Metabolic effects of high sucrose and saturated oil feeding on insulin resistance in Sprague-Dawley rats

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In this study, we explored the effects of long-term consumption of a high-sugar high-fat diet on glucose tolerance and insulin resistance in rats. Rats were fed with either standard rat chow diet (NC group) or high-sugar high-fat diet (HSHF group) for 16 weeks. The HSHF group showed significantly higher fasting insulin level than NC group. Following intraperitoneal glucose challenge, blood glucose and insulin levels in the NC and HSHF groups increased. However, the magnitude of the response in NC group was low compared to HSHF group. Insulin resistance was higher in HSHF group and insulin sensitivity decreased significantly (P < 0.05) in HSHF group in contrast to NC group. Low-density lipoprotein-cholesterol (LDL-C) and triglyceride (TG)/high-density lipoprotein-cholesterol (HDL-C) levels showed significant increase in HSHF group, while triglyceride and total cholesterol levels did not show any difference. The study demonstrated that feeding high-sugar high-fat diet to the experimental Sprague-Dawley rats for 16 weeks increased possibility of insulin resistance in them but did not turn them hyperglycemic or diabetic. Thus, they prove to be a suitable animal model to explore various aspects of insulin resistance.

Keywords: Glucose tolerance, Metabolic syndrome, Cholesterol, Obesity, Diet, Hyperglycemia, Diabetes, HDL, LDL, Triglycerides

Insulin resistance, characterized by reducing insulin sensitivity to insulin-dependent processes, is a common metabolic disorder that plays a critical role in the pathophysiology of diabetes, hypertension, and coronary heart disease. It results in reduced glycogenesis and glucose disposal in target tissues but increased gluconeogenesis; proteolysis in muscle; lipolysis in adipose tissue; and production of free fatty acids. Further, it contributes to defective insulin signaling pathways with receptor or post receptor defects, leading to insulin resistance in target organs such as liver, adipocytes and skeletal muscles. Fasting hyperglycemia as a result of insulin resistance in liver is a phenomenon of uncontrolled basal hepatic glucose output. However, postprandial hyperglycemia is a result of insufficient insulin secretion by β-cells after taking a meal, impaired hepatic glucose production, and defective glucose uptake by peripheral insulin-sensitive tissues especially skeletal muscle.

In type 2 diabetes mellitus (T2DM), insulin resistance is a consequence of complex interactions between genetic and environmental factors including inappropriate diet, physical inactivity and obesity. Different diets may lead to metabolic disorders. High energy intake from fat can alter insulin resistance and boost the risk of T2DM, but cannot cause frank hyperglycemia (fasting blood glucose level of 150-200 mg/dL) or diabetes. Several studies have reported that diets high in fat and/or refined sugar resulted in insulin resistance. Many other researchers have also reported that insulin resistance develops after using a high-fat and/or sucrose diet for as little as three weeks to two months. It was demonstrated earlier that refined sugar causes a bigger problem than the high fat diet during glucose tolerance tests, while the mixture of high fat and sucrose showed a worse response. High-fat and high-sucrose diets have been associated with abnormalities such as hyperlipidemia, glucose intolerance, hypertension and atherosclerosis. It can lead to insulin resistance and diabetes in some diabetogenic models of rodents as well or in rats with destroyed β-cell mass. On the other hand, the literature on non-diabetogenic animal models such as Wistar rats showed that feeding high-calorie diet (59% of total calories) for 21-23 days generated
insulin resistance by reducing the glucose disposal rate; while body weight, blood glucose, and insulin levels did not change noticeably over the study. Koshinaka et al. showed in a 4-wk study on Wistar rats that consumed high-fat diet, insulin resistance was induced and phosphatidylinositol 3-kinase pathway that mediates cellular response to insulin was impaired, and as a result, insulin-stimulated glucose transport in skeletal muscle and adipose tissue was decreased. Feeding a high fat diet (40%) containing either 30% w/w sucrose or 30% w/w starch for longer periods (8-9 wk) in Wistar rats resulted in increased body weight in both groups, while insulin levels during an oral glucose tolerance test and epididymal and perirenal fat pad weights in high-fat, high-sucrose fed rats increased remarkably. Studies in Sprague-Dawley rats by Sevilla et al. indicated that 24 wk feeding high-fat diet reduced glucose transport in the skeletal muscle and adipose tissue with decreased glucose transporter (GLUT4) gene expression. Moreover, Huang et al. reported the effect of an 8-wk study with increase in body weight, fasting blood glucose and plasma cholesterol levels. Likewise, Srinivasan et al. showed that consumption of high-fat diet by the same strain of rats in short term (4 wk) led to a significant increase in body weight, insulin, triglyceride, cholesterol levels and mild fasting hyperglycemia and impaired oral glucose tolerance test. Although the symptoms observed by taking various diets in rats are diverse and enormous studies have been carried out on this issue, it is still interesting to use inexpensive animal models to easily investigate various aspects of prediabetic status such as insulin resistance or even the susceptibility of diet to induce type 2 diabetes, atherosclerosis or metabolic syndrome. In this experiment, Sprague-Dawley rats, which have been widely used to explore the effects of different diets, fed on ad libitum high-sugar and high-fat diet for 16 wk so as to investigate the effects of the excess dietary calorie intake of oil palm-based fat and sugar solution on the glucose homeostasis, insulin sensitivity, and serum lipid profile.

