBank database. The primary nucleotide sequences are shown in Table 2 [14].

Table 2 Primer nucleotide sequences for real-time PCR

Genes		Primer	Length of PCR products (bp)	GenBank accession no.
PPAR γ 2	Sense	5'-ACTCTGGGAGATCCTCCTGTTG-3'	68	Y12882
	Antisense	5'-GAAGTGCTCATAGGCAGTGCAT-3'		
GAPDH	Sense	GCC GAG GGC CCA CTA AAG	70	BC059110
	Antisense	TGC TGT TGA AGT CAC AGG AGA CA		

Optimization of cDNA amplification products was performed using conventional PCR with a program at a temperature of 95°C for 5 minutes, 95°C for 1 minute, 58°C for 1 minute, and 72°C for 1 minute with 34x cycles. The temperature was maintained at 72°C for 5 minutes and 12°C for 5 minutes. The optimization program for real-time (RT)-PCR was at a temperature of 95°C for 5 minutes, 95°C for 1 minute, 60°C for 30 seconds and 72°C for 1 minute with 39x cycles. The melt curve was maintained at 65-95°C for 5 seconds, and then the plate was read. The average change in the level of gene expression ($2^{-\Delta\Delta CT}$) PPAR γ -2 was analyzed according to [15].

Immunohistochemistry of insulin-producing cells

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

Statistical analysis

Data from this analysis are presented as the 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 before and after treatments was analyzed by paired-samples t-test. One-way ANOVA followed by Duncan's multiple range test (DMRT) was used for statistical analyses of the gene expression of PPAR, total number of Langerhans islets and insulin-producing cells (p-values of less than 0.05 indicated significant differences). Statistical analyses were performed using SPSS version 17 software.

Results

Blood glucose

The average fasting blood glucose levels in rats fed a high fructose and high fat diet before and after being treated with probiotic kefir, synbiotic kefir and simvastatin are shown in Table 3.

Table 3 The average blood glucose in rats 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+ probiotic kefir	112.81 ±9,54 ^a	114.34 ±18,81 ^a
HFHF+synbiotic kefir	116.98 ±6,76 ^a	105.08 ±11,93 ^a
HFHF+simvastatin	221,37 ±6,76 ^a	82,35 ±11,93 ^b

Different letters in the same row indicate significant differences (P<0,05)

Table 3 shows that in negative controls (normal rats, only receiving standard diet), blood glucose levels were still within the normal glucose range, and there was no difference before and after treatment. Rats that only received high-fructose and high-fat feed (positive control) showed higher glucose levels after being given the diet for 5 weeks (after treatment) compared to before treatment, although not significantly. Goat milk kefir enriched with porang glucomannan could reduce blood glucose levels, but the decrease was not significant, which was only 11.9 mg/dL. Simvastatin treatment significantly reduced blood glucose levels in rats fed a HFHF diet, which was approximately 139.02 mg/dL.

Hemoglobin (Hb)A1c

Based on Table 4, HbA1c levels in rats after treatment with synbiotic kefir were lower ($p<0.05$) than those before treatment. However, other groups of rats, including those who received probiotic kefir treatment, did not show any significant difference before and after treatment.

Table 4 The average HbA1c in rats before and after treatment

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+probiotic kefir	26,02±4,79 ^a	35,44±18,99 ^a
HFHF+synbiotic kefir	28,89±4,12 ^a	23,56±3,47 ^b
HFHF+simvastatin	22,72±4,64 ^a	33,61±16,45 ^a

Different letters in the same row indicate significant differences ($P<0,05$)

Free fatty acid (FFA)

Table 5 shows that the average plasma FFA levels in rats after various treatments were higher ($p<0.05$) in all groups of rats than before treatment, although the increase in FFA after kefir treatment was not significant.

Table 5 The average plasma FFA in rats before and after various treatments

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

Different letters in the same row indicate significant differences ($P<0,05$)

Tumor necrosis factor alpha (TNFα)

Based on Table 4, there was no decrease in TNFα levels in rats after various treatments, except in rats treated with synbiotic kefir.

