Chronic Consumption of Fructose Dysregulates Genes Related to Glucose and Lipid Metabolism in Prostate Tissue
(Pengambilan Kronik Fruktosa Mengganggu Kawal Atur Gen-gen Berkaitan dengan Metabolisme Glukosa dan Lipid dalam Tisu Prostat)

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ABSTRACT

Fructose is commonly used as a taste enhancer in many processed foods. Excessive fructose consumption is highly associated with obesity and development of cancer particularly prostate cancer. This study aimed to investigate the biochemical and molecular changes in the prostate tissue of rats treated with 20% fructose for six months. A total of 18 rats weighted 200-250 g were divided into two groups, where each group consisted of 9 rats. Control group is given normal diet, while the treated group was given normal diet and 20% fructose in drinking water. After six months of treatment, both groups were sacrificed for further analysis. Body weight, blood pressure and glucose were measured. Lipid profiles were determined using quantitative colorimetric assay. Transcripts level of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), leptin (LEP), angiopoietin 1 (ANGPT1), microRNA (miR)-34a, miR-10b and miR-192 were determined using quantitative PCR, while the protein levels of 11β-HSD1 and leptin were determined using ELISA. The results showed that 20% fructose diet significantly increased blood glucose level as compared to the control (p<0.05). The transcript levels of LEP and miR-192 were significantly lower in the fructose-treated group as compared to the control (p<0.05). There was a significant linear relationship between prostate LEP and serum LDL/VLDL level as well as between the level of prostate LEP and serum total cholesterol level (p<0.05). Thus, our results showed that chronic consumption of fructose could down-regulate LEP and miR-192 expression in prostate tissue, and promote higher accumulation of fat in the tissue. Additionally, downregulation of miR-192 has been reported to be associated with the pathogenesis of prostate cancer. Thus, it can be concluded that long-term fructose diet leads to higher blood glucose level and down-regulation of LEP and miR-192 expression in prostate tissue.

Keywords: Chronic consumption; fructose; leptin; miR-192; prostate; obesity

ABSTRAK

Fruktosa lazimnya digunakan sebagai penambah perisa dalam kebanyakan makanan yang diproses. Pengambilan fruktosa secara berlebihan sangat berkait rapat dengan keobesan dan pembentukan kanser terutamanya kanser prostat. Kajian ini bertujuan untuk mengkaji perubahan biokimia dan molekul dalam tisu prostat tikus yang dirawat dengan fruktosa 20% selama enam bulan. Sejumlah 18 ekor tikus dengan berat 200-250 g dibahagikan kepada dua kumpulan dengan setiap kumpulan mengandungi 9 ekor tikus. Kumpulan kawalan diberi diet normal, sementara kumpulan rawatan diberi diet normal dan 20% fruktosa dalam minuman. Selepas enam bulan rawatan, kedua-dua kumpulan dikorbankan untuk analisis selanjutnya. Berat tubuh, tekanan darah dan aras glukosa diukur. Profil lipid ditentukan menggunakan asai kolorimetrik kuantitatif. Aras transkripsi 11β-hidroksisteroid dehidrogenase jenis 1 (11β-HSD1), leptin (LEP), angiopoietin 1 (ANGPT1), mikroRNA (miR)-34a, miR-10b dan miR-192 ditentukan menggunakan kuantitatif PCR, sementara aras protein 11β-HSD1 dan leptin ditentukan menggunakan ELISA. Keputusan menunjukkan bahawa diet fruktosa 20% secara signifikan meningkatkan aras glukosa darah berbanding kawalan (p<0.05). Aras transkripsi LEP dan miR-192 adalah lebih rendah secara signifikan bagi kumpulan yang dirawat dengan fruktosa berbanding kawalan (p<0.05). Terdapat hubungan linear yang signifikan antara aras LEP tisu prostat dengan aras LDL/VLDL serum (p<0.05) dan antara aras LEP tisu prostat dengan aras kolesterol total dalam serum (p<0.05). Dengan itu, kajian ini menunjukkan bahawa pengambilan fruktosa untuk tempoh yang lama mengurangkan ekspresi LEP dan miR-192 pada tisu prostat serta menggalakkan pengumpulan lemak pada tisu. Malah, ekspresi miR-192 yang rendah dilaporkan mempunyai kaitan dengan patogenesis kanser prostat. Maka, dapat disimpulkan bahawa diet fruktosa jangka panjang boleh mengakibatkan peningkatan aras gula dalam darah dan mengurangkan ekspresi LEP dan miR-192 pada tisu prostat.