Materials and Methods

Animals and diets—Male Sprague-Dawley rats aged 6-8 wk (initial weight 200-250 g) were obtained from a local supplier (Saphire Enterprise Sdn. Bhd, Serdang, Malaysia). All rats were individually housed in plastic cages with stainless steel covers and maintained on a 12-h L:D cycle at a room temperature of 23±2 °C. All animals were treated according to the ethical guidelines of the Faculty of Veterinary Medicine, Universiti Putra Malaysia, Malaysia with the reference number of UPM/FPV/PS/3.2.1.551/AUP-R48. They were acclimatized to the laboratory condition for one week with free access to standard rat chow and water. After that, rats were given free access to experimental diets for 16 wk. The diets were the standard rat chow (51.93 g carbohydrate, 3.03 g lipid, 20.50 g protein and 4.17 g crude fiber/100 g diet and 3.77 kcal/g energy) given to a normal control group (NC, n=10) and the high-sugar high-fat diet given to a HSHF group (n=10). The high-sugar high-fat diet (4.29 kcal/g, 30% and 70% of total calories from fat and carbohydrate, respectively) was prepared by mixing 15% (w/w) of palm oil-based margarine (Unilever Co. Malaysia) with standard rat chow and 30% of refined coarse sucrose (CSR Co. Malaysia) dissolved in drinking water. The mixture was prepared daily and unused feed in a period of 24 h was removed so as to prevent lipid oxidation and rancidity. Body weight and fasting blood glucose levels were recorded monthly. Lipid profiles were measured in experimental groups at baseline (0 wk) and at the end of 16 wk. Intraperitoneal glucose tolerance test (IPGTT), homeostasis model assessment of insulin resistance (HOMA-IR) and intraperitoneal insulin tolerance test (IPITT) were performed at the end of the study as well.

Intraperitoneal glucose tolerance test (IPGTT)—The IPGTT was carried out during week 16, with minor modifications to the method described by Zhang et al. After an overnight fasting, blood samples were drawn via saphenous vein and fasting blood glucose was determined as 0 time using the Accu-Chek Instant Plus blood glucose monitor (Accu-Check, Roche Diagnostic Corporation, USA). Then the animals were intraperitoneally injected with 30% glucose solution (2 g/kg body wt.) (Sigma-Aldrich, St. Louis, MO, USA). After the glucose injection, the blood samples (20 μL) were taken at 30, 60 and 120 min time points and blood glucose was measured by blood glucose test strip using the Accu-Chek Instant Plus blood glucose monitor. Blood samples were analyzed for measuring the plasma insulin level using ELISA Rat/Mouse insulin kit (Millipore Corporation, USA).

Analysis of AUC and HOMA-IR—The area under the curve (AUC) was calculated using the Trapezoidal rule. The Homeostasis model assessment of insulin
resistance (HOMA-IR) was calculated as explained by Hosker et al., with the use of fasting insulin and fasting glucose levels obtained from IPGTT to characterize insulin sensitivity and insulin secretion capacity. HOMA-IR=(FIxFG)/22.5, where FI is fasting insulin (µU/ml) and FG is fasting glucose (mmol/L).

