



High Fiber and Beta Carotene from Sweet Potatoes and Pumpkin Improve Insulin Resistance by Inhibition of Sterol Regulatory Binding Protein 1c in Liver of Hypertriglyceridemic Rats

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Abstract

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BACKGROUND: High sterol regulatory binding protein 1c (SREBP-1c) gene expression increases triglyceride synthesis, which induces insulin resistance. Short-chain fatty acids (SCFAs) from fiber fermentation and beta carotene may inhibit SREBP-1c gene expression.

AIM: The aim of this study was to evaluate the high fiber and beta carotene diet on improving insulin resistance in hypertriglyceridemic rats.

METHODS: A total of 25 Wistar male rats were divided into five groups: (1) Normal control (NC); (2) hypertriglyceridemia control (HC); (3) hypertriglyceridemia rats with treatment 1 (HT1); (4) hypertriglyceridemia rats with treatment 2 (HT2); and (5) hypertriglyceridemia rats with treatment 1 (HT3). The HT1, HT2, and HT3 received fiber 1.0 g; 2.0 g; and 3.1 g and beta carotene 725.7 µg; 1451.5 µg; and 2177.2 µg per day, respectively, for 6 weeks. The HC received high fat and fructose diet and the NC received a standard diet. The levels of triglyceride were analyzed using the colorimetric method before and after treatment. At the end of the study, the expression of SREBP-1c was identified by a quantitative polymerase chain reaction.

RESULTS: High fat and fructose diet increased the levels of triglyceride (36.53 ± 1.27 vs. 119.79 ± 7.73), but high fiber and beta carotene diet can reduce triglyceride levels in HT1 (94.58 ± 4.53 vs. 77.70 ± 7.97); HT2 (115.58 ± 4.76 vs. 66.90 ± 3.11); and HT3 (103.87 ± 7.47 vs. 62.06 ± 4.45). The decreased triglyceride levels were related to low SREBP-1c gene expression, especially in the liver. Low SREBP-1c gene expression was correlated with homeostatic model assessment of insulin resistance index with $r = 0.414$; $p < 0.05$ in the liver and $r = 0.158$; $p > 0.05$ in white adipose tissues.

CONCLUSION: High fiber and beta carotene diet can improve insulin resistance through inhibition of SREBP-1c gene expression.

Introduction

High dietary fiber intake has been known to reduce the risk of type 2 diabetes mellitus (T2DM) [1]. Fiber, especially soluble fiber, has been reported to improve insulin resistance and metabolic profiles in T2DM patients [2], [3]. In our previous study showed that *Dioscorea esculenta* can improve the homeostatic model assessment of insulin resistance (HOMA-IR) through increasing insulin receptor substrate 1 (Irs1) expression and reducing plasma glucose levels [4]. Administration of high fiber snacks that made of *D. esculenta*, arrowroot, pumpkin, and cassava for T2DM patients can also reduce insulin resistance (HOMA-IR) [5]. Fiber or resistant starch can be fermented by colonic bacteria to produce short-chain fatty acids, mainly butyric acids, propionic acids, and acetate acids short-chain fatty acid (SCFAs) [6].

Butyrate can prevent insulin resistance through its actions in peripheral tissues [7] and acetate has an important role in regulating insulin sensitivity and body weight through its effects on glucose homeostasis and lipid metabolism [8]. Oral administration of SCFAs reduces the expressions of gene that related to lipid metabolism [9]. One of the genes related to lipid metabolism is sterol regulatory element-binding protein 1c (SREBP-1c), which is the main transcriptional regulator of fatty acid and triglyceride synthesis in the liver [10]. Hepatic SREBP-1c mediates the effect of insulin stimulation on fatty acid synthesis [11]. SREBP-1c controls the synthesis of enzymes involved in the synthesis of sterol, fatty acid, and triglyceride and has implications for insulin resistance in skeletal muscle and the pathogenesis of beta-cell dysfunction [12]. SREBP-1c expression is mainly in the liver, white adipose tissue, adrenal gland, skeletal muscle, and brain [13]. Ruiz *et al.* [14] reported that SREBP-1c contributed to hepatic lipid accumulation and insulin resistance.