Kata kunci: Fruktosa; leptin; miR-192; keobesan; pengambilan kronik; prostat

Fructose is a ketonic monosaccharide that is absorbed directly into the bloodstream in the digestive tract and it can be found in various edible plants (Rumussen 1992). Lots of food products are added with fructose for its sweet taste, such as high-fructose corn syrup. Even though fructose can be a taste enhancer for many processed food, but excessive fructose consumption may cause type 2 diabetes (Kolderup & Svibus 2015), development of visceral adiposity, elevation of low density lipids (LDL) cholesterol and triglycerides (Bursac et al. 2014), obesity (Bocarsly et al. 2010) and increased risk of cardiovascular disease (Johnson et al. 2007).

Fructose metabolism occurs rapidly in the liver, producing glyceraldehyde and dihydroxyacetone phosphate that later congregates with the glycolytic pathway (Basciano et al. 2005). Thus, even though glucose consumption is low, fructose can always enter the glycolytic pathway and produce glucose, glycogen, lactate and pyruvate, which promoting the overproduction of triglycerides via *de novo* lipogenesis and subsequently leads to obesity (Kolderup & Svihus 2015).

Apart from obesity, fructose intake may have effects on the regulation of leptin. Leptin is a circulating hormone encoded by LEP gene (MIM 164160), secreted by white adipocytes. Under normal condition, adipose tissue produces leptin to suppress appetite and regulate food intake. Thus, leptin plays a key role in regulating body weight. As body weight and fat mass rise, the level of leptin in plasma proportionately increases (Li 2011). In an *in vitro* study of prostate cancer, high level of leptin in a long-term period was presented to augment the proliferation of prostate cancer cell lines namely LNCaP, DU145 and PC-3 by stimulating cell survival pathways (Noda et al. 2015), triggering proliferation as well as angiogenesis (Frankenberry et al. 2004).

Another factor that was reported to link with obesity is 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) (Aida Azlina et al. 2009; Paulsen et al. 2007). It is a reductase enzyme encoded by hydroxysteroid 11β-dehydrogenase 1 (HSD11B1) gene [MIM 600713]. HSD11B1 is highly expressed in key metabolic tissues like liver and adipose tissue (Aida Azlina et al. 2009; Lee et al. 2013). Its function is to catalyse the inactivation cortisol into cortisone (Sjostrand et al. 2010). As a result, overexpression of HSD11B1 could lead to the accumulation of active cortisol in tissues, which is the main glucocorticoid in tissue and subsequently promoting visceral adiposity (Spencer & Tilbrook 2011).

Previously, reports also showed that obesity is also associated with the up-regulation of angiopoietin 1 gene (ANGPT1) that involves in anti-apoptosis, cell growth and differentiation (Ribeiro et al. 2012). ANGPT1 protein was not only expressed in adipose tissues, it has also been found in both prostate tumour cells and its capillaries, in which it worked as a sprouting angiogenesis inducer (Satoh et al. 2008).

To understand the regulation of genes related to obesity, we included epigenetic roles in particular microRNA (miRNA). There are several miRNAs that involved in obesity (Azmir et al. 2014; Chartoumpekis et al. 2012; Dehwah et al. 2012; Viesti et al. 2014; Williams & Mitchell 2012) and cancer development (Ibrahim et al. 2015) including miR-10b, miR-34a and miR-192 (Khella et al. 2013; Senanayake et al. 2012; Sun et al. 2016). These small non-coding RNAs work by regulating the expression of their target genes in adipocytes and consequently influence the differentiation of adipocytes (Arner & Kulyte 2015).