**Intraperitoneal insulin tolerance test (IPITT)**—The intraperitoneal insulin tolerance test (IPITT) was carried out with a slight modification of the method described by Wendel et al. so as to test the ability of the body to use insulin. After an overnight fast, blood glucose levels of rats were measured in the tail vein at 0 time points using glucometer, following an intraperitoneal injection of 1.5 IU/kg insulin (Humulin, Eli Lilly and Company Indianapolis, USA) into both NC and HSHF groups. Then, blood glucose values were measured at 30, 60, 120 minutes after insulin injection using the glucometer (Accu-Check, Roche Diagnostic Corporation, USA).

**Blood serum and plasma preparation**—About 3-5 ml blood was drawn via cardiac puncture into plain and heparin vacutainer tubes from overnight fasted rats that were anesthetized. Prepared blood samples were kept instantly at 4 °C. The blood in plain tube was stored at room temperature for a few minutes to be coagulated before being cooled. Samples were centrifuged at 1500 G for 10 min (Eppendorf, Germany) to separate the plasma from the red blood cells in heparin tubes, and to separate serum in plain tubes. The plasma and serum samples were divided into storage tubes according to their required amount of biochemical tests. They were quickly stored at -20 °C for further analysis.

**Biochemical measurements**—Glucose was measured using the glucometer (Accu-Check, Roche Diagnostic Corporation, USA). Plasma insulin levels were measured with a rat insulin kit (Millipore, Bedford, MA, USA) by enzyme-linked immunosorbent assay (ELISA). Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-C) levels in blood serum were determined by enzymatic methods using standard reagents and automatic analyzers (ADVIA 2400 Chemistry Systems, Siemens Healthcare Diagnostics, USA). Total cholesterol measurement (Siemens Diagnostics) was based on an enzymatic method using cholesterol esterase and cholesterol oxidase conversion followed by a Trinder endpoint. The triglyceride method (Siemens Diagnostics, Medfield, MA, USA) was based on Fossati three-step enzymatic reaction with a Trinder endpoint. The direct HDL-C measuring method was based on the elimination of cholesterol from non-HDL particles and measuring HDL-C by a Trinder reaction after releasing cholesterol from HDL particles. The concentration of LDL-C was calculated according to the formula of Friedewald et al., when TG <4.5 (mmol/L): LDL-C (mmol/L) = TC - [(TG/2.18) + HDL-C].

**Statistical analysis**—The data were analyzed as a completely randomized experimental design using the General Linear Model (GLM) of SAS Version 9.1 (Statistical Analysis Systems Institute Inc., 1992). All time dependent values were analyzed by repeated-measures ANOVA. The Tukey’s HSD and least significant difference (LSD) tests were used to differentiate the means. Differences with \( P < 0.05 \) were considered significant. The data were checked for normality using PROC Univariate and the results in the tables are presented as means ± standard error of the means.

**Results**

Animal weight in each group increased significantly \( (P <0.05) \) after 16 wk (Table 1). There was also a significant difference \( (P >0.05) \) in body wt. between NC and HSHF groups. Fasting blood glucose level decreased significantly \( (P <0.05) \) in HSHF group when compared to baseline level but it was not significantly different \( (P >0.05) \) compared to the NC group at week 16. Rats in the NC group did not show significant \( (P >0.05) \) change in blood glucose levels over this period. The results of Table 1 showed that fasting plasma insulin level almost doubled in the group fed HSHF diet after 16 wk. As demonstrated in Fig. 1a, the blood glucose concentrations during IPGTT, which did not differ between groups at zero time, increased dramatically in the NC group after 30 min post-glucose injection, and start to reduce and reach to the pre-treatment levels after 120 min. In the HSHF group, after glucose injection, the blood glucose concentration increased and reached to the peak at 30 min post injection. At this point and time 60 and 120 min, the values were significantly \( (P <0.05) \) higher than the NC rats. Plasma insulin levels were also determined during the IPGTT at 0, 30, 60, 120 min of experiment (Fig. 1b). Insulin level in the NC group increased minimally and peaked at 30 min and reached to the normal level after 120 min. Rats fed HSHF diet produced significantly
higher fasting insulin levels in response to glucose tolerance tests compared to NC rats. Insulin level augmented sharply in HSHF group, peaked at 30 min and reached to a level significantly ($P < 0.05$) higher than that of NC group. Plasma insulin levels were significantly ($P < 0.05$) higher in HSHF group at 0, 30, 60, 120 min post-glucose injection as compared to NC group. Area under the curve for glucose and insulin graph in IPGTT was significantly higher ($P < 0.05$) in HSHF group compared to NC group.