Insulin resistance can be assessed using the HOMA-IR [15], which can be improved by the administration of beta carotene from pumpkin (*Cucurbita maxima*) [16]. Beta carotene is a member of carotenoids and as an antioxidant that can prevent oxidative stress and is reported to help prevent the development of T2DM, which is characterized by insulin resistance [17]. Oxidative stress has been known to be strongly associated with impaired glucose metabolism and is reported as one of the key players in the progression of insulin resistance and T2DM [18]. Therefore, this study evaluates the benefits of a high fiber and antioxidant diet on the relationship between SREBP-1c gene expression and triglyceride levels and HOMA-IR in hypertriglyceridemia rats.

Materials and Methods

Study design

A total 25 Wistar male rats, aged 8 weeks, body weight 180–200 g were divided into five groups: (1) Normal control (NC); (2) hypertriglyceridemia control (HC); (3) hypertriglyceridemia rats with treatment 1 (HT1); (4) hypertriglyceridemia rats with treatment 2 (HT2); and (5) hypertriglyceridemia rats with treatment 3 (HT3). The HT1, HT2, and HT3 received fiber 1.0 g; 2.0 g; and 3.1 g and beta carotene 725.7 µg; 1451.5 µg; and 2177.2 µg per day, respectively. A high fat and fructose diet was used to induce hypertriglyceridemia for 7 weeks, and the rats with plasma triglyceride levels were >70.79 mg/dL which were considered hypertriglyceridemia [19]. High fat and fructose diet was made by substituting sucrose into fructose and corn starch into trans-fat [20], [21].

The rats were acclimatized using a modified AIN-93M formulation (L-cystine was replaced by DL-methionine and choline bitartrate by choline chloride) and water *ad libitum* [22] for 7 days in individual cages. The rats were kept under standard conditions (light/dark cycle 12:12 h and room temperature 22–25°C). In 100 g, normal diet contains 61.94 g corn starch, 14 g casein, 10 g sucrose, 4 g corn oil, 5 g alpha-cellulose, 3.5 g mineral mixture, 1 g vitamin mixture, 0.3 g DL-methionine, 0.25 g choline chloride, and 0.008 g tetrabutylhydroquinone. The HT1, HT2, and HT3 intervention diets were made with corn starch substitution using sweet potatoes and pumpkin and contain fiber 6.88 g; 13.77 g; and 20.65 g and beta carotene 4838.2 µg; 9676.5 µg; and 14514.7 µg, respectively, per 100 g diet. These treatment diets were given for 6 weeks. The content of fiber and beta carotene in diet was examined by the Center for Food and Nutrition Studies, Universitas Gadjah Mada. All procedure described involving animals in this research was approved by the Ethics Committee of the Integrated Research and Testing Laboratory,

Universitas Gadjah Mada, Yogyakarta, Indonesia, with approval number 00065/04/LPPT/ 2017.

Biochemical analysis

Blood samples were taken from orbital sinuses before and after the intervention. To obtain the plasma, the ethylenediaminetetraacetic acid (EDTA)-blood samples were centrifuged for 15 min, 3000 rpm, at room temperature. Plasma-EDTA was used to analyze triglyceride levels using the colorimetric method (DiaSys, Holzheim, Germany). HOMA-IR was determined using formula: fasting plasma insulin (ng/mL) × fasting plasma glucose (mg/dL)/405 [23].

Isolation of RNA and quantitative polymerase chain reaction (q-PCR)

Total RNA was isolated from frozen liver and white adipose tissue using TRizol reagent (Invitrogen, USA). Reverse transcription with 1 µg total RNA was done based on the protocol from Revert Aid First Strand cDNASynthesis Kit (Thermo Scientific, USA). The q-PCR analysis was done using SsoFast Eva Green Supermix (Bio-Rad, United Kingdom) and the total reaction for q-PCR was 10 µL. The results were normalized against beta-actin. The primer sequences used in this study are shown in Table 1. The thermocycling conditions in this reaction were: 5 min at 95°C, 1 min at 95°C, followed by 62.2°C at 1 min for SREBP-1c. The q-PCR cycles were set at 40 cycles.

