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Beet (*Beta vulgaris*) Suppressed Gene Expression and Serum Fatty Acid Synthase in High Fat and Fructose-induced Rats

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Abstract

BACKGROUND: The expression and activity of fatty acid synthase (FAS) enzymes determine *de novo* fatty acid synthesis, which can be enhanced by a high-fat and high fructose diet or inhibited by some bioactive compound diets. Beets are a great source of therapeutic compounds that have the potential to improve health and prevent disease.

AIM: This study examined the effects of beets on liver FAS gene expression and FAS levels.

METHODS: A total of 25 male Wistar rats divided into five groups: (1) Standard diet (n); (2) high fat and fructose diet (HFFD); (3) HFFD have given beet 6%-contained standard diet (B1); (4) HFFD have given beet 9%-contained standard diet (B2), and (5) HFFD have given beet 12%-contained standard diet (B3). The HFFD was given to rats in the 2, 3, 4, and 5 group diets for 8 weeks? and then 3, 4, and 5 groups received beet-contained standard diet for 6 weeks. At the end of the intervention, FAS levels were measured (please specify where it was measured from) using the ELISA method, liver FAS gene expression was analyzed by quantitative polymerase chain reaction, and triglyceride (TG) levels were determined by the colorimetric method.

RESULTS: The beet-substituted diet significantly suppressed the hepatic FAS gene expression and decreased the serum FAS levels in rats previously given HFFD ($p < 0.05$). The expression of the FAS gene showed a significant positive correlation with the levels of FAS serum ($p < 0.05$), and also with the hepatic TG levels but not significant ($p > 0.05$). Substitution of beet 9% in diet gives the best effect in hepatic FAS gene expression and the serum FAS levels.

CONCLUSIONS: The diet contained beet 9% was seen as a necessary physiological dose to improve the effects of high-fat and diet fructose diet through suppressing FAS gene expression and a decreased serum FAS levels.

Introduction

Fatty acid synthase (FAS) enzyme catalyzes *de novo* synthesis of fatty acids that can be stored, used membrane assembly and repair, or secreted in the form of lipoprotein triglycerides (TG). In *de novo* lipogenesis (DNL), FAS catalyzes saturated fatty acids biosynthesis from simple precursors with the primary product palmitate (C16:0). However, stearate (C18:0) and shorter fatty acids may also be produced. The substrates of FAS are acetyl-CoA, malonyl-CoA, and NADPH. The liver can uptake, synthesize, store, secrete, and catabolize fatty acids and TG. FAS enzyme may be a target of therapeutics to treat fatty liver and dyslipidemia, and dietary fat and fructose had been known effect to regulate of FAS in the liver [1].

Some previous studies showed that consuming high fat (HF) and high fructose affects high TG synthesis. Rats fed high fructose and saturated fatty acid diet for 10 weeks had an increase in the levels of total cholesterol and TG. Compared to the control group, they also had proportions of monounsaturated FAs such as palmitoleic acid [16:1(n-7)] and oleic acid [18:1(n-9)] higher, but the proportions of some polyunsaturated n-6 FAs, such as linoleic acid [18:2(n-6)] and arachidonic acid [20:4(n-6)] lower [2]. Zaki *et al*. [3] reported that there was an increase of hepatic TG in rats given a diet with HF, high fructose (HFr), and HF and high fructose (HFHFr) mean values by 83%, 50%, and 112%, respectively, compared to those in the control group. Fructose has lipogenic properties that can trigger TG synthesis [4] and consumption of fructose had effects significantly on the liver and skeletal muscle, increased weight, triggers lipogenesis, and metabolic disorders [5].

Meanwhile, metabolic disorders due to malnutrition such as hyperlipidemia can be prevented or treated with nutritional therapy containing bioactive compounds. Some studies reported the potential of red beet (*Beta vulgaris* rubra) in health-promoting and disease prevention. According to Clifford *et al*. [6], beet is a dietary source of health that potentially provide therapeutic substances for several pathological disorders such as cardiovascular disease. Several studies demonstrated the constituents of beet have potent antioxidant, anti-inflammatory, and vascular-protective *in vitro* and *in vivo* human and animal

