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Evaluating of High Fructose Diet to Induce Hyperglycemia and its Inflammatory Complications in Rats

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Abstract: It has been reported that a diet enriched in fructose would present animal models used to emulate diabetes mellitus type 2 in human. This study aimed to examine the effect of high fructose induction on the blood glucose, C-reactive protein, interleukin-6, interleukin-4 and body weight among high fructose diet-induced rats. The HFD was induced through drinking water in 21% (w/v) concentration among male *Sprague-Dawley* rats. The high fructose diet administration was unable to induce hyperglycemia, hypertriglyceridemia or any classic inflammatory markers. Also, no histological inflammation was observed. It was concluded that healthy *Sprague-Dawley* rats fed high fructose diet for 2.5 months could not develop signs of diabetes mellitus type 2.

Key words: High fructose diet, hyperglycemia, inflammatory biomarkers, *Sprague-dawley* rats

INTRODUCTION

Non-genetic environmental factors including diet and food constituents are able to prevent or induce several diseases (Stark *et al*., 2000; Dimo *et al*., 2002). Therefore, elevating of effective dietary compounds is necessary. Fructose is widely used as sweetener in food processing (Dai and McNeill, 1995). It is demonstrated that normal rats fed with fructose-enriched diet develop hypertension accompanied with metabolic abnormalities including hyperglycemia, insulin resistance, hyperinsulinemia and hypertriglyceridemia (Dai and McNeill, 1995; Vrana and Kazdova, 1986; Suzuki *et al*., 1997; Katakam *et al*., 1998; Lee *et al*., 2002; Bunnag *et al*., 1997). Feeding of a High Fructose Diet (HFD) can provide a type 2 diabetic dietary model associated with insulin resistance (Thorburn *et al*., 1989; Tobey *et al*., 1982) and hypertriglyceridemia (Tobey *et al*., 1982; Zavaroni *et al*., 1982). The glucose metabolism and glucose uptake pathways are disturbed through an overload of fructose. These perturbed metabolisms caused enhanced rate of lipogenesis and triacylglycerol synthesis through high concentration of glycerol and acyl molecules from fructose catabolism which leads to insulin resistance observed in both human and animal models (Basciano *et al*., 2005). The high cost of human trials and limited control conditions, emphasize the need for finding appropriate animal models to examine the efficacy of various compounds (Stark *et al*., 2000). Potential nutritional animal models are useful in future experiments to evaluate various nutraceuticals in preventing Diabetes Mellitus Type 2 (DMT2). The present study was designed to assess the effects of high fructose-induced rat models as reproducible animal models of pre-diabetic state in humans.

Diabetes Mellitus (DM) is an autoimmune disease that resulted from dysfunction of beta cell's insulin secretion (Matsuzaki *et al*., 2007). Besides, proinflammatory cytokines caused autoantigen presentation which leads to beta cell dysfunction and DM (Mathews *et al*., 2005). An increase in circulating levels of C-Reactive Protein (CRP) and interleukin-6 (IL-6) is known to decrease insulin sensitivity and raise DM type 2 complications (Jain *et al*., 2007). Diabetes mellitus is a sign of ongoing cytokine-mediated acute phase response initiated by the body's innate immune system. Evidence suggests that inflammation play a crucial intermediary role in pathogenesis of DM (Jialal *et al*., 2004; Wellen and Hotamisligil, 2005; Cho *et al*., 2006). Intranuclear NFêB activity and expression of CRP and IL-6's mRNA levels are associated with hyperglycemia (Mohanty *et al*., 2000; Dhindsa *et al*., 2004).

MATERIALS AND METHODS

Animal maintenance and experimental setup: Male *Sprague-dawley* rats, weighing 200-250 g (aged six to eight weeks) were obtained and housed in the animal house unit of Faculty of Medicine and Health Sciences, University Putra Malaysia. The rats were kept at room temperature (28-35°C) in a controlled room with a 12-h

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light: 12-h dark cycle. The normal rat chow and tap water were provided *ad libitum* during the experiment. Animals were in stabilization to acclimatize to animal house environment for one week before beginning of the experiment. Also, rats body weight was recorded every other week. The study was approved by the animal ethics committee of the University Putra Malaysia (UPM/FPSK/PADS/UUH/F04).