Table 1: Primer sequence

Gene	Primer	Ref.
SREBP-1c (rat)	Forward 5'-CTGTCGTCTACCATAAGCTGCAC-3'	Liu et al. [24]
	Reverse 5'-ATAGCATCTCCTGCACACTCAGC-3'	
Beta-actin	Forward 5'-ACGGTCAGGTCATCACTATCG-3'	
	Reverse 5'-GGCATAGAGGTCCTTACGGATG-3'	

Statistical analysis

All data value presented as mean ± standard error of mean (SEM). One-way ANOVA was used to analyze triglyceride levels and index of HOMA-IR between intervention groups. Paired t-test was used to analyze the changes of triglyceride levels and index HOMA-IR before and after intervention. Pearson correlation was used to analyze the correlation between triglyceride levels and index of HOMA-R with SREBP-1c gene expression both in liver and adipose tissue. Difference considered as statistically significant at $p < 0.05$.

Results

This study showed that high fat and fructose diet increased the levels of triglyceride and insulin

resistance (high HOMA-IR), in other hands, high fiber and beta carotene diet can reduce it in the hypertriglyceridemia rats (Figure 1a and 1b). The triglyceride levels of the rats with high fat and fructose diet were significantly higher than those in normal rats, reducing of triglyceride levels in HT2 and HT3 groups was significant. Whereas, the HOMA-IR value in HT2 group was smaller than HT1 and HT3 although it was not significantly different.

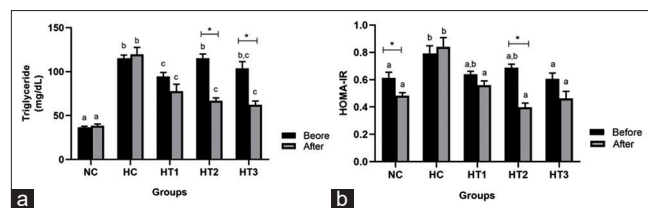


Figure 1: (a and b) Changes of triglyceride levels and HOMA-IR before and after treatment. NC: Normal control; HC: Hypertriglyceridemia control; HT1: Hypertriglyceridemia rats with treatment 1; HT2: Hypertriglyceridemia rats with treatment 2; HT3: Hypertriglyceridemia rats with treatment 3. Superscript^{a,b}, and^c indicate $p < 0.05$ according to one-way ANOVA test followed by Tukey test. Superscript^{a,b} indicate no difference between^a nor^b. Superscript^{b,c} indicate no difference between^b nor^c. *Mark indicate $p < 0.05$ according to paired t-test

Decreased triglyceride levels in rats after consumption of high fiber and beta carotene diet were seen to relate with the SREBP-1c gene expression in the liver ($r = 0.689$; $p < 0.05$) and the adipose tissue ($r = 0.264$; $p > 0.05$) (Figure 2).

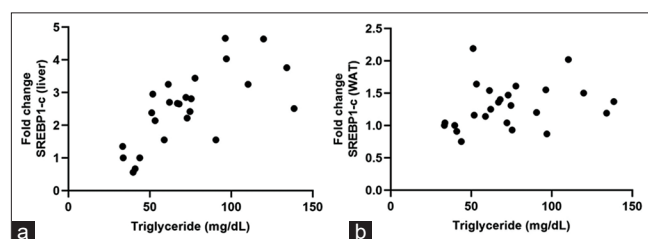


Figure 2: (a and b) Correlation between triglyceride levels and SREBP-1c gene expression both in liver ($r = 0.689$; $p < 0.05$) and white adipose tissue (WAT) ($r = 0.264$; $p > 0.05$)

This study showed that significant correlation between HOMA-IR with SREBP-1c gene expression in the liver ($r = 0.414$; $p < 0.05$), but it was not significant in the adipose tissues ($r = 0.158$; $p > 0.05$) after 6 weeks administration of high fiber and beta carotene diet (Figure 3).