models. In human studies, it has been shown that beet supplementation lowers blood pressure, weakens inflammation, prevents oxidative stress, maintains endothelial function, and restores cerebrovascular hemodynamics. El-Hawary *et al*. [7] identified eight phenolic compounds, including apigenin and luteolin from *B. vulgaris* that have antioxidant activities. Apigenin reported decreased levels of plasma free fatty acid, total cholesterol, apolipoprotein B, and markers of liver dysfunction as well as increased hepatic steatosis and hepatomegaly, without altering dietary intake and adiposity in obese rats induced by a high-fat diet. The effect of apigenin is caused by upregulation of gene expression that regulates several metabolic pathways such as lipolytic and lipogenic genes and decreased activity of enzymes responsible for the synthesis of TG and cholesterol esters in the liver [8]. Meanwhile, luteolin is a flavonoid that has been reported to suppress hepatic lipogenesis and lipid absorption, thereby improving hepatic steatosis and attenuating hepatic lipotoxicity by increasing the expression of PPARg protein in adipose tissue in mice fed high-fat feed. In addition, luteolin also upregulates the expression of genes that control lipolysis and the tricarboxylic acid cycle before the formation of lipid droplets leads to decreased adiposity [9]. Therefore, we evaluated the effect of beet on FAS gene expression and the level of FAS serum in rats with HF and high fructose diet.

Methods

Animals and experimental studies

Twenty-five male Wistar rats (*Rattus novergicus*) (4 weeks old; 100–150 g) were obtained from the Faculty of Pharmacy, Gadjah Mada University. All rats were individually in cages and maintained under standard conditions (12:12-h light/dark cycle and 22–25°C room temperatures). The rats were acclimated for 7 days by a standard diet and water *ad libitum*, and then they were divided into five groups. Group 1 received standard diet (n); Group 2 given HF and fructose diet (HFFD); whereas Groups B1, B2, and B3 after received HFFD for 8 weeks treated by beet-substituted standard diet with percentage 6%, 9%, and 12%, respectively, for 6 weeks. The standard diet was prepared in accordance with the modified-AIN-93M formulation; the HF and fructose and the intervention diets were done by modification of standard diet with composition in Table 1. The beet was obtained from the traditional market, washing and blending before to substitute in the diet. The consuming diets were calculated by weighing the diet and the remaining in the next day and the bodyweight of the rats were monitored weekly. At the end of the experiment, after fasting about 8 h, the animals were sacrificed, and blood was collected from vena retro orbital to analyze levels of FAS. The hepatic tissue was collected to analyze gene expression of FAS and TG level. This study has been approved by the Ethical Commission of Integrated Research and Testing Laboratory (LPPT) Gadjah Mada University Yogyakarta, number: 00011/04/LPPT/V/2019.

Table 1: Composition of diets

Substance (g/kg Diet)	Standard diet (n)	High fat and fructose diet	Intervention diet with 6% beet	Intervention diet with 9% beet	Intervention diet with 12% beet
Cornstarch	621	300	561	531	501
Casein	140	140	140	140	140
Sucrose	100	-	100	100	100
Trans fat	-	214	-	-	-
Fructose	-	250	-	-	-
Bit	-	-	60	90	120
Alfa cell (Fiber)	50	50	50	50	50
Mineral mix (AIN'93M-MX)	35	35	35	35	35
Vitamin mix (AIN'93-VX)	10	10	10	10	10
Methoinine	1.8	1.8	1.8	1.8	1.8
Choline chloride	2.5	2.5	2.5	2.5	2.5
Tert-butylhydroquinone	0.008	0.008	0.008	0.008	0.008

Laboratory analyses

To obtain serum, 2 mL vena retro-orbital blood was collected in a microtube and centrifuged for 15 min at 3000 rpm. The top layer as the serum was taken and used to determine the FAS using the Enzyme-Linked Immunosorbent Assay (ELISA) method based on the FineTest kit protocol. Hepatic TG levels were measured by colorimetric method, using TG Diasys Kit. The Expression of FAS gene was analyzed by quantitative polymerase chain reaction (qPCR) on a Bio-Rad CFX96 instrument, and β-actin was used as a standard control of the expression. mRNA isolation from hepatic tissue was done using Tri-RNA solution (Favorgen) based on the manufacturer's protocol. The RNA concentration was quantified by nanodrop, and conversion of RNA to cDNA was done by using Revertaid first-strand cDNA synthesis kit (ThermoScientific, USA). The cDNA was mixed with 2X Fast Q-PCR Master Mix (SYBR, no ROX) (SMOBIO), and qPCR was done using the following protocol; early denaturation at temperature 95°C for 5 min, denaturation 95°C for 1 min and annealing 60.2°C for 1 min, and elongation 72°C for 1 min with 40 cycles. The primary sequences of FAS and β-actin (purchased from Genetica Science) are listed in Table 2.