Numerous dietary approaches in an animal model have been shown to induce metabolic syndrome which is characterized by obesity, hyperlipidaemia, hypertension and increased risk of cardiovascular disease (Mamikutty et al. 2014; Wong et al. 2016).

In the present *in vivo* study, we investigated the effect of long-term fructose consumption in an animal model (Mamikutty et al. 2014) on the biochemical changes and genes related to obesity including LEP, HSD11B1, ANGPT1, miR-34a, miR-10b and miR-192 in the peripheral blood and prostate tissues.

MATERIALS AND METHODS

STUDY DESIGN

This study was approved by Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) (reference no: FP/FISIO/2013/NORHAZLINA/25-SEPT./530-SEPT.-2013-MAY-2016). A total of 18 male Wistar rats weighted between 200 – 250 g were divided into two groups, consisted of 9 rats each. The treatment group was given 20% D-fructose in drinking water, while the control group was given distilled water, for six months (Mamikutty et al. 2014). Both groups received normal rat pallet *ad libitum*. Each rat was kept in a separate cage in a well-ventilated room at 22°C with 12 h light and dark cycle. Body weight, blood pressure and food consumptions were first measured prior to the treatment followed by weekly monitoring until before they were sacrificed. After six months of treatment, the rats were sacrificed under anaesthesia by maximal bleeding through cardiac puncture after 6 h fasting.

SAMPLE COLLECTION

Blood was collected in plain tubes and was placed at room temperature for 2 h before being centrifuged at 8000 × g and serum was then collected. Prostate tissues were cut and divided into several parts (for ELISA and RNA extraction) before being snapped freeze and stored in -80°C for further analysis.

FASTING BLOOD GLUCOSE DETERMINATION

After 6 h of fasting, a blood sample was collected to determine fasting blood glucose using Accu Check

Performa glucometer (Roche, US). The blood glucose level was presented in mg/dL.

QUANTITATIVE COLORIMETRIC ASSAY

The level of total cholesterol, triglycerides, high-density lipoprotein (HDL) and low-density lipoprotein/very low-density lipoprotein (LDL/VLDL) level in serum were determined using quantitative colorimetric assay (BioAssay Systems, USA) following manufacturer's protocol. Only non-haemolysed serum observed by yellowish fluid was used in this assay. Optical density (OD) reading was done at 570 nm. The total cholesterol, HDL and LDL/VLDL concentration (mg/dL) were then calculated by normalizing the sample's and standard's OD reading with the blank's OD reading. The normalized sample's OD reading was then divided with the normalized standard's OD reading and multiplied by 100.

ENZYME-LINKED IMMUNOSORBENT ASSAY

An enzyme-linked immunosorbent assay (ELISA) was performed to measure the concentration of 11β-HSD1 and leptin in both serum and prostate tissue employing Rat Leptin ELISA Kit and Rat 11β-HSD1 ELISA Kits. Tissue samples were rinsed in ice-cold PBS (0.01M, pH7.4) to eliminate blood thoroughly and were cut into small pieces. Tissues were weighed and then homogenized in PBS with a glass homogenizer on ice. The PBS volume used was 1 mL for 100 mg tissue. The suspension was subjected to four times of freeze and thaw cycles to break the cell membrane. The homogenates were then centrifuged for at 5000 × g for 5 min before the supernatant was collected.

Serial dilutions of standard were included in each run, ranging from 0, 0.156, 0.313, 0.625, 1.25, 2.5, 5, up to 10 ng/mL. The ELISA for both leptin and 11β-HSD1 were done based on the assay protocols provided. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm.

QUANTITATIVE PCR (qPCR) FOR HSD11B1, LEP, ANGPT1

Total RNA was isolated from a prostate tissue sample using miRNeasy Mini kit (Qiagen). Then, the extracted total RNA was converted into cDNA using Quantinova cDNA conversion kit (Qiagen) following manufacturer's protocol. Subsequently, qPCR was used to relatively quantify the expression of HSD11B1, LEP and ANGPT1 genes. All results will be normalized with hypoxanthine guanine phosphoribosyl transferase (HPRT) as the housekeeping gene. The initial activation step was set at 95°C for 15 min., followed by 40 cycles of denaturation at 95°C for 15 min, annealing at 55°C for 30 s and extension at 72°C for 30 s. The fold changes were calculated using the ΔΔCt comparative quantification method. Fold change of more than 1 indicates up-regulation, whereas a fold change of less than 1 indicates down-regulation (Chartoumpekis et al. 2012).