Rats were divided into two groups (N = 6). Normal rats (N) and High Fructose-induced (HFD) rats. The high fructose diet consisting 21% (w/v) of fructose (HmbG, D-fructose, France) concentration was delivered *ad libitum* through drinking water for 10 weeks (Dai and McNeill, 1995; Yadav *et al*., 2007).

Blood glucose measurement and cytokine assay: Rats were fasted overnight to measure blood glucose level. Blood was obtained via tail incision and an Advantage Accu-check glucometer (Roch, Germany) was used. To determine inflammatory biomarkers, rats were anesthetized through a slight diethyl ether exposure. Blood (3-5 ml) was collected via retro orbital venous plexus and centrifuged at 3000 rpm for 10 min (Hettich, EBA 20, Germany). C-reactive protein, IL-6 and IL-4 levels of plasma were measured by the Enzyme Linked Immunosorbent Assay (ELISA) method using an available commercially kit (IBL, Germany). Blood samples were collected at the beginning (to measure blood glucose) and at the end of the 10th week of HFD induction (for blood glucose and inflammatory markers measurement).

Histopathology of selected organs: At the end of the HFD induction, rats were sacrificed by exposure to an excess amount of diethyl ether. The animals were dissected and major organs such as pancreas, liver and kidney were collected and fixed into 10% neutral buffered formalin for histological studies. The hematoxylin and eosin (H and E) method was applied for staining.

Statistical analysis: Data were subjected to statistical analysis using one-way ANOVA to compare the means of inflammatory biomarkers. MANOVA was used to compare the blood glucose concentrations and body weight between groups.

RESULTS

The body weight showed a progressive increase in normal and HFD-Induced (HFDI) groups during the HFD induction period (Table 1). At the last week of regimen with high concentration of fructose (week 10), rats body weight raised between 82-102 g which was 23% and 28.6% in normal and HFD groups, respectively.

Table 1 shows the effect of HFD on blood glucose level of rats. Before administrating the diet which was enriched in high amounts of fructose, the blood glucose of rats on the HFD diet (6.3±0.81) were similar to the normal group (6.2±0.90). The blood glucose measurement at the end of the HFD induction period exhibited a moderate increase by 11% in HFDI group compared to the normal one. Although the blood glucose level of rats under HFD regimen has shown increase within experimental period in comparison with the normal group, changes were not significantly different (Table 1).

Table 1: Effect of the HFD on body weight, blood glucose and triglycerides in rats

	N	HFDI
Body weight (g)		
1st wk	246.50±11.91	279.70±11.91
5th wk	264.50±15.69	335.70±15.69
10th wk	303.70±20.87	361.70±20.87
Blood glucose (mmol/L)	6.30±01.01	6.80±00.90
Blood triglycerides (mmol/L)	0.93±00.03	1.05±00.03

Values are mean±SEM, N = 6 rats per group, N = Normal, HFDI = High Fructose-Induced. There were no differences in values between different groups

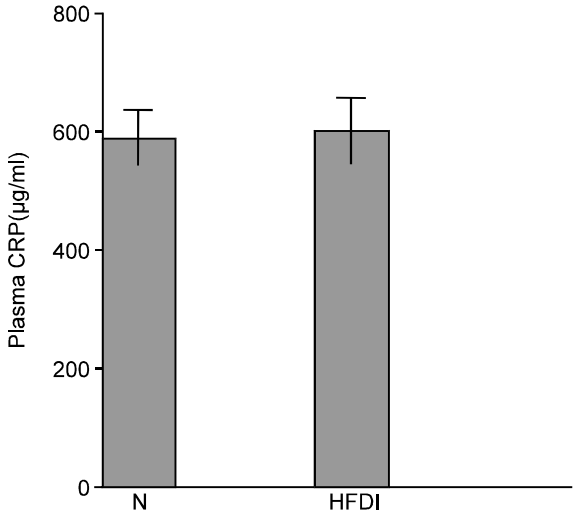

Fig. 1: Values are mean±SEM, N = 6 rats per group, N = Normal, HFDI = High Fructose-Induced, C-reactive protein level of normal and HFD-induced rats during the experiment. There were no differences in values between different groups

There was a slight increase in Triglyceride (TG) level of rats under the diet compared to the normal rats. Increased TG level was seen after 10 weeks HFD induction from 0.9±0.03 mmol/L in normal rats to 1.0±0.03 mmol/L in rats fed with HFD (+0.16 mmol/L). However, there was no significant difference in the changes (Table 1).