Table 6 The average plasma TNFα in rats before and after treatment

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

Different letters in the same row indicate significant differences ($P<0,05$)

PPARγ-2 gene expression

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

Table 7 Average relative expression of PPAR γ 2 genes in white adipose tissue from rats receiving various treatments

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

Different letters in the same column indicate significant differences ($p < 0,05$)

Normal control rats had an average ΔCT of - 3.07, an average $\Delta\Delta CT$ of 0.00 and an average of changes in the gene expression of PPAR γ 2 ($2^{-\Delta\Delta CT}$) of 1.00.

Based on Table 8, the pattern of the changes in the expression of PPAR γ 2 genes in liver tissue was similar to that of white adipose tissue, in which rats treated with kefir added or without glucomannan showed lower changes in gene expression ($p < 0.05$) compared to rats without kefir treatment. Likewise, the simvastatin-treated rats also had lower changes in PPAR γ 2 gene expression ($p < 0.05$) than the HFHF rats.

Table 8 Average relative expression of PPAR γ 2 genes in liver tissue from rats receiving various treatments

Treatments	PPAR γ 2 gene expression		
	ΔCT	$\Delta\Delta CT$	$2^{-\Delta\Delta CT}$
HFHF	0,00 $\pm$ 1,59 ^a	-6,28 $\pm$ 1,59 ^a	123,46 $\pm$ 120,56 ^a
HFHF+ probiotic kefir	1,88 $\pm$ 1,12 ^b	-4,41 $\pm$ 1,12 ^b	27,25 $\pm$ 21,01 ^b
HFHF+ synbiotic kefir	3,84 $\pm$ 0,89 ^c	-2,44 $\pm$ 0,89 ^c	6,33 $\pm$ 3,78 ^b
HFHF+ simvastatin	2,77 $\pm$ 1,97 ^{bc}	-3,51 $\pm$ 1,99 ^{bc}	19,79 $\pm$ 17,89 ^b

Different letters in the same column indicate significant differences ($p < 0,05$)

Normal control rats had an average ΔCT of 6.29, $\Delta\Delta CT$ of 0.00 and an average of changes in gene expression of PPAR γ 2 ($2^{-\Delta\Delta CT}$) of 1.00.

Table 7 and Table 8 showed that the change in PPAR γ 2 gene expression in liver tissue was higher than that in adipose tissue. In the rats fed a high-fructose and high-fat (HFHF) diet without kefir supplementation, the highest changes in PPAR gene expression ($p < 0.05$) were observed in both adipose and liver tissue.

Immunohistochemical (IHC) of β -cells

The average number of Langerhans islets and insulin-producing beta cells with various treatments is shown in Table 9.

Table 9 The average number of Langerhans islets and insulin-positive β -cells in rats with various treatments

Treatments	Langerhans ^{ns}	Insulin-positive β -cells ^{ns}
Normal control	3.04 $\pm$ 0.78	96.95 $\pm$ 94.19
HFHF	2.47 $\pm$ 0.76	46.08 $\pm$ 2,59
HFHF+ probiotic kefir	2.90 $\pm$ 0.71	71.74 $\pm$ 22.42
HFHF+ synbiotic kefir	3.55 $\pm$ 0.65	82.14 $\pm$ 45.27
HFHF+ simvastatin	3.05 $\pm$ 0.82	107.35 $\pm$ 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 not significantly different (Table 9).

Immunohistochemical (IHC) staining of pancreatic tissue showed that insulin-producing beta cells showed a brown color when using rat anti-insulin antibodies (Fig.1). Figure 7 shows that HFHF rats were rarely observed on Langerhans islets and had very weak intensity of IHC staining on insulin-producing beta cells, and there were few insulin-producing beta cells (Fig. 1), although the number of Langerhans islets and beta cells in all treatment groups was not significantly different (Table 9). Rats that received kefir treatment showed intense beta cell staining intensity as in negative control rats (normal normal rats). The HFHF rats given simvastatin showed less strong IHC staining intensity compared to kefir treatment.