QUANTITATIVE PCR FOR miR-10b, miR-34a AND miR-192

For miRNA expression study, total RNA was changed into cDNA using miScript II RT kit (Qiagen). The expression of miRNAs that include rno-miR-10b, rno-miR34a and rno-miR-192 were also relatively quantified using qPCR. For miRNA expression study, RNU6 was used as the housekeeping gene. The initial activation step was set at 95°C for 15 min., followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 55°C for 30 s and extension at 70°C for 30 s. The results were calculated as a $2^{-\Delta\Delta Ct}$ method (Chartoumpekis et al. 2012).

STATISTICAL ANALYSIS

Statistical Package for the Social Sciences (SPSS) Software version 20.0 was used for statistical analysis. Data were not normally distributed, hence non-parametric tests were used to compare between study groups. Wilcoxon Signed Rank Test was used to compare body weight and blood pressure before and after six months of fructose treatment in both control and fructose treated groups respectively. The differences of body weight, blood pressure, biochemical parameters and gene as well as microRNA expression between control and treated groups were analysed using Mann-Whitney U Test. Simple linear regression analysis was utilized to explore the relationship between two numerical variables. A p value of less than 0.05 was regarded as statistically significant.

RESULTS

Body weight and blood pressure of each rat were measured before and after six months of fructose treatment (Table 1). A significant increase in body weight was observed in both control and fructose-treated rats group ($p<0.05$). However, an increase in systolic blood pressure was seen only in the fructose-treated group ($p<0.05$).

Subsequently, the changes in the body weight and blood pressure were compared between the control and fructose-treated groups (Table 1). Median body weight of the control group (558.2 ± 77.58 g) was observed to be higher than the fructose-treated group (501.0 ± 59.00 g) but statistically, it was not significant. There was no statistically significant difference between the median changes in systolic blood pressure among the fructose-treated group (124.0 ± 15.36 mmHg) as compared to the control group (121.0 ± 19.11 mmHg). A similar result was observed in the median change in the diastolic blood pressure among the fructose treated group (90.5 ± 20.93 mmHg) as compared to the control group (78.0 ± 15.09 mmHg) (Table 1).

Comparing blood biochemical parameters of both groups following 6 months of treatment, only blood glucose level in fructose group showed significantly increased ($p<0.05$) (Table 2). No significant effect of fructose treatment was observed in 11β-HSD1, leptin and lipid profile levels as compared to the control group.

TABLE 1. Body weight and blood pressure measurement of pre- and post-treatment of rats with fructose diet and control groups

	Control (*n*=9) Median (SD)			Treated (*n*=9) Median (SD)		
	Month 0	Month 6	P-value	Month 0	Month 6	P-value
Body weight (g)	209.3 (28.59)	558.2 (77.58)	0.008[a]	202.2 (40.52)	501.5 (59.00)	0.008[a]
Blood pressure:						
Systolic (mmHg)	127.0 (26.69)	121.0 (19.11)	0.953	112.5 (18.95)	124.0 (15.36)	0.025[a]
Diastolic (mmHg)	86.0 (24.94)	78.0 (15.09)	0.859	78.0 (16.41)	90.5 (20.93)	0.237

Data are shown as median (standard deviation, SD)
[a]*p*<0.05 median body weights and systolic blood pressure were significantly increased compared to before treatment

TABLE 2. Biochemical parameters of the rats treated with fructose and control groups (*n*=9)