Figure 1-3 shows the effect of HFD administration for 10 weeks on plasma CRP, IL-6 and IL-4 levels of rats. The plasma levels of CRP were not elevated in HFDI rats compared to those of normal. C-reactive protein level of normal and HFDI groups was shown in Fig. 1. The CRP

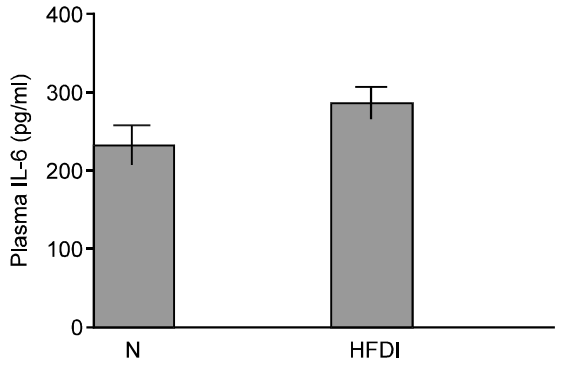

Fig. 2: Values are mean±SEM, N = 6 rats per group, N = normal, HFDI = High Fructose-Induced, Interleukin-6 level of normal and HFD- induced rats during the experiment. There were no differences in values between different groups

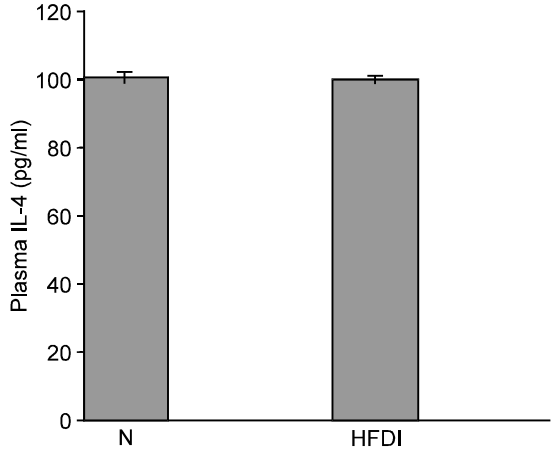

Fig. 3: Values are mean±SEM, N = 6 rats per group, N = normal, HFDI = High Fructose-Induced, Interleukin-4 level of normal and HFD-induced rats during the experiment. There were no differences in values between different groups

level of HFDI group was only 10 μg/ml higher than normal which raised from 589.0±28.33 μg/ml in normal to 600.3±32.83 μg/ml in HFDI group. The administration with high concentration of fructose could slightly elevate the IL-6 level (Fig. 2) from 232.7±14.44 pg/ml in normal to 285.0±12.42 pg/ml in HFDI rats. The IL-4 level (Fig. 3) of normal rats was similar to HFDI while the IL-4 levels were 100.3±0.09 pg/ml and 99.7±0.06 pg/ml in HFDI and normal rats, respectively. However, the changes were not significantly different.

Figure 4-6 show pancreas, liver and kidney sections of normal and HFDI groups after 10 weeks of high fructose administration. Hematoxylin and eosin staining of the pancreas micrographs did not exhibit any morphological changes in Langerhans islets among acinar cells. Even

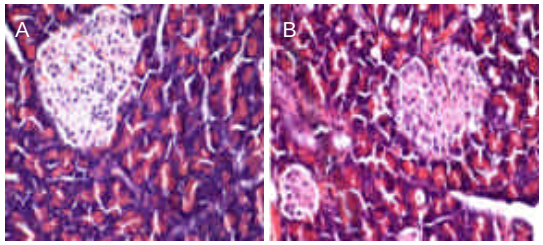

Fig. 4: Histological structure of rat pancreas sections. (A) Normal pancreas sections, (B) Pancreatic section of HFD-induced rat (Hematoxylin and Eosin staining at x400 magnification)