Table 2: Primer sequences for quantitative polymerase chain reaction

Gen	Primer	Polymerase chain reaction product
Fatty acid synthase	Forward: ACCTCATCACTAGAAGCCACCAG Reverse: GTGGTACTTGGCCTTGGGTTTA	116 bp
Beta aktin	Forward: TGTGGATTGGTGGCTCTATC Reverse: AGAAAGGGTGTAAAACGCAG	149 bp

Statistical analysis

The expression of FAS gene and FAS serum levels was analyzed using the One-Way ANOVA test and followed with Tukey honest significance difference

test. Whereas post- and pre-group design of FAS serum levels was analyzed using Paired Sample t-Test. The correlation between FAS serum levels and expression of FAS gene, as well as hepatic TG levels, was analyzed with the spearman correlation test. The limitation for statistical significance was set at $p < 0.05$. The results are expressed as the means ± standard deviation (SD).

Results

Serum FAS

The HFFD significantly increased the serum FAS levels in HFFD group ($p < 0.05$), whereas the beet-substituted diet significantly reduced serum FAS levels in Groups B1, B2, and B3 groups which received a diet containing beet 6%, 9%, and 12%, respectively ($p < 0.05$), for 6 weeks (Table 3) (Any paragraph explaining the results illustrated in a table should be placed under its table).

Table 3: The serum FAS levels in rats before and after given by beet-substituted diet

Group	n	Serum FAS (pg/mL)		Δ Serum FAS	p*
		Before	After		
Normal (N)	5	526.93 ± 25.29	547.29 ± 7.37[a]	20.36 ± 18.90	0.074
HFFD	5	564.80 ± 24.44	592.72 ± 29.76[b]	27.92 ± 14.35	0.012
B1	5	566.61 ± 39.37	489.58 ± 12.78[c]	−77.03 ± 32.58	0.006
B2	5	576.95 ± 40.89	549.55 ± 28.72[a]	−27.40 ± 18.80	0.031
B3	5	570.23 ± 22.50	551.62 ± 28.76[ab]	−18.61 ± 7.46	0.005
P		0.135	0.00		

Values are presented as mean±SD. [a,b,c]indicate $p < 0.05$ in One-Way ANOVA test followed by Tukey honest significance difference test. [a,b]indicate no difference either a or b. p in a row means the difference FAS serum before and after administration in the same group. P in the last column indicates the differences in FAS serum between the group. FAS: Fatty acid synthase.

The levels of FAS serum with hepatic TG had a positive correlation, but it was not statically significant ($p > 0.05$) (Figure 1).

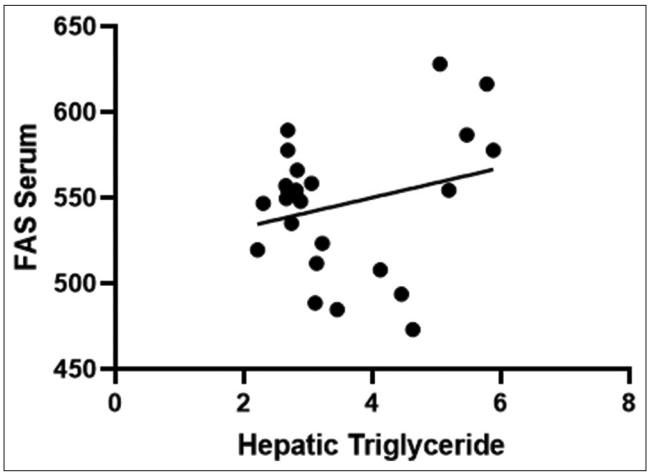

Figure 1: Correlation between the levels of fatty acid synthase serum and hepatic triglyceride

The beet-substituted diet also affects the hepatic FAS gene expression where the rats in B1, B2, and B3 groups showed a statistically significant reduced FAS gene expression than in rats with HFFD ($p < 0.05$). Compare to the N group, the hepatic FAS gene expression in the B1 was significantly lower; however, it in the B2 and B3 were not quite different (Figure 2).

