Beet (Beta vulgaris) Improve Blood Glucose and AKT2 Gene Expression in High Fat and Fructose-induced Rats

M. Windi Dona Fitri, Arta Farmawati, Sunarti Sunarti

Department of Biochemistry, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract

BACKGROUND: Diet components significant effects on glucose homeostasis. A diet contains high saturated fat and fructose induces insulin resistance and enhanced blood glucose. In contrast, food containing flavonoids such as beet can improve glucose homeostasis via modulation of gene expression, for example, AKT2, involving glucose metabolism.

AIM: This study was to evaluate the benefit of beet on AKT2 gene expression and fasting glucose (FG).

METHODS: Twenty Wistar male was divided into five groups: Normal were fed a normal diet, group HFFD was given a diet containing high fat and fructose, and three groups (HFB1, HFB2, HFB3) were given a diet containing high fat and fructose for 8 weeks and continuous fed beet-contained normal diet for 6 weeks. The percentage of beet in the diet for each 6%, 9%, and 12%, respectively.

RESULTS: The FG was measured before and after the intervention, whereas the gene expression of AKT2 at skeletal muscle tissue was determined after the intervention. A diet high in fat and fructose increased FG levels, and a beet-contained diet decreased it.

CONCLUSIONS: The beet 9% substituted diet can improve glucose homeostasis from the effects of a high fat and fructose diet, and the expression of the AKT2 gene may have a role in the process.

Introduction

The composition of the diet has significant and clinically relevant effects on circulating glucose, and it is influenced by food components, such as form, kind, and amount. High saturated fat and high fructose have been known can induce insulin resistance in tissues and enhanced blood glucose. The rats fed a diet containing high fat or high fat and also a high fructose diet had enhanced blood glucose. The rats fed a diet containing high polyphenol diet can influence gene expression, for example., AKT2, involving glucose metabolism. In mice and humans, loss of function mutations in AKT2, including skeletal muscle, liver, and adipose tissue, were reported to relating glucose intolerant and insulin resistance because specifically in skeletal muscle, were reported to relating glucose homeostasis significantly in the high-fat diet-fed mice. An ethyl acetate fraction of Molineria latifolia containing high polyphenol can be reported to escalate the effectors’ expression of insulin signaling such as GLUT4, hexokinase 2, IRS1, IRS2, and AKT2 via phosphorylation of IRS1/Akt pathway in tissues of skeletal muscle of high-fat diet-induced diabetic male rats. AKT2, a serine/threonine kinase, has a critical role in transduce insulin stimulation into metabolic responses in target tissues, including skeletal muscle, liver, and adipose tissue.

Beetroot (Beta vulgaris L.) contains a lot of phenolic substances and others, for example., betalains, that can improve health. Beetroot has antioxidative properties and hepatoprotective activity, and some studies showed that beetroot or products from its derivative can glycemia control. Lorizola et al. reported that supplementation beet stalks and leaves containing flavonoids could ameliorate glucose homeostasis significantly in the high-fat diet-fed mice. An ethyl acetate fraction of Molineria latifolia containing high polyphenol can be reported to escalate the effectors’ expression of insulin signaling such as GLUT4, hexokinase 2, IRS1, IRS2, and AKT2 via phosphorylation of IRS1/Akt pathway in tissues of skeletal muscle of high-fat diet-induced diabetic male rats. AKT2, a serine/threonine kinase, has a critical role in transduce insulin stimulation into metabolic responses in target tissues, including skeletal muscle, liver, and adipose tissue.

In mice and humans, loss of function mutations in AKT2, specifically in skeletal muscle, were reported to relating glucose intolerant and insulin resistance because the most postprandial glucose is used by the skeletal muscle. At the transcript level, the AKT2 regulates the expression of the GLUT1 gene to modulate the availability of glucose and abrogating the expression of AKT2 impaired glucose uptake by the cell. It showed
that the AKT2 has an important role in a pathway that directly integrates glucose, GLUT1 expression, and glucose availability to maintain the viability of AKT2-dependent cells [10].

