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Research Article

Effects of prebiotics, probiotics, and synbiotics on the body weight, blood glucose, triglyceride and TNF-α of diet-induced obesity rats

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Abstract

High-fat diet leads to obesity-associated chronic low-grade inflammation. Prebiotics, probiotics, and synbiotics produced short-chain fatty acids (SCFA), bonded to *G protein-coupled receptors* (GPR)-41 and GPR-43 decreased triglyceride deposits in adipocytes and liver, decreased fatty acid oxidation, increased glucose regulation and insulin sensitivity thus reduced the risk of obesity and metabolic syndrome. This study conducted in order to evaluate the effects of prebiotics, probiotics, and synbiotics on the body weight, blood glucose, triglyceride, and TNF-α used rats model, which were fed by a high-fat diet. Thirty-eight 6-8 weeks old male rats were fed by high-fat diet for three weeks, then rats were randomly divided into four groups, high-fat diet (HFD), a high fat diet with prebiotics supplementation (HFD+ PRE), a high fat diet with probiotics supplementation (HFD+PRO), and high-fat diet with synbiotics supplementation (HFD+SYN) for three weeks. Blood samples and body weight were measured at the third and sixth week. There was no effect of prebiotics, probiotics, and synbiotics on body weight, triglyceride levels, blood glucose, and TNF-α in rats fed a high-fat diet compared to control. These results suggested that supplementations gave inconsistent results with other studies and needed further researches.

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INTRODUCTION

The prevalence of obesity in adulthood had doubled since 1980, estimated in 2015, one third of the world's population suffered from obesity (Chooi, Ding, & Magkos, 2018). Consumption of high fat diet was one of the causes of obesity (Xu & Xue, 2016).

Obesity caused inflammation of adipose tissue called meta-inflammation (Reilly & Saltiel, 2017), released pro-inflammatory cytokines interleukin (IL)-12, IL-17, IL-1β, tumor necrosis factor (TNF)-α, interferon (IFN)-γ thus promoted M-1 polarization (Li et al., 2018), so TNF-α was one of the cytokines closely related to metabolic syndrome (Indulekha, Surendar, & Mohan, 2011).

TNF-α, IL-6, leptin, and free fatty acids (FFA) caused insulin receptor substrate (IRS)-1 and (IRS)-2 degradation, promoted insulin resistance (Kwon & Pessin, 2013) characterized by the decrease of glycogenesis and glucose uptake, and lipolysis (Samuel & Shulman, 2016).

The effects of prebiotics, probiotics, and synbiotics in obesity are increasingly being studied. Supplementations produced short-chain fatty acids (SCFA), bonded to *G protein-coupled receptors* (GPR)-41 and GPR-43 decreased triglyceride deposits in adipocytes and liver, decreased fatty acid oxidation, increased glucose regulation and insulin sensitivity (Winer, Luck, Tsai, & Winer, 2016) (Tunapong et al., 2018) (Markowiak & Ślizewska, 2017).

However, other studies mentioned opposite results (Luo, Yperselle, Rizkalla, Rossi, & Bornet, 2000) (Million et al., 2012), so that supplementations required further researches before established it as an additional therapy for obesity.

METHODS

Animals

Adult Male Wistar rats (Marques et al., 2016) (n = 18), 6-8 weeks, were obtained from the Biochemistry Department (Universitas Airlangga, Indonesia). All rats were caged with a 12 h light/dark cycle, fed by standard diet, and water *ad libitum*. After a week of adaptation, all rats were fed by a high-fat diet for three weeks. Then, rats were randomly divided into four groups, high-fat diet (HFD); high-fat diet + prebiotics supplementation (HFD+ PRE); high-fat diet + probiotics supplementation (HFD+PRO); and high-fat diet + synbiotics supplementation (HFD+SYN) for three weeks. Bodyweight, triglyceride, blood glucose, and TNF-α were measured at the end of the third and sixth week when the highest effects occurred. Ethical approval was obtained from the Health Research Ethical Clearance Commission, Universitas Airlangga Faculty of Dental Medicine No. 534/HRECC.FODM/VII/2019.