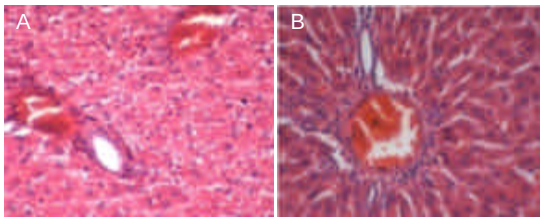

Fig. 5: Histological structure of rat pancreas sections. (A) Normal liver sections, (B) Liver section of HFD-induced rat (Hematoxylin and Eosin staining at x400 magnification)

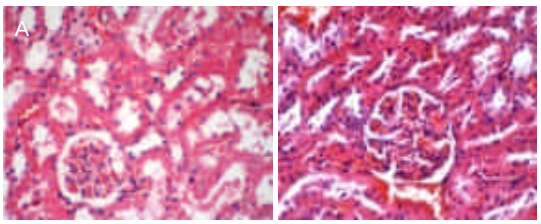

Fig. 6: Histological structure of rat pancreas sections. (A) Normal kidney sections, (B) Kidney section of HFD-induced rat (Hematoxylin and Eosin staining at x400 magnification)

after 10 weeks under this diet, no necrosis or damaged Langerhans islets were observed. Besides, results from liver portal tracts did not illustrate any inflammatory infiltration such as neutrophils presence within surrounding scanty connective tissues, even after 10 weeks under the HFD. Moreover, the HFDI rats showed similar kidney histology as in normal group. No tubular foaming changes were observed, as well.

DISCUSSION

A practical procedure to understand the etiology and pathogenesis of diseases is study on animal models. The benefits of using animal models in research works involved the ability to access its multifactorial genetics and complications. However, correlations between animal diseases and that in human kind are well