Methods

Animals and experimental studies

Approval of the present research was obtained from the Ethical Committee of Integrated Research and Testing Laboratory, Universitas Gadjah Mada (Approval number: 0011/04/LPPT/V/2019). Wistar male rats at the age of 1 month (n = 20), weighing ±150 g, were obtained from Pharmacy Faculty, Universitas Gadjah Mada. Each rat was kept in cages in a room with a temperature of 25°C and the cycle of light/dark12:12 h. The rats were anesthetized to get skeletal muscle tissue using intraperitoneal injection of ketamine and then were randomly assigned to five groups consisted of four rats for each group. Group N was fed a normal diet, group HFFD was fed a diet containing high fat and high fructose, and three groups (HFB1, HFB2, and HFB3) were fed a diet containing high fat and high fructose for eight weeks and continuously fed a beet-contained normal diet for 6 weeks. The substitution percentage of beet in the diet for each 6%, 9%, and 12%, respectively. All rats were given distilled water ad libitum during the study. The formulation of the diet showed in Table 1.

| Table 1: Diet composition |
|---------------------------|
| Substance (g/kg diet) | Standard diet (N) | High fat and fructose 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 | - |
| Alpha cell (Fiber) | 50 | 50 | 50 | 50 | 50 |
| Mineral mix | 35 | 35 | 35 | 35 | 35 |
| Vitamin mix (AIN/93-MX) | 10 | 10 | 10 | 10 | 10 |
| Methionine | 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

The levels of fasting glucose (FG) were measured before and after the intervention, whereas the gene expression of AKT2 at skeletal muscle tissue was determined after the intervention. The serum obtained from fasting (8–10 h without diet) whole blood that taken from sinus orbitalis, enter the tube and stay for 2 h at room temperature, and then centrifuged at 3000 rpm for 15 min. The top layer solution is serum. The rats were anesthetized to get skeletal muscle tissue using intraperitoneal injection of ketamine and sacrificed. The levels of serum glucose were measured by the colorimetric method according to the protocol in glucose Diasys Kit. The steps of analysis of AKT2 gene expression using quantitative polymerase chain reaction (qPCR) are as follows: The total RNA of rats skeletal muscle was isolated with a TriRNA (Favorgen) based on instructions manufacturer. The RNA concentration was determined by Nanodrop, and the synthesis of cDNA using kit from Revertaid First Strand cDNA Synthesis Kit (Thermo scientific). The gene expression of AKT2 was analyzed using SSoAdvanced Universal SyBR Green Supermix (Biorad). Primer pairs are listed in Table 2. The qPCR protocol was as follows: Early denaturation for 5 min at temperature 95°C, denaturation for 1 min at 95°C, annealing for 1 min at 58°C and elongation for 1 min 72°C with 40 cycles. The primary sequences of AKT2 and β-actin (purchased from Genetica Science) were listed in Table 2.

Table 2: Primers of reverse transcription PCR analysis

| Gene | Primer sequence (Product) |
|------|---------------------------|
| AKT2 | F : 5’GGAGGTCATGGAGCATCGGTTC3’ R : 5’GTGTTGAAAGGCGGCCAGACG3’ | 80 bp |
| Beta actin | F : 5’GTGTTGACTGGTGCGCTTATAC3’ R : 5’AGAAAGGGTGTTAAAAGCGAG3’ | 149 bp |

Statistical analysis

The results are presented as mean±standard deviation. The FBG and AKT2 gene expression were determined by one-way ANOVA and next to a post hoc multiple comparison test. Comparative analysis of pretest-posttest data using paired sample t-test.

Results

Changes in the levels of blood glucose of fasting rats after received a diet high in fat and fructose (before rats get a beet-contained diet) and after given a beet-contained diet were presented in Table 3. The HFFD, HFB1, HFB2, and HFB3 groups had FBG levels significantly higher than those in the N groups (p < 0.05). The beet 9%-contained diet can significantly reduce the FBG levels.