Discussion

All rats fed a high fat and high fructose diets demonstrated a risk factor for metabolic syndrome with fasting blood glucose > 100 mg/dL [2]. After kefir treatments (probiotic and synbiotic kefir) in this study, there were no decrease in blood glucose in rats fed HFHF diet, except for simvastatin treatment. 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 [16]. In contrast to a previous study by [17], skim milk kefir given at a dose of 3.6 ml/day for 4 weeks could significantly reduce blood glucose levels by 111.00 mg/dL. In the present study, the decrease in low blood glucose was possibly because synbiotic kefir was still not enough to play a role in reducing blood glucose in rats that consumed HFHF diets during the experiment. In the study by [17], diabetic rats were not fed a HFHF diet. The low dose of glucomannan in kefir and the difference in the conditions of the subjects may not cause a significant reduction in blood glucose levels.

The decrease in blood glucose by simvastatin treatment in this study is in accordance with a previous study by [18], in which mice fed a high-fat diet and treated with rosuvastatin showed lower blood glucose, which might be due to improved glucose uptake, but beta cell activity is inhibited through lowered insulin

levels and inhibited Ca^{2+} signaling in beta cells, resulting in lowered insulin secretion. Double effects on glucose homeostasis by rosuvastatin are due to increased insulin sensitivity, while beta cell activity is inhibited. In another study by [19], glucose uptake in adipose tissue was upregulated in pravastatin-treated mice fed a high fat/high sucrose diet and db/db mice. In contrast to studies by [20, 21], simvastatins can increase the risk of 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 levels. Atorvastatin at a high dose causes worsening of glycemic control in patients with DM [22]. According to [23], individual types of statins may have different effects on glucose metabolism. 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.

Porang glucomannan added to kefir could improve glucose metabolism to reduce glycosylated hemoglobin. According to [24], the synergistic effects of these two components, probiotics and prebiotics, make it a more effective supplement than probiotics or prebiotics separately. In another study by [25], the fructose diet was rapidly metabolized by the liver, causing changes in carbohydrate and lipid metabolism as well as hepatic inflammation, which led to the development of hyperglycemia, insulin resistance, hyperinsulinemia, and hypertriglyceridemia as major risk factors for diabetes complications. 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 [26]. However, the results indicate that the administration of fermented milk containing the probiotic *Lactobacillus rhamnosus* GG (150 g/kg standard diet) can reduce the increase in glycosylated hemoglobin (HbA1c) in rats induced by diabetes by feeding high fructose feeds [26]. Additionally, the 24 individuals with T2DM had significantly decreased HbA1c by 7.7% after glucomannan noodle intervention [10].

Probiotic and synbiotic kefir in the present study 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 [27]. The lower dose of glucomannan from porang tuber in the present study compared to the previous study by [27] resulted in no decrease in plasma FFA. According to [28, 27], increased levels of circulating FFAs can stimulate fibrinogen synthesis in the liver. Elevated plasma fibrinogen is characteristic of insulin resistance in the liver (insulin may regulate the synthesis of fibrinogen). Glucomannan from konjac, which is fermented in the colon, can decrease FFA production, including propionate, leading to a decrease in fibrinogen synthesis. Therefore, colonic production and absorption of SCFAs (propionate) from soluble fiber may contribute to this fiber's metabolic effects [27]. The various physiological processes, including the control of lipolysis and lipogenesis in adipose tissue, inflammation, endocrine signaling and the composition and characteristics of cellular membranes may be affected by each kind of FFA. The progress of insulin resistance and coagulatory damage may result from the physiological changes caused by changed plasma FFA levels or profiles [29].

In the present study, 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 disorders is in accordance with a previous study using chitosan fiber [30], 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 α and plasminogen activator inhibitor-1 (PAI-1).

In other study, supplementation with a 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 the levels of TNF α and IL-6 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 [31]. 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 [32].

Normally, PPAR γ 2 is most abundantly expressed in adipocytes and plays major adipogenic and lipogenic roles in the tissue [33]. Because the rats in the present study received a high-fat and high-fructose diet, it was possible to cause fatty liver. According to [34, 35], in non-alcoholic fatty liver disease (NAFLD) patients and experimental animals there was an increase in the expression of PPAR γ in the liver. In addition, in mice fed a high-fat diet showed a high PPAR γ expression in the liver [36].