established. Different characteristics in animals compared to human and lack of any exact similar model to study are some of the major concerns that render the inconclusiveness of the outcome observed (Chattopadhyay *et al*., 1997). Few established methods are available to induce experimental diabetes in animal model. An approach in developing diabetic experimental animal model is through dietary modification which has been identified as one of the major factors that is associated with the development of diabetes mellitus type 2. In animal model of experimental diabetic diet containing higher levels of fructose has been reported to contribute to a metabolic disturbance in animal models and result in hyperglycemia and hyperlipidemia (Kasim-Karakas *et al*., 1996). Animals on HFD showed an increased body weight compared to ND which can be the consequence of high fructose daily consumption. Gradual weight elevation was observed throughout the study period with a higher rate in HFD induced rats. Excess fructose consumption is a cause of weight gain. It is the result of higher plasma TG which is made from excess fructose in the liver (Basciano *et al*., 2005). There are many studies demonstrated the association between high fructose diet with elevation of plasma glucose, insulin and triglycerides in animal models. A 10% (w/v) fructose solution given for 12 weeks period, increased blood glucose and TG level in 200 g-male Wistar rats (Dai and McNeill, 1995). In another report, a 10% (w/v) of HFD supplementation for four weeks has been shown to induce insulin resistance in male *Sprague-dawley* rats (Carranza *et al*., 2004). Yadav *et al*. (2007) showed that a 21% (w/v) concentration of fructose in diet conferred a significant increase in blood glucose and TG after eight weeks on the diet regimen. In a more recent study, a high fructose diet (10% w/v) for eight weeks has been shown to induce diabetes mellitus type 2 in two-month old male *Sprague-dawley* rats (Berkane *et al*., 2008). Absorbed fructose is metabolized rapidly by the liver. The exposure of the liver to large quantities of fructose leads to high stimulation of lipogenesis and TG accumulation. In such condition, insulin sensitivity is reduced and hepatic insulin resistant, occurs. In contrast with other carbohydrates, fructose can continuously enter the related pathways to produce glucose and glycogen and promote the over production of TG. These negative effects of fructose metabolism have summoned its usage on DM induction in animal models (Mayes, 1993). To be sure of any hyperglycemic signs among HFD groups, rats TG level was determined. Similar studies that showed elevated blood glucose in rat fed HFD, had observed a higher TG level, as well (Islas-Andrade *et al*., 2000). In the present study, the average TG level was 1.09±0.39 mmol/L which is low to be considered as diabetic. The normal way for diagnosing of DM emphasizes blood glucose as the best test to identify DM among asymptomatic subjects. Blood glucose level $\geq$11.1 mmol/L (200 mg/dL) is enough for diagnosis of DM (Braunwald *et al*., 2008). In the immune regulation of DM, T helper cells play a key role in initial inflammatory process. Increased cytokines secreted by Th1 cells and reduced cytokines secreted by Th2 cells, gradually cause imbalance in proinflammatory/ anti inflammatory level of the system and lead to early inflammation in diabetics (Raz *et al*., 2005). In the present study, three major inflammatory biomarkers, CRP, IL-6 and IL-4 were measured. C-reactive protein is a sensitive biomarker of inflammatory system and used mainly as a major marker of inflammation and vascular inflammatory processes in DM. It had been shown that CRP levels are elevated in either diabetic patients or rats (Jialal *et al*., 2004; Wellen and Hotamisligil, 2005; Cho *et al*., 2006; Crook *et al*., 1993; Rodriguez-Moran and Guerrero-Romero, 1999). Interleukin-6, one of the proinflammatory cytokines in vascular inflammation (Kern *et al*., 2001; Singh *et al*., 2005), involved in the regulation of glucose metabolism in skeletal muscles and adipose tissues (Grunfeld and Feingold, 1991; Löfgren *et al*., 2000). Since IL-6 is a major cytokine of the acute phase response, increased levels of IL-6 could reduce insulin sensitivity (Halse *et al*., 2001; Fernandez-Real *et al*., 2001). Elevated levels of IL-6 in the blood of many diabetic patients has been reported in many previous studies (Jain *et al*., 2007; Hussain *et al*., 1996; Pickup *et al*., 1997; Pradhan *et al*., 2001) which is suggested to be the result of an inflammatory response of DM. Interleukin-4 is a major Th2 secreted cytokine with anti inflammatory properties. Interleukin-4 is an important immunomodulatory cytokine. Due to its essential role in protective immunity and inflammatory diseases, it is recognized markers in DM. Inflammatory biomarkers of rats on the HFD were similar to the normal group without exhibition of any inflammatory or hyperglycemic alteration. No immune response was observed in the HFD group during the experimental period and no change in their measured inflammatory biomarkers, as well. All histological sections of rats on the HFD were similar to the normal group. No inflammatory or hyperglycemic alters was exhibited. Also, no inflammatory invasion and its associated immune responses were seen in order to the HFD administration during the experimental period. There were no significant increases in the blood glucose, TG level, body weight and inflammatory markers in the HFDI rats compared to the normal rats. Also, no hyperglycemic histological changes were seen. This observation indicates that hyperglycemia can not be induced by HFD in male *Sprague-dawley* rats for duration of 10 weeks. Besides, Stark *et al*. (2000), illustrated that feeding rats with a high fructose (53% w/v) and high sucrose (10% w/v) diet was not able to assert hyperglycemia, hyperinsulinemia, hypertriglyceridemia and hypercholesterolemia hence

unable to develop diabetes mellitus type 2 models among rats, although many other researchers have shown a positive results. The present study did not show the potential of HFD in inducing hyperglycemia. It may be due to the differences in the characteristic of the rat's species used in various studies. Diverse breeds are not physiologically similar. They can show different reactions based on their sensitivity to various environmental factors. Since *Sprague-dawley* rats are from diverse breeds, their colonies can appear genetically variable throughout the world. It is acceptable that studies carried out in different countries, may not exhibit similar findings despite similar experimental conditions. The age of rats may also be a contributing factor. Different metabolic reactions happen to similar diet at different age of the rats. It was shown in a previous study that blood glucose level of the young rats can be altered by the HFD (Han *et al*., 1997). The length of exposure can also be another factor that may alter experimental outcomes, includes exposure time to HFD, metabolic profiles of the rat during experiment and time of measurements. However, it was found that healthy animals were able to adapt to the HFD after 12 weeks without developing metabolic disorders (Stark *et al*., 2000). The results of the present study allow for the conclusion that high fructose diet for duration of 10 weeks was unable to induce hyperglycemia and its relevant histological and inflammatory changes in male *Sprague-dawley* rats.

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