Results of IPITT (Fig. 2) exhibited decreased insulin sensitivity in a HSHF group after 16 wk study. Before insulin injection (at zero time) blood glucose levels were not significantly ($P > 0.05$) different. However, glycemic values of NC rats decreased rapidly after insulin injection, and began to reduce and reached to pre-treatment values after 120 min. In the HSHF group, after insulin injection, the glycemic level peaked at 30 min and decreased slowly within 120 min of the experiment compared to the NC group. The blood glucose level of HSHF group was significantly ($P < 0.05$) higher at 30, 60 and 120 min post-insulin injections compared to the NC group.

As illustrated in Fig. 3, HOMA-IR analysis exhibited significant increase ($P < 0.05$) in the insulin resistance index of animals in the HSHF diet as
compared to the NC group. Calculation of area under the curve for glucose and insulin graph in IPGTT and even glucose graph in IPITT showed that the HSHF diet is able to induce glucose intolerance, reduced insulin sensitivity and insulin resistance in animals after 16 wk compared to the rats fed standard rat chow.

Animals in the NC group showed no significant difference (P >0.05) in lipid profile levels (TG, TC, HDL) throughout the experimental period (Table 1). At the end of week 16, TG and TC serum levels in HSHF group increased significantly (P <0.05) over the time but the values were not statistically different from NC group at the end of the study. Serum LDL-C levels of animals increased significantly (P <0.05) in HSHF group after 16 wk, and it was significantly (P <0.05) higher than the NC group. Serum HDL-C levels and HDL-C/TC ratio values significantly (P <0.05) decreased in HSHF group after 16 wk when compared to baseline levels and their values were significantly (P <0.05) lower than that the NC group at week 16. The TG/HDL-C level also increased significantly (P <0.05) compared to the NC group.

Discussion

Small animal model in a controlled environment is a logical procedure to evaluate the influence of excess dietary ingredients on induction of insulin resistance and diabetes. Among all, rodents, particularly, rats have shown a good response to dietary factors. No animal model accurately simulates human physiology and, hence outcome of studies on insulin resistance too, bound to differ. Few conventional methods are available to induce insulin resistance in animal models. One of these methods is dietary modification which has been recognized as one of the major factors in the development of insulin resistance and diabetes. Most of the energy of the diets is mainly provided from fat and sugar, the main constituents in western foods. According to the Subcommittee on Laboratory Animal Nutrition Committee on Animal Nutrition Board of Agriculture National Research Council, USA, the suggested fat concentration of dietary lipid for growing male and female rats is 5% and for dietary carbohydrate concentration is about 50% by weight. Hence, the diet which was used in our study was labeled as high-sugar high-fat diet resembling the western diet. Significant weight increase in HSHF and NC animals was observed after 16 wk which might be the consequence of ad libitum daily consumption of diets, high rate of lipogenesis and TG accumulation in the liver. Previous studies have shown an increase in body wt. of animals on high calorie diets as well.

Elevated fasting blood glucose level (more than 6.1 mol/L), which is considered as a regular sign of diabetes, was not noticed at the end of 16 wk and the values were still in the normal ranges. This result was expected since several studies report glucose intolerance, with normal fasting glucose levels after using high fat or high fructose diets. In contrast, Han et al. demonstrated glucose intolerance with hyperglycemia in some of fasted animals in which the period of feeding the animals was longer than this study (32 wk) while the diet composition was comprised of similar saturated fatty acids (32% of calories as lard oil and 18% corn oil).