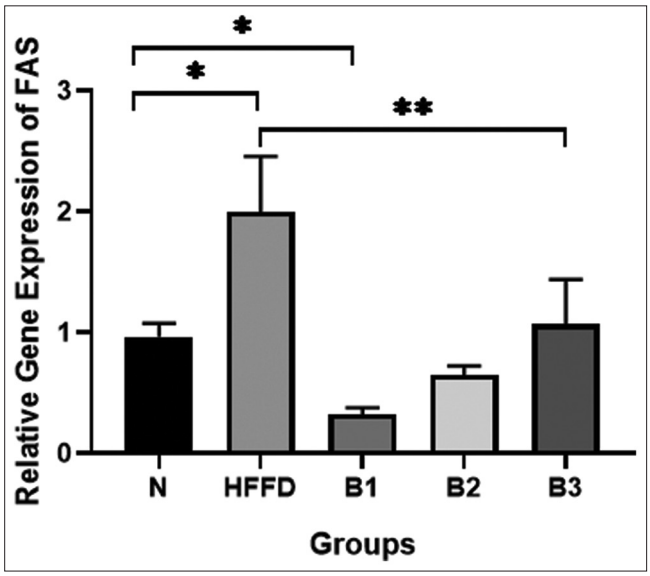

*Figure 2: Expression of hepatic fatty acid synthase gene. Data are shown as a mean ± SD (n = 5). *Significant difference ($p < 0.05$) compared with the normal group. **Significant difference ($p < 0.05$) compared with HFFD group. Significance difference according to ANOVA followed by the Tukey HSD: Honest significant difference test. N: Normal; HFFD: High fat and fructose diet; B1: HFFD with beet 6 g/rat/day; B2: HFFD with beet 9 g/rat/day; B3: HFFD with beet 12 g/rat/day*

The expression of FAS gene showed a positively significant correlation with the levels of FAS serum ($p < 0.05$) (Figure 3), and also with the hepatic triglyceride levels but not significant ($p > 0.05$) (Figure 4).

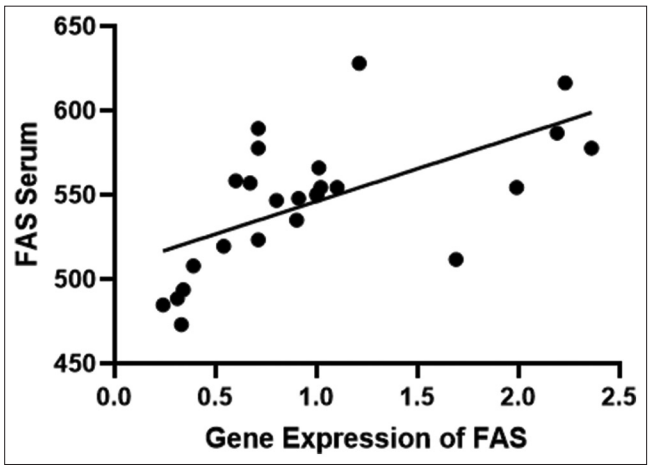

Figure 3: Correlation between fatty acid synthase (FAS) serum and expression of mRNA FAS. R and p values for the correlation should be added under the figure

Discussion

This study showed that the HFFD increased the serum FAS levels, and beet-substituted diet can reduce it

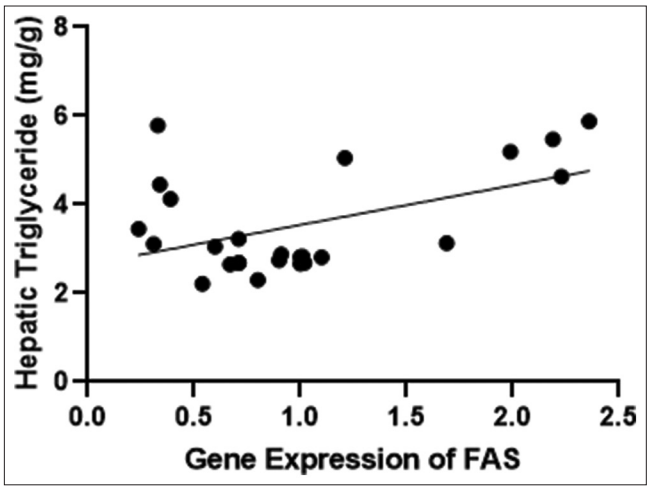

Figure 4: Correlation between the expression of fatty acid synthase gene and hepatic triglyceride levels was positive but not significant. R and p values for the correlation should be added under the figure