In the present study, the change in gene expression was the lowest in rat tissue that was treated with synbiotic kefir, although this difference was not significant compared to probiotic kefir treatment. It is possible that kefir-containing probiotics synergize with the prebiotic glucomannan and play a role in the downregulation of PPAR γ 2 expression in white adipose and hepatic tissue. The result in the present study was similar to that of a previous study [37], in which mice fed a high-fat diet supplemented with 0.2% kefir powder for 8 weeks lowered PPAR γ gene expression in the epididymal fat. In another study, 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 reduced PPAR γ expression in adipose tissue [38]. Decreased levels of PPAR- γ and GLUT4 mRNA after high fructose treatment were also enhanced by *Lactobacillus reuteri* GMNL-263 administration [39]. It was further emphasized by [31], besides reducing PPAR γ expression, the mixed bacterial cellulose and glucomannan from konjac also lowered the protein expression of PPAR γ by reducing the size of cells in the adipose tissue of high-fat diet-fed mice.

Consumption of dietary fibers, especially mixed bacterial cellulose/konjac glucomannan, 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 malondialdehyde (MDA) in the liver. Moreover, supplementation with these fibers regulated the levels of leptin and adiponectin and inhibited the protein expression of PPAR γ by reducing the size of cells in the adipose tissue of high-fat diet-fed mice [31]

The highest changes in PPAR γ 2 gene expression in both adipose and liver tissue of rats treated HFHF without kefir in the present study were in accordance with the results in a previous study [40], who found that the PPAR γ expression level was significantly higher in rats fed a high-fat diet than in rats fed a normal diet, which is mainly related to fat formation. PPAR γ 2 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 [33]

There was no change in the number of Langerhans islets and insulin-producing beta cells in all treatments, indicating that a high-fructose high-fat diet received during the experiment did not cause β -cell damage. This was also evidenced by unchanged average fasting blood glucose levels in HFHF rats before and after being treated with kefir (Table 2). According to [41], individuals with type 2 diabetes have decreased β -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 [42]. 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. A high fat and high fructose diets in the present study had not yet led to diabetes but only caused prediabetes because blood glucose levels ranged from 100 mg/dL to 125 mg/dL, which is at risk of becoming diabetic (≥ 126 mg/dL), whereas normal blood glucose was < 100 mg/dL [43, 44].

Immunohistochemical staining showed that rats fed HFHF diet without kefir addition had the weakest color intensity. However, rats fed HFHF diet with probiotic or synbiotic kefir showed a strong color intensity as in normal rats (Fig. 1). These result indicate that the probiotic microorganisms in kefir have an important role in improving insulin-producing β -cells. This was supported by a previous studies on diabetic rats treated with konjac extract (containing glucomannan) alone showed less strong in improving insulin-producing β -cells than the rats treated with combination of konjac and inulin extract [45].

Conclusions

The metabolic syndrome caused by the habit of consuming high-fat and high-fructose diets can be improved by consuming synbiotic kefir, through decreasing HbA1c, TNF α , and gene expression of PPAR γ 2 and preventing the increase in FFA. Therefore, synbiotic kefir containing porang glucomannan is expected to be a suggestion for the food industry to develop synbiotic-based functional foods that have the potential to improve metabolic syndrome.

Abbreviations

HFHF: High-fat high-fructose; PPAR γ : Peroxisome proliferator activated receptor gamma; HbA1c: Hemoglobin A1c; TNF α : Tumor necrosis factor alpha; FFA: Free fatty acid; IHC: Immunohistochemical.

Declarations

Ethics approval

All treatments which given to animal experiments in this study were already approved by the Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine Universitas Gadjah Mada, Indonesia (Approval number: KE/FK/95/EC/2015).

Data Availability

The original data used to present results of this study are available upon request.

Conflict of Interest

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

Authors' Contributions

EH had a great contribution on research planning, discuss and review paper. S and N had a contribution to overall planning, preparing and performing this research.

Consent for publication

All authors support submission to this journal.

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Figures

Figure 1

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