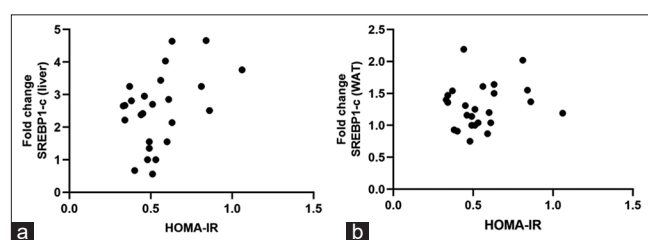


Figure 3: (a and b) Correlation between HOMA-IR and SREBP-1c gene expression both in liver ($r = 0.414$; $p < 0.05$) and white adipose tissue (WAT) ($r = 0.158$; $p > 0.05$)

The effect of high fiber and beta carotene diet on SREBP-1c gene expression in the liver was more

pronounced than in adipose tissue. The effect was significantly found only in HT2 in the liver (Figure 4).

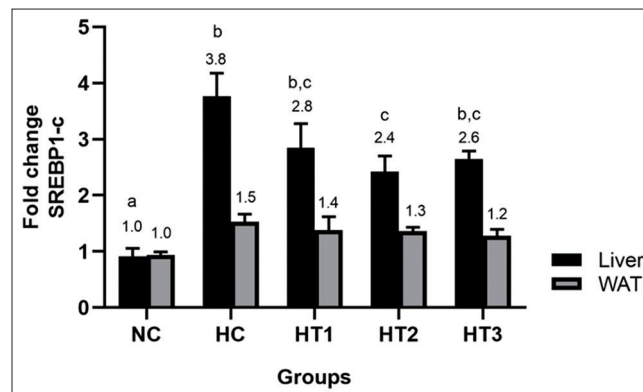


Figure 4: SREBP-1c gene expression in liver and white adipose tissue. NC: Normal control; HC: Hypertriglyceridemia control; HT1: Hypertriglyceridemia rats with treatment 1; HT2: Hypertriglyceridemia rats with treatment 2; HT3: Hypertriglyceridemia rats with treatment 3. Superscript^{a,b}, and^c indicate $p < 0.05$ according to one-way ANOVA test followed by Tukey HSD test. Superscript^{b,c} indicate no difference between^b nor^c

Discussion

High fat and fructose intake had been reported to increase fasting triglyceride levels and develop insulin resistance in animals' studies [25]. In this study, a high fat and fructose diet also increased levels of fasting triglycerides and HOMA-IR index (Figure 1a and 1b). According to Tranchida *et al.* [26], consumption of high-saturated fatty acids is related to hyperinsulinemia in rats. On the other hand, fructose affects the homeostasis of lipid metabolism in the liver [27].

Hyperinsulinemia is a symptom of insulin resistance that is, directly and indirectly, contributes to T2DM [28], [29]. Hyperinsulinemia can induce SREBP-1c, which is a master regulator of lipogenic gene expression in the liver and contributes to hepatic lipid accumulation and insulin resistance [14]. SREBP-1c regulates the synthesis of enzymes involved in the synthesis of sterols, fatty acids, and triglycerides and is reported to be involved in T2DM, insulin resistance in skeletal muscle, and the pathogenesis of beta-cell dysfunction [12]. HOMA-IR is commonly used to assess insulin resistance. After a high fiber and beta carotene diet, the levels of triglyceride and HOMA-IR index decreased (Figure 1a and 1b).

A meta-analysis of the effectiveness of dietary fiber in T2DM showed that there was a statistically significant relationship between high dietary fiber intake and reduction in the relative risk of T2DM [1]. Previous research reported that a diet rich in pro-vitamin A carotenoids can help prevent the development of T2DM which is characterized by insulin resistance [17]. In this study, the greatest reduction in HOMA-IR index was

found in (HT2) rats on a diet containing 13.77 g fiber and 9676.5 µg beta carotene per 100 g or the rats consuming around 2.07 g fiber and 1451.5 µg beta carotene per day. Beta carotene is a plant pigment that has biological antioxidant properties and as a nutritional precursor of Vitamin A. Our previous research showed that beta carotene from pumpkin (*C. maxima*) can improve of HOMA-β cell function in hypercholesterolemia rats. The best improving HOMA-β was seen in rats that received 0.64 g pumpkin powder/200 BW [16].