	Median (SD)		P value
	Control	Fructose Treated	
Blood glucose (mg/dL)	11.10 (2.33)	14.50 (6.34)	0.030*
11β-HSD1 ng/mL	5.29 (5.29)	4.15 (4.56)	0.337
Leptin ng/mL	1.00 (0.51)	1.15 (0.68)	1.000
Triglyceride (mg/dL)	60.50 (23.23)	61.53 (54.23)	1.000
Total cholesterol (mg/dL)	99.66 (15.78)	83.14 (21.67)	0.748
HDL (mg/dL)	37.15 (14.91)	33.05 (4.24)	0.749
LDL/VLDL (mg/dL)	17.17 (8.59)	18.56 (17.72)	0.262

Data are shown as median (standard deviation, SD)
p<0.05 median blood glucose levels of the fructose-treated group were significantly increased compared to control group after a 6-month treatment

Interestingly, even though there were no significant changes in blood levels of 11β-HSD1, leptin and triglyceride, the expression of LEP and miR-192 of the fructose-treated group were lower compared to the control (*p*<0.05) (Table 3).

The results from simple linear regression analysis showed a linear relationship between LEP and LDL/VLDL level (Figure 1) (b=0.05, 95% CI 0.024, 0.076, *p*=0.002). This indicated that for every increase in the LEP level, there was an increase in the level of LDL/VLDL. A similar trend was observed between LEP and total cholesterol level whereby there was also a significant linear relationship between these two parameters (Figure 2) (b=0.031, 95% CI 0.004, 0.058, *p*=0.027).

DISCUSSION

Chronically, fructose consumption leads to obesity, insulin resistance, type 2 diabetes mellitus and hypertension (Kolderup & Svihus 2015; Lakhan & Kirchgessner 2013; Mamikutty et al. 2014). Fructose has the same chemical formula as glucose and readily absorbed in the gut via facilitated diffusion. Metabolism of fructose differs from that of glucose, where it is rapidly converted into glucose, glycogen, lactate, and fat in the liver. Our study adopted animal model established by Mamikutty et al. (2014). In the present study, 20% fructose treatment for a period of six months did not lead to significant differences in body weight and lipid profile when compared to the control

TABLE 3. Concentration of 11β-HSD1, leptin, triglyceride and expression levels of HSD11B1, LEP, ANGPT1, miR-10b, miR-34a and miR-192 in fructose-treated and control groups after a 6-month treatment

	Median (SD)		P value
	Control	Treated	
11β-HSD1	12.8 (3.27) ng/mL	10.94 (4.76) ng/mL	0.596
Leptin	0.45 (0.13) ng/mL	0.33 (0.19) ng/mL	0.134
Triglyceride	90.28 (51.56) ng/mL	155.28 (112.56) ng/mL	0.211
HSD11B1	1 (0)	0.85 (0.64)	1.000
LEP	1 (0)	0.55 (1.11)	0.009*
ANGPT1	1 (0)	0.92 (0.31)	0.348
miR-10b	1 (0)	0.94 (0.77)	0.383
miR-34a	1 (0)	1.07 (12.51)	0.671
miR-192	1 (0)	0.45 (0.40)	0.004*

Data are shown as median (standard deviation, SD).
p<0.05 Expression of LEP gene and miR-192 were down-regulated in fructose-treated group compared to control after a 6-month treatment
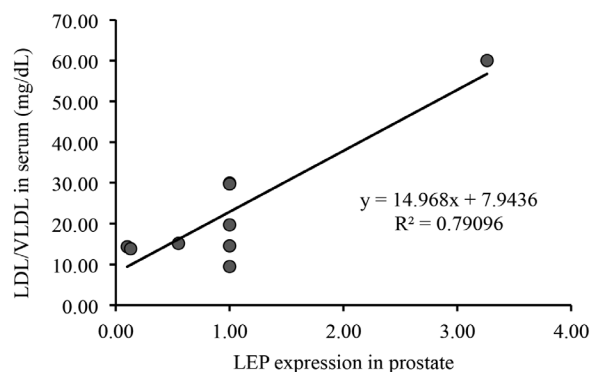
FIGURE 1. Simple linear regression analysis between LEP expression level in prostate tissue and LDL/VLDL serum level showed positive correlation after a 6-month treatment
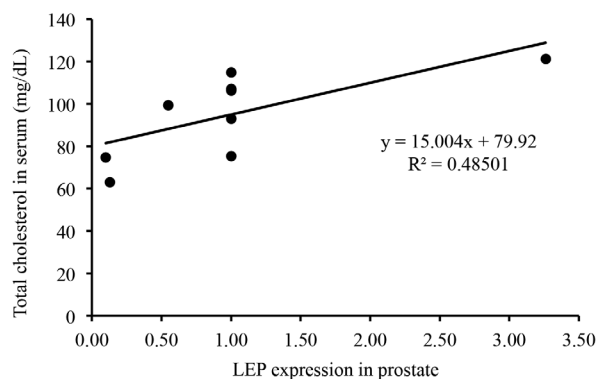