Standard diet

Standard diet used comfeed 593® (PT. Charoen Pokphand Indonesia). Feed compositions were protein (15%), fat (3%), fiber (8%), and ash (6%). Every rat was given a standard diet everyday *ad libitum* intended to gain the rat's nutritional needs that couldn't be obtained by providing a high-fat diet.

High-fat diet

Cow brain 1 g/rat/day (Abdel-Hafez, Othman, & Seleim, 2011) and egg yolk powder 0.5 g/rat/day diluted with aquadest. Rats were fed by using gastric tube 2 ml/rat/day (Alioes, Sukma, & Sekar, 2019). High-fat diet administration was given every day at 9.00 am.

Supplementations

Prebiotics consisted of mix FOS and GOS (0.5 g/kg body weight/day) (Kao, Spitzer, Anthony, & Lennox, 2018). Probiotics consisted of 1 x 10^{10} CFU/ml mix strain *Lactobacillus casei,*

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Lactobacillus rhamnosus, Lactobacillus acidophilus, and *Bifidobacterium spp.* (10 ml/kg body weight/day) (Nimgampalle & Kuna, 2017). Both were obtained from the Faculty of Sains and Technology (Universitas Airlangga, Indonesia). Synbiotics were made by mixing both ingredients. Supplementation was administered by using a gastric tube right after high-fat diet administration to reduce stress.

Bodyweight measurement

The rat was put on the plastic bowl on the weighing scales, and carefully adjusted the weight.

Blood collection

Two cc blood was collected in a red-topped tube, put in the cool box, and transferred to the laboratory. Blood samples were centrifuged at 2,000 x g, 4º C for 10 minutes to obtain blood serum.

Serum analysis

Triglyceride and blood glucose were performed in a spectrophotometer using Rajawali commercial kit no. 116392® GPO PAP method and no. 112191® GOD PAP method. TNF-α was measured in the ELISA-Sandwich method used Elabscience® reagen.

Data analysis

Pre and post-test data were analyzed using a *paired t-test* (if data were normally distributed) or *Wilcoxon test* (if data weren't normally distributed). Comparative tests between the control group and supplementation groups used *independent t-test* (if data were normally distributed) or *Mann Whitney* (if data weren't normally distributed). Statistical tests used SPSS version 22, p-value < 0,05 was considered as a significant value.

RESULTS

Supplementations effect on body weight

A high-fat diet increased body weight before and after the intervention. The supplementation of prebiotics, probiotics, and synbiotics could not control the increase of body weight caused by a high-fat diet ($p < 0,01$) so that until the third week of treatment, there was still an increase in body weight (Table 1). There were no significant differences in body weight between the control and treatment groups (Table 2)

Supplementations effect on blood glucose

Supplementation of prebiotics, probiotics, and synbiotics did not change glucose levels. There was only a downward trend of glucose level in HFD+PRE and HFD+SIN groups (Table 3). There were no significant differences in blood glucose levels between the control and treatment groups (Table 4).

Table 1. Effect of supplementations on body weight within groups

Groups	Pre-supplementations	Post-supplementations	p-value
	Mean ± SD	Mean ± SD	
HFD	174.57 ± 29.478	235.29 ± 41.299	0,011
HFD + PRE	176.71 ± 24.336	224.71 ± 26.329	0,001
HFD + PRO	159.44 ± 25.870	213.89 ± 51.910	0,001
HFD + SIN	190.78 ± 31.352	253.44 ± 48.161	0,001

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Table 2. Effect of supplementations on body weight among groups

Groups	Δ Body Weight	p-value
	Mean ± SD	
HFD	60,71 ± 44,195	0,337
HFD + PRE	48,00 ± 19,079	
HFD	60,71 ± 44,195	0,461
HFD + PRO	54,44 ± 30,373	
HFD	60,71 ± 44,195	0,628
HFD + SIN	62,67 ± 38,380	