Table 3: The mean of the levels of blood glucose of fasting rats after induction and after intervention

| Groups | N | Average of FBG levels (mg/dL) | AFBG |
|--------|---|-------------------------------|------|
| HFB3   | 4 | 103.1 ± 2.50                 | 127 ± 3.74 |
| HFB2   | 4 | 103.3 ± 2.50                 | 127 ± 3.74 |
| HFB1   | 4 | 103.5 ± 2.50                 | 127 ± 3.74 |
| HFFD   | 4 | 103.7 ± 2.50                 | 127 ± 3.74 |

*Normal control group: HFFD. Rats received high fat and fructose. HFB1: HFFD fed by beet 6%-contained diet; HFB2: HFFD fed by beet 9%-contained diet; HFB3: HFB3 fed beet 12%-contained diet. Data are presented in mean ± standard deviation. Superscript # and #: P < 0.05 according to One Way ANOVA and Tukey test; Superscript a/b: There is no difference in either a or b; P*: Difference of FBG levels between groups; P**: Difference of FBG levels before and after beet intervention at same groups. Sign # shows that significant. FBG: Fasting blood glucose.

There was no significant correlation between the expression of the AKT2 gene and the FG because
glucose metabolism involved many pathways, such as
the previous study (Figure 1). This study showed that
the effect of beet on AKT2 gene expression was not
significant, although the AKT2 gene expression in the
HFFD group was lowest, but the AKT2 gene expression
in the HFB2 group closely to those in the N group
(Figure 2).

Discussion

This present study showed that the rats in HFFD, HFB1, HFB2, and HFB3 groups had FG between 130,135 g/dL, and it more than the normal
group (N). According to the previous studies that
the levels of blood glucose of fasting rats fed a diet
containing high fat and fructose for six weeks were
higher than rats fed the standard diet [11]. Haroun
et al. [12] also reported that rats fed a diet containing
high fat only or fructose alone for 5 weeks had
higher glucose levels than normal control rats.

Another study showed that the combination of a high-
fat diet and high fructose beverages induced fasting
hyperglycemia after 6 months [13]. In some studies,
using animals showed that high-fat diet impaired
blood sugar tolerance that was associated with decreased
basal and insulin-stimulated glucose metabolism,
whereas, in the human, a high-fat diet reduced insulin
sensitivity. In this study, we used saturated fat, and
saturated fat is more deleterious with respect to fat-
induced insulin insensitivity than monounsaturated
and polyunsaturated fat [14]. Insulin insensitivity or
insulin resistance is also reported to be associated with
high fructose consumption. Fructose-induced insulin
resistance can be mediated by an increase of reactive
oxygen species that mediates a proinflammatory
cascade to lead to an increase of adipogenesis, release
of inflammatory cytokines, and decrease in adiponectin
cause insulin resistance [15], [16].

After getting a beet-contained diet, the FBG in
HFB1, HFB2, and HFB groups decreased, especially
in the HFB2 group that gets a beet 9% contained
diet, seen significantly. It showed that beet 9% as a
physiological dose having the best effects to maintain
glucose homeostasis. The result of this present study,
in accordance with the previous research by Lorizola
et al. [17] reported the rats treated with the stem and leaf
parts of beets had a decrease in blood glucose levels
compared to the untreated rat group. The result of this
study accordance with the study Gezginci-Okbayoglu
that reported that extract of B. vulgaris L var. cicla can
increase GLUT2 via AKT2 and defence of antioxidant
in the liver lead to improve hyperglycemia [18]. AKT is
involved in the metabolism regulation of glucose and
the pathway of intracellular insulin transduction and
metabolism of energy in the liver [19].