Observed dyslipidemia is affected by the higher energy intake, physical inactivity, dietary fat composition and genetic predisposition. This experiment indicated that an extreme intake of a diet containing high sugar plus palm-based margarine is not only able to effect on glucose metabolism, but also cause alterations in lipid metabolism. Observed disorders in lipid metabolism in this experiment represent as key factor for the development of atherosclerosis and subsequent coronary heart disease. The increase in LDL-C concentration indicates consumption of a diet rich in saturated fatty acids that decrease the LDL receptor-mediated catabolism. Since the number of LDL receptors in cell membranes reduces when there is sufficient LDL-C in
intracellular space, uptake of LDL-C by target cells decrease consequently. Besides, increased activity of lipoprotein lipase in hyperlipidemic animals leads to increased production of LDL-C from VLDL-C. The insignificant increase of total cholesterol between both groups was in contrast with the findings of Dobrian et al., in which a moderately high fat diet (32% kcal from fat) significantly increased serum total cholesterol and triglyceride level and decreased HDL level in Sprague-Dawley rats as compared with control group after 10 wk. Their results suggested that hypercholesterolemia is linked to the diet. So, the difference between the used diets, source and breeding of Sprague-Dawley rats can be considered as possible reasons for the results obtained in the present study. Since each of the TC and TG levels are not the only effective determinant of atherosclerosis disease, HDL-C/TC and TG/HDL-C ratios have been considered as the main predictors of cardiovascular disease (CVD) and increase the risk of heart attack. The significant decrease in HDL-C/TC and the increase in TG/HDL-C values indicated that the exerted hyperlipidemia is considered as a crucial risk factor in the initiation and development of atherosclerotic lesions and succeeding cardiovascular complications.

Diets high in saturated fats and carbohydrates are linked with glucose intolerance, obesity, coronary heart disease, and type 2 diabetes mellitus. The present results have shown that feeding Sprague-Dawley rats with the experimental HSHF diet reduced insulin sensitivity and exerted insulin resistance, similar to prediabetic status rather than induction of fasting hyperglycemia or type two diabetes mellitus. Studies by Barnard et al. have also documented that high-sucrose, high-fat diet that is similar to the typical USA diet can induce metabolic syndrome in rats.

In addition, experiments on rats have clearly revealed that diets rich in simple sugars are able to reduce insulin sensitivity. The prediabetic phase that frequently develops before the diagnosis of type 2 diabetes is a consequence of three common conditions of insulin resistance: impaired glucose tolerance, impaired fasting glycaemia and elevated fasting insulin levels. To better understand the glucose homeostasis in both human and rodent models some techniques have been used to detect the incidence of insulin resistance, including glucose tolerance tests (GTT), and insulin tolerance tests (ITT). Glucose tolerance is a function of glucose-stimulated insulin secretion, hepatic glucose production and tissue insulin sensitivity. IPGTT was performed on the overnight fasting animals and reflected inhibition of glucose production by the liver and insulin-stimulated glucose clearance. In this study, the HSHF rats showed fasting normoglycaemia and hyperinsulinemia before the glucose injection at 0 time. Glycaemia in IPGTT increased dramatically in both groups post-glucose injection. However, in NC rats, blood glucose normalized faster to pre-treatment values while in HSHF rats values were considerably higher during the experiment. Calculation of area under the curve for glucose and insulin graph in IPGTT showed significant decrease in glucose clearance rate in contrast to NC rats. It is indicated that HSHF diet is able to induce glucose intolerance, reduced insulin-stimulated glucose uptake in animals after 16 wk. This may be due to the decrease in the number of insulin receptors exerted by high fat diet that, in consequence, reduce the activity of the glucose transport system and the intracellular glucose metabolism.

As expected, the plasma insulin level in IPGTT increased minimally after a glucose load in NC rats, whereas insulin level increased markedly in HSHF rats during IPGTT. The rise in plasma insulin level during IPGTT reflects an increased rate of insulin released into the blood in response to glucose absorption and hyperglycaemia. The consequent decline in insulin level after 2 h most likely represents a moderation in the rate of insulin release and an increased rate of insulin removal of blood. The HSHF group also showed decreased insulin sensitivity and marked deficiencies in glucose clearance as indicated by elevated blood glucose levels post-insulin injection in IPITT. This clearly suggests that the applied HSHF diet containing palm oil-based margarine plus sucrose in this experiment has the ability to impair glucose and lipid homeostasis and decrease insulin sensitivity that is perhaps because of peripheral insulin resistance caused by higher levels of circulating free fatty acids.

Overall, long-term consumption of high calorie diets can lead to metabolic syndrome in experimental animals depending on diverse contributing factors such as type of diet, length of consumption, the amount of calorie intake, animal species, their metabolic profile and source of animal breeding. Taking into account all the above factors as well as the observed findings, it is concluded that HSHF diet...
containing plant-based fat content and sugar drink is able to induce hyperinsulinemia, glucose intolerance, body weight and insulin resistance in a relatively long-term consumption in Sprague-Dawley rats. However, there was no rise in fasting glucose concentration or even a relative deficiency in insulin secretion, which are considered as regular signs of type 2 diabetes mellitus.

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References