(Table 1). Previous study by Chanmin *et al*. [10] reported that a high-fat diet causes a significant hyperlipidemia and hepatic lipid accumulation. In addition, a high-fructose diet able to support enhanced hepatic in DNL through raising the enzyme levels involved in DNL. It has a stronger effect than a high-fat diet. Fructose delivered to the liver through a portal vein is much higher as compared to other tissues, and elevated protein levels of all enzymes involved in DNL during its conversion into TG (this paragraph needs to be revised). As like as other carbohydrates, fructose is metabolized into two carbons intermediate acetyl-CoA which activates the lipogenic transcriptional factors SREBP1c and ChREBP in the liver, stimulating every step of DNL [11]. DNL is the conversion of circulation carbohydrates into fatty acids that can be used to synthesize TG. In the liver, it is highly regulated at the transcription level that major involved SREBP-1c [12]. Maithilikarpagaselvi *et al*. [13] reported that high-fructose increased the expression of SREBP1c and FAS in the rat liver. FAS is an essential DNL enzyme that is involved in the lipid metabolism. Original FAS from the liver is reported in the blood circulation and associated with low-density lipoprotein (LDL) cholesterol. LDL may serve as a carrier for FAS in the circulation [14]. This data were in accordance with our results that showed there was a positive correlation between the levels of FAS serum and the expression of FAS gene ($p < 0.05$) (Figure 3).

In addition, the levels of FAS gene expression showed a significant positive correlation with fat content in the liver. It showed that FAS plays an important role in the synthesis of fat in the liver [15]. In the present work, a positive correlation existed between FAS gene expression of and hepatic TG levels, although it was not significant (Figure 4). FAS expression and its enzymatic activities reported an increase in the liver of murine models of obesity and non-alcoholic fatty liver diseases. FAS plays an important role as a key rate-limiting enzyme in palmitate synthesis, where palmitate can be modified by elongase and desaturase to produce various fatty acids. The rate of incorporation of DNL-synthesized FA into very low-LDL (VLDL)-TG is positively correlated with increased plasma VLDL-TG content. Therefore, increased FAS expression increases the availability of DNL-synthesized FA for TG synthesis and VLDL production and can lead to hypertriglyceridemia [16].

Our study showed that the beet-substituted diet could suppress FAS gene expression. The effect of this beet substitution may be related to its content of apigenin and luteolin in the red beet. Apigenin can regulate the expression of lipolytic and lipogenic genes and decrease the activity of enzymes responsible for the hepatic TG synthesis [8], and luteolin can suppress hepatic lipogenesis and lipid absorption, increase the expression of PPARg protein in adipose tissue, upregulates the expression of genes controlling lipolysis [9]. According to Jung *et al*. [8], long-term supplementation of flavonoid antioxidants for rats induced by high-fat diets can improve the condition the liver dyslipidemia and steatosis by controlling TG synthesis and cholesterol esterification and decreased expression of lipogenic and lipolytic genes in the liver. On other hand, the antioxidant FYGL extracted from *Ganoderma lucidum* reduces TG and total cholesterol content in liver cells by increasing the phosphorylation of AMP-activated protein kinase and acetyl-CoA carboxylase, which causes inhibition of FAS expression [17]. El-Hawary *et al*. [7] it is reported that apigenin and luteolin in red beet have antioxidant activities. Therefore, the effects of red beet in lowing FAS gene expression can be related to the antioxidant properties of apigenin and luteolin.

Conclusions

The beet-substituted diet can suppress the FAS gene expression and decrease serum FAS levels in rats induced by high-fat and high fructose diets. The diet contained beet 9% seen as a physiological dose that needed to improve the effects of high-fat and fructose diet.

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