Table 3. Effect of supplementations on blood glucose within groups

Groups	Pre-supplementations	Post-supplementations	p-value
	Mean ± SD	Mean ± SD	
HFD	176.29 ± 29.607	147.86 ± 15.453	0,118
HFD + PRE	179.86 ± 47.386	135.43 ± 22.315	0,0502
HFD + PRO	168.44 ± 28.426	172.67 ± 21.413	0,105
HFD + SIN	168.56 ± 33.201	144.56 ± 11.865	0,755

Table 4. Effect of supplementations on blood glucose among groups

Groups	Δ Blood glucose	p-value
	Mean ± SD	
HFD	-28,43 ± 41,299	0,949
HFD + PRE	-44,43 ± 48,100	
HFD	-28,43 ± 41,299	0,550
HFD + PRO	4,22 ± 39,280	
HFD	-28,43 ± 41,299	0,668
HFD + SIN	-24,00 ± 39,446	

Table 5. Effect of supplementations on triglyceride within groups

Groups	Pre-supplementation	Post-supplementation	p-value
	Mean ± SD	Mean ± SD	
HFD	17,365 ± 3,077	21,285 ± 6,111	0,271
HFD + PRE	17,259 ± 5,646	17,003 ± 3,466	0,499
HFD + PRO	17,347 ± 6,882	20,546 ± 8,318	0,400
HFD + SIN	17,251 ± 1,450	30,568 ± 23,600	0,058

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Table 6. Effect of supplementations on triglyceride among groups

Groups	Δ Trigliserida *Mean* ± SD	p-value
HFD	9.29 ± 23.556	0,848
HFD + PRE	-20.00 ± 90.618	
HFD	9.29 ± 23.556	0,169
HFD + PRO	13.33 ± 34.681	
HFD	9.29 ± 23.556	
HFD + SIN	-19.22 ± 25.044	0,900

Table 7. Effect of supplementations on TNF-α within groups

Groups	Pre-supplementation *Mean* ± SD	Post-supplementation *Mean* ± SD	p
HFD	17,3649 ± 3,07651	21,2849 ± 6,11110	0,176
HFD + PRE	17,2589 ± 5,64610	17,0030 ± 3,46623	0,866
HFD + PRO	17,4369 ± 6,88219	20,5463 ± 8,31814	0,314
HFD + SIN	17,2514 ± 1,44980	30,5678 ± 23,60047	0,008

Table 8. Effect of supplementations on TNF-α among groups

Groups	Δ TNF-α *Mean* ± SD	p
HFD	3,9200 ± 7,32187	0,915
HFD + PRE	-2,559 ± 6,59710	
HFD	3,9200 ± 7,32187	0,742
HFD + PRO	3,1094 ± 7,79678	
HFD	3,9200 ± 7,32187	0,266
HFD + SIN	13,3163 ± 23,73930	

Supplementations effect on triglyceride

High-fat diet administration for 3 weeks had not been able to give an effect of increasing triglyceride levels. Most are still in the normal range of 82.70 ± 7.60 (Mesomya, Hengsawadi, & Cuptapun, 2001) (Ihedioha, Noel-uneke, & Ihedioha, 2013) (Table 5), so there was no difference between the control and treatment groups in reducing triglyceride levels (Table 6).

Supplementations effect on TNF-α

Gastric sonde to administer a high-fat diet caused an increase in TNF-α levels. This was also seen in the control group. The supplementation of prebiotic, probiotic, and synbiotic had not been able to reduce TNF-α levels due to gastric sonde installation; even there was a significant increase in the HFD + SIN group (p = .008) (Table 7). No significant differences were found between the control and treatment groups in TNF-α levels (Table 8).

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DISCUSSION

Effects of Probiotics, Prebiotics, and Synbiotics on Body Weight

Probiotics had been widely used for the treatment of diarrhea and inflammatory bowel disease, but some researchers revealed other uses of probiotics on weight loss (Karimi et al., 2015) (Paturi et al., 2015) (Nicolucci et al., 2017). Debates continued to emerge regarding the anti-obesity effect; another meta-analysis study actually stated the opposite, said that probiotics actually caused weight gain (Million et al., 2012). The weight gain effect might be influenced by the type of bacterial strain.