The AKT2 was reported to modulate the
availability of glucose through expression regulation
of GLUT1 at the level of transcript and abrogating
expression of AKT2 impaired glucose uptake by the
cell [10]. In the present research, the effect of beet-
substituted diet on the gene expression of AKT between
groups was not statistically significantly different,
but the rats get beet substituted diet indicated AKT
gene expression higher than those in the HFFD rat
group, and its expression closely to normal rat group
was beet 9% substituted diet (Figure 2). It suggested
that substitution of beet 9% in the diet can meet the
physiological need to improve the effects of a diet
containing high fat and fructose related to glucose
homeostasis. In low doses (beet 6%) may allow less
effect on ligands, whereas a higher dose (beet 12%)
over a long period of time may trigger prooxidant
activity. Flavonoids given in high amounts can act as
prooxidants. Fang et al. [20] reported that flavonoids
become prooxidant activity occurs by triggering
OH in the presence of Cu + and H2O2. In addition,
the expression of the AKT2 gene has a negative
expression with FG, but it was not statically significant.

It is because glucose metabolism involved many
pathways. Although the skeletal muscle AKT2 has
been believed to play an important role in homeostasis glucose, however, the mice’s skeletal muscle only lacking AKT2 showed cannot stimulate insulin resistance or inhibit the uptake of glucose. It showed that besides skeletal muscle AKT2, another signaling molecule might be involved in perturbation tolerance of glucose and sensitivity of insulin in vivo [10].

Conclusion

The beet 9% substituted diet can improve glucose homeostasis from the effects of a diet containing high fat and fructose, and the expression of the AKT2 gene may have a role in the process.

Acknowledgments

We want to thank Abrory Agus Cahya Pramana, Dianandha Septiana Rubi, and Salma Nadiyah for their research assistant and discussion. This study was supported by The Ministry of Research and Technology Republic of Indonesia.