FIGURE 2. Simple linear regression analysis between LEP expression level in prostate tissue and total cholesterol serum level showed positive correlation after a 6-month treatment

group. This finding is in agreement with Bursac et al. (2014), in which differences in the body weight of the fructose-treated group (male Wistar rats, treated with 60% fructose over 9 weeks) when compared to the control was not significant. Bursac et al. (2014) also reported that there was a statistically higher level of triglycerides and leptin concentration in the fructose treated group but with no significant differences in blood glucose level as compared to the control. Thus, it can be postulated that higher fructose intake is associated with more accumulation of visceral fat. In 2008, the Third National Health and Nutrition Examination Survey showed that 10% of daily calories intake came from fructose. Thus, compared to the study by Bursac et al. (2014), a lower percentage of fructose diet (20%) was chosen in the present study. Choosing an inflated (60%) fructose diet as in Bursac et al. (2014) study does not mimic the real-life situation although it is not impossible. Furthermore, adding 60% of fructose in daily meals seems to be very unusual.

In the existing study, even though there were no significant alterations in physical and biochemical parameters between both treated and untreated groups, significant changes were found at the molecular level. Interestingly, after six months of intervention, no significant changes were observed in circulating leptin, however, mRNA level of LEP in the prostate tissue was considerably lower in the fructose-treated group as compared to the control. Elevated leptin level was observed in plasma samples after two months of 60% fructose treatment (Bursac et al. 2014). Under normal condition, LEP is expressed to suppress appetite when an individual has already consumed sufficient amount of food. Thus, in the present study, significantly lower LEP expression in the fructose-treated group showed that long-term (six months) fructose diet could dysregulate the expression of key genes at the mRNA level in glucose and lipid metabolism pathway, thus promoting to the progression of metabolic syndrome. This is because LEP was reported to play a key role in the development of obesity and somatic mutation or variant in LEP was linked with early-onset obesity (Saeed et al. 2015; Shabana & Shahida 2016).

LEP expression level was further analyzed and a significant linear relationship between prostate LEP and serum LDL/VLDL level as well as between prostate LEP and serum total cholesterol level were discovered. Thus, our results showed that long-term fructose diet could down-regulate the expression of LEP in prostate tissue leading to the accumulation of fat higher in prostate tissue compared to the blood circulation.

Apart from LEP mRNA level, the miR-192 expression level in prostate tissue was also significantly reduced in the fructose-treated group than in control group. In concordance with our finding, reduced miR-192 expression was also detected in the development of obesity (Chartoumpekis et al. 2012). The involvement of miR-192 in obesity can be explained through its target genes, zinc finger E-box (*zeb1* and *zeb2*) that have been associated with adipogenesis and obesity (Saykally et al. 2009). More alarmingly, there were also previous studies that reported the association between the down-regulation of miRNA-192 with cancer development (Khella et al. 2013; Senanayake et al. 2012) including prostate cancer (Sun et al. 2016). Thus, our finding showed that long-term fructose diet could dysregulate miR-192 in the prostate with the possibility to cause not only obesity but also increase the risk of prostate cancer development.

CONCLUSION

Chronic fructose consumption may lead to downregulation of the mRNA level of LEP and miR-192 in prostate tissue. Thus, it can be postulated that a longer duration of fructose diet leads to prostate adiposity and consequently increase the risk of prostate cancer. Further functional study of the targeted genes is required to confirm this hypothesis.

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