This study used a combination of several different species and genera of bacteria because it was more effective than a single strain (Chapman, Gibson, & Rowland, 2011), but other studies revealed that *Lactobacillus acidophilus* actually caused weight gain (Arora et al., 2012) (Million et al., 2012), whereas *Bifidobacterium spp* has the opposite effect (Ji et al., 2019), so there was no anti-obesity effect.

Another lack of the trial was a relatively short time of high-fat diet administration. An increase in body weight was estimated to continue within a period of 0-6 months. The first two months were the fastest, the next two months slowed down, and the lowest effect occurred in the last two months (Hafizur, Raza, Chishti, Shaukat, & Ahmed, 2015). In this study, rats were given a high-fat diet only three weeks, so the treatment did not provide the expected results because the weight gain effect still occurred. After 3 weeks of administration, weight was within the normal range (Nistiar, Racz, & Novakova, 2012) and likely to increase until six months ahead.

Supplementation of prebiotics, probiotics, and synbiotics reduced body weight due to increase secretion of anorexic hormones GLP-1 and PYY due to SCFA binding to their receptors, which decreased appetite (Tolhurst et al., 2012) (Fukui et al., 2018). The gastric tube was used to administer a high-fat diet, so there is no effect of decreasing appetite because the number of diets given every day was constant.

Effects of Probiotics, Prebiotics, and Synbiotics on Triglycerides

Supplementations reduced triglyceride levels (Choi et al., 2016) (Miao et al., 2016) through AMPK phosphorylation (den Besten et al., 2013), which triggers lipid oxidation (Jeon, 2016). However, based on existing reference values, rat triglyceride levels were within the normal range of 82.70 ± 7.60 (Mesomya et al., 2001) (Ihedioha et al., 2013). 3 weeks administration period was not enough to induce hypertrygliceride, which began to increase at week 9 (Marques et al., 2016).

Effects of Probiotics, Prebiotics, and Synbiotics on Blood Glucose

Decreased blood glucose levels caused by SCFA, caused AMPK phosphorylation leading to GLUT-4 translocation to the plasma membrane, which increased glucose uptake to cells (Jeon, 2016).

Glucose and triglyceride levels only experienced a downward trend, because rats were not yet in hypertriglyceridemia and hyperglycemic condition, so supplementation had only a slight effect when compared to the opposite situation, according to the other study stated that the magnitude of the effect was determined by hyperglycemic conditions before the intervention (Ruan et al., 2015).

Effect of Probiotics, Prebiotics, and Synbiotics on TNF-α levels

Gastric sonde used to administer high-fat diets or supplementations of prebiotics, probiotics, and synbiotics caused an increase in cortisol,

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and TNF-α levels indicated a response to stress (Lalive et al., 2002) (Walker et al., 2012). There was an upward trend in almost all groups. The increase of TNF-α was not a marker of adipocyte tissue inflammation but only a response to stress due to gastric sonde installation. In addition, the installation of gastric sonde could also cause death due to aspiration into the respiratory tract. A total of 4 rats died as a result of gastric distension, which triggered aspiration into the lungs (Damsch et al., 2011).

Use of TNF-α for monitoring the therapeutic effect of prebiotic, probiotic, and synbiotic was inappropriate because TNF-α levels do not accurately correlate with changes in fat mass (Bedoui et al., 2005) (Wu et al., 2016), lipid levels (Reinehr et al., 2005), and blood glucose (Choi et al., 2004). While other pro-inflammatory cytokines CRP, are more significantly correlated with BMI, blood pressure (Koenig et al., 1999), triglycerides (Yudkin et al., 1999), glucose (Bahceci et al., 2005), and obesity (Marques -vidal et al., 2012) (Fernandez-Berges et al., 2014).

CONCLUSION

Previous studies suggested that supplementations gave inconsistent results and needed further researches to establish a standard regarding dosage, time of administration, bacterial strains, and type of prebiotics before took prebiotics, probiotics, and synbiotics as an alternative therapy for the obesity problem. A high-fat diet and supplementation might have been too short of making any significant effect. Diet administration through oral (*ad libitum*) or gastric sonde should take into consideration, related to outcomes that influenced the results and implementation to humans.

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