References

1. Huang BW, Chiang MT, Yao HT, Chiang W. The effect of high-fat and high-fructose diets on glucose tolerance and plasma lipid and leptin levels in rats. Diabetes Obes Metab. 2004;6(2):120-6. https://doi.org/10.1111/j.1462-8902.2004.00323.x PMid:14746577
2. Lin CL and Lin JK. Epigallocatechin gallate (EGCG) attenuates high glucose-induced insulin signaling blockade in human hepG2 hepatoma cells. Mol Nutr Food Res. 2008;52(8):930-9. https://doi.org/10.1002/mnr.200700437 PMid:18496818
3. Kang GG, Francis N, Hill R, Waters D, Blanchard C, Santhakumar AB. A review of dietary polyphenols and gene expression in molecular pathways associated with Type 2 diabetes mellitus. Int J Mol Sci. 2020;21(1):140. https://doi.org/10.3390/ijms21010140 PMid:31878227
4. Vidal PJ, López-Nicolás JM, Gandía-Herrero F, García-Carmona F. Inactivation of lipooxygenase and cyclooxygenase by natural betalains and semi-synthetic analogues. Food Chem. 2014;154:246-54. https://doi.org/10.1016/j.foodchem.2014.01.014 PMid:24518339
5. Vujić JJ, Ćebović TN, Čanadanović-Brutner JM, Četković GS, Čanadanović VM, Djilas SM, et al. In vivo and in vitro antioxidant effects of beetroot pomace extracts. Journal of Funct Foods. 2014;6(1):168-75. https://doi.org/10.1016/j.jff.2013.10.003
6. Gilchrist M, Winyard PG, Fulford J, Anning C, Shore AC, Benjamin N. Dietary nitrate supplementation improves reaction time in Type 2 diabetes: Development and application of a novel nitrate depleted beetroot juice placebo. Nitric Oxide. 2014;40:67-74. https://doi.org/10.1016/j.niox.2014.05.003 PMid:24858657
7. Lorizola IM, Miyamoto JÉ, Vieira AL, Sunmero BR, Bezerra RM, Torsoni MA, et al. Beet (Beta vulgaris L) stalk and leaf supplementation improves glucose homeostasis and insulin resistance markers in liver of mice exposed to a high-fat diet. Res Sq, 2020;1:26. https://doi.org/10.21203/rs.3.rs-40368/v1
8. Minic M, Rocha N, Harris J, Groeneveld MP, Leiter S, Wareham N, et al. Constitutive activation of AKT2 in humans leads to hypoglycemia without fatty liver or metabolic dyslipidemia. J Clin Endocrinol Metab. 2017;102(8):2914-21. https://doi.org/10.1210/jc.2017-00768 PMid:28541532
9. Jaiswal N, Gavin MG, Quinn WJ, Luongo TS, Gerler GF, Baur JA, et al. The role of skeletal muscle AKT in the regulation of muscle mass and glucose homeostasis. Mol Metab. 2019;28:1-13. https://doi.org/10.1016/j.molmet.2019.08.001 PMid:31444134
10. Jensen PJ, Gunter LB, Carayannopoulos MO. AKT2 modulates glucose availability and downstream apoptotic pathways during development. J Biol Chem. 2010;285(23):17673-80. https://doi.org/10.1074/jbc.m109.079343 PMid:20356836
11. Chayati I, Sunarti, Marsono Y, Astuti M. Anthocyanin extract of purple corn improves hyperglycemia and insulin resistance of rats fed high fat and fructose diet via GLP1 and GLP1R mechanism. J Food Nutr Res. 2019;7(4):303-10. https://doi.org/10.12691/jfnr-7-4-7
12. Haroun MA, Elsayed LA, Rushed LA, Mohammed MA. The effect of high fat diet and high fructose intake on insulin resistance and GLP-1 in experimental animals. Med J Cairo Univ. 2011;79(2):2332.
13. Lozano I, van der WR, Bietiger W, Seyfritz E, Peronet C, Pinget M, et al. High-fructose and high-fat diet-induced disorders in rats: Impact on diabetes risk, hepatic and vascular complications. Nutr Metab (Lond). 2016;13:15. https://doi.org/10.1186/s12986-016-0074-1 PMid:26918024
14. Lichtenstein AH, Schwab US. Relationship of dietary fat to glucose metabolism. Atherosclerosis. 2000;150(2):227-43. https://doi.org/10.1016/s0021-9150(99)00504-3 PMid:10856515
15. Khiatan Z, Kim DH. Fructose: A key factor in the development of metabolic syndrome and hypertension. J Nutr Metab. 2013;2013:682673. https://doi.org/10.1155/2013/682673 PMid:23762544
16. Ang BR, Yu GF. The role of fructose in Type 2 diabetes and other metabolic diseases. J Nutr Food Sci. 2018;8:14.
17. Lorizola IM, Furlan C, Portovedo M, Milanski M, Botelho PB, Bezerra R, et al. Beet stalks and leaves (Beta vulgaris L.) protect against high-fat diet-induced oxidative damage in the liver in mice. Nutrients. 2018;10(7):872. https://doi.org/10.3390/nu10070872 PMid:29976910
18. Gezginici-Oktayoglu S, Sacan O, Bolkent S, Ipıcı Y, Kabasakal L, Sener G, et al. Chard (Beta vulgaris L. var. cicla) extract ameliorates hyperglycemia by increasing GLUT2 through AKT2 and antioxidant defense in the liver of rats. Acta Histochem. 2014;116(1):32-9. https://doi.org/10.1016/j.acthis.2013.04.016 PMid:23746671
19. Liu Y, Liang X, Zhang G, Kong L, Peng W, Zhang H. Galangin
and pinocembrin from propolis ameliorate insulin resistance in HepG2 cells via regulating AKT/mTOR signaling. Evid Based Complement Alternat Med. 2018;2018:971842. https://doi.org/10.1155/2018/7971842
PMid:30420897

20. Fang X, Gao W, Yang Z, Gao Z, Li H. Dual anti/prooxidant behaviors of flavonoids pertained to Cu (II) catalyzed tyrosine nitration of insulin receptor kinase domain in the antidiabetic study. J Agric Food Chem. 2020;68(22):6202-11. https://doi.org/10.1021/acs.jafc.0c01676
PMid:32395994