Many studies showed the benefit of dietary fiber. Chen *et al.* [2] reported that regular consumption of soluble dietary fiber can significantly improve insulin resistance and metabolic profiles in T2DM patients. In our previous study showed that a combination *D. esculenta* containing high fiber and resistant starch, with *Eubacterium rectale* or only *D. esculenta* can reduce the levels of plasma glucose and HOMA-IR index through increased of Irs1 expression [4]. Fiber-rich snacks made from *D. esculenta*, arrowroot, pumpkin, and cassava can also reduce insulin resistance (HOMA-IR) [5]. The benefit of fiber that reduces insulin resistance may be related SCFAs, especially butyric acid, propionic acid, and acetate acid, which is fiber fermentation product by colonic bacteria. According to McNabney and Henagan [7], butyrate could prevent insulin resistance through its actions in peripheral tissues and dietary strategy by increasing butyrate levels may be used to treat T2DM. Whereas, acetate plays an important role in regulating body weight and insulin sensitivity through effects on lipid metabolism and glucose homeostasis [8].

In this study, improving insulin resistance could also be caused by beta carotene in pumpkin. Beta carotene has been known as an antioxidant that can prevent oxidative stress. High fat and fructose diet have been reported to trigger oxidative stress, which can stimulate insulin resistance. Moreno-Fernández *et al.* [30] reported that high fat and glucose diet increased oxidative stress, the levels of fasting plasma glucose, and insulin. Oxidative stress is a factor that is strongly associated with impaired glucose metabolism and insulin resistance. High fat and fructose consumption causes oxidative stress in mice that showed increased activities of superoxide dismutase, catalase, and glutathione peroxidase enzymes [31]. Excess nutrition, including fat and glucose, promotes endoplasmic reticulum stress, which contributes to increased oxidative stress. Oxidative stress is one of the key players in the development of insulin resistance and T2DM [18]. According to Hurrle and Hsu [32], free fatty acids accelerate mitochondrial fission and increase the production of reactive oxygen species (ROS). ROS interferes with the transduction of insulin receptor signals which reduces the expression of glucose transporter Type 4 transporters in the cellular membranes, therefore, causing insulin resistance.

Insulin resistance can be associated with suppression of the Irs1 gene transcription caused by

binding of SREBP-1c with the promoter of Irs1 [13] and causes abnormal insulin signaling. In this study, although not statistically significant, there was a positive correlation between HOMA-IR and SREBP-1c gene expression in the liver and adipose tissues (Figure 3). SREBP-1c is mainly expressed in the liver, white adipose tissue, adrenal glands, skeletal muscle, and brain [9]. In this study, the high fat and beta carotene diet can reduce SREBP-1c gene expression which is stronger in the liver than in adipose (Figure 4). This means that SREBP-1c gene expression in the liver is more easily influenced by the nutritional components. Hashidume *et al.* [33] reported that the administration of a soy protein diet decreased in hepatic SREBP-1c mRNA.

Decreased expression of SREBP-1c gene may be an effect SCFAs resulting from the fermentation of dietary fiber by colonic bacteria. A previous study reported that oral administration of SCFAs decreased RNA expression of lipid metabolism-related genes, including SREBP-1c in liver [9]. SREBP-1c is a member of SREBPs and as the main transcriptional regulator of fatty acids and triglyceride synthesis through inducing mRNAs encoding enzymes that catalyze various steps in the fatty acids and triglyceride synthesis pathways in the liver [10]. Karasawa *et al.* [11] reported that hepatic SREBP-1c controls plasma lipoprotein rich in triglycerides and mediates the effects of insulin stimulation on fatty acid synthesis. Insulin increases the mRNA amount of SREBP-1c in the isolated hepatic cell of rats. In this study, triglyceride levels correlated with SREBP-1c gene expression in liver and white adipose tissue (Figure 2) but were not statistically significant. Overall, data in the present study provide information about the beneficial effects of a high fiber and beta carotene diet in reducing triglyceride levels and improving insulin sensitivity. This mechanism might be related to the suppression of SREBP-1c gene expression, especially in liver.

Conclusion

High fiber and beta carotene diet can reduce triglyceride levels and improve insulin sensitivity through suppression of SREBP-1c gene expression, especially in liver.

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