Leptin, Adiponectin and Insulin as Regulators for Energy Metabolism in a Rat Model of Metabolic Syndrome
(Leptin, Adiponektin dan Insulin sebagai Pengawalatur untuk Metabolisme Tenaga dalam Model Tikus Sindrom Metabolik)

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ABSTRACT

Leptin, adiponectin, and insulin are pivotal regulators for lipid and glucose metabolism. This study aimed to investigate the changes in these hormones in a rat model of metabolic syndrome (MetS) induced by high-carbohydrate high-fat (HCHF) diet. Twelve-week-old male Wistar rats were divided into two experimental groups. The normal group was given standard rat chow with tap water. The HCHF group was given HCHF diet with 25% fructose-supplemented drinking water to induce MetS. Body composition of the animals was measured using dual-energy X-ray absorptiometry. Blood was collected at week 0, 8, 12, and 16 for the measurement of blood glucose and hormone levels. Our findings demonstrated that HCHF diet significantly increased fat mass, percentage of fat, and decreased lean mass in the animals starting from week 8. The levels of blood glucose, leptin, and insulin were significantly higher but the level of adiponectin was significantly lower in the HCHF rats compared to the normal rats. In conclusion, hormones play a key underlying role in regulating lipid and glucose metabolism in MetS.

Keywords: Adiponectin; glucose; insulin; leptin; lipid

ABSTRAK

Hormon leptin, adiponektin, dan insulin merupakan pengawalatur untuk metabolisme lipid dan glukosa. Kajian ini bertujuan untuk mengkaji perubahan aras hormon dalam model tikus sindrom metabolik (MetS) yang diaruhkan oleh diet tinggi karbohidrat tinggi lemak (HCHF). Tikus jantan Wistar berumur 12 minggu dibahagikan kepada dua kumpulan. Kumpulan normal diberi makanan tikus piawai dan air paip. Kumpulan HCHF diberi diet HCHF dan air minuman yang ditambah dengan 25% fruktosa untuk mengaruh sindrom metabolik. Komposisi badan haiwan diukur menggunakan absorptiometer sinar-X. Darah haiwan dikumpul pada minggu 0, 8, 12, dan 16 untuk mengukur aras glukosa darah dan hormon. Keputusan kajian menunjukkan diet HCHF meningkatkan jisim lemak, peratusan lemak dan menurunkan jisim tanpa lemak secara signifikan dalam haiwan pada minggu ke-8. Aras glukosa darah, leptin dan insulin adalah lebih tinggi tetapi aras adiponektin adalah lebih rendah dalam tikus HCHF berbanding tikus normal. Kesimpulannya, hormon memainkan peranan dalam mengawalatur metabolisme lipid dan glukosa dalam MetS.

Kata kunci: Adiponektin; glukosa; insulin; leptin; lipid

INTRODUCTION

Central obesity, hyperglycaemia, hypertension, hypertriglyceridemia, and low high-density lipoprotein (HDL) cholesterol are medical abnormalities that define metabolic syndrome (MetS) (Alberti et al. 2009). These abnormalities resulted from over-nutrition and physical inactivity, which together are stronger predictors of cardiovascular disease and type 2 diabetes mellitus than individual conditions. The underlying factors that unify this syndrome include insulin resistance, glucose intolerance, adiposity, adipose tissue dysfunction, and disordered lipid metabolism. Adipose tissue is an endocrine organ capable of synthesizing a wide variety of biologically active molecules, known as adipokines. The changes in adipokine secretion are often closely associated with MetS.

Leptin is an adipokine that has a major role in the regulation of energy homeostasis, appetite and metabolism in the state of energy excess or deficiency, and neuroendocrine function (Kelesidis et al. 2010). Adiponectin is another adipokine that has potent effects on glucose and lipid metabolism in skeletal muscle, cardiac muscle, and liver. Specifically, adiponectin regulates food intake, improves glucose uptake, fatty acid oxidation, as well as reduced glycogen synthesis, glucose production, and triglyceride content in these organs (Karbowska & Kochan 2006). Previous studies have consistently shown that leptin and adiponectin were respectively in direct and inverse correlation with body mass index (BMI), waist circumference, fat mass, insulin resistance, hyperinsulinemia, triglycerides, and low-density lipoprotein (LDL) cholesterol (Bazanelli et al.

2013; Izadi et al. 2013; Matsubara et al. 2002; Milewicz et al. 2010).

The co-occurrence of obesity and elevated circulating leptin levels are often caused by leptin resistance resulting in an imbalance between energy intake and expenditure (Morris & Rui 2009; Myers et al. 2010). Leptin resistance also causes the reduction of leptin-induced fatty acid oxidation and increases triglyceride content, resulting in the development of lipotoxicity (Harris 2014). On the contrary, adiponectin displays anti-atherogenic, anti-inflammatory, and insulin-sensitizing effects. Adiponectin alleviates obesity-induced ectopic lipid accumulation and lipotoxicity by promoting fatty acid oxidation within the cells. Adiponectin also increases insulin sensitivity by reducing hepatic gluconeogenesis (Yamauchi et al. 2002). Therefore, leptin and adiponectin exert reciprocal effects in mediating the lipid and glucose metabolism.

Insulin is traditionally identified as a regulatory hormone for glucose homeostasis (Roberts et al. 2013). The stimulus for insulin secretion is the rise of blood glucose after a meal. Hence, glucose concentration is maintained within a very narrow range in the bloodstream. This is achieved by the regulation of glucose synthesis by the liver as well as glucose uptake by peripheral tissues such as skeletal muscle, liver, and adipose tissue. Additionally, insulin also exhibits control over lipid metabolism by attenuating lipolysis and concomitantly enhancing triglyceride storage in adipocytes (Chakrabarti et al. 2013). Insulin resistance is a hallmark feature of the MetS, characterized by the body not responding effectively to insulin. Hence, individuals with insulin resistance are also associated with the disturbance of lipid and glucose metabolism.

Leptin, adiponectin, and insulin may be the hormones of interest mediating the abnormalities associated with MetS. The aim of this study was to evaluate the effects of high-carbohydrate high-fat (HCHF) diet on the level of these hormones that regulate lipid and glucose metabolism.

MATERIALS AND METHODS

STUDY DESIGN

All animal experimentations were reviewed and approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC) (Code: PP/FAR/2015/IMA/20-MAY/679-JUNE-2015-MAY-2017). Age-matched twelve-week-old male Wistar rats (n=14) weighed 200 – 250 g were obtained from Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia (Kuala Lumpur, Malaysia). The rats were housed individually at Animal Laboratory, Department of Anatomy, Universiti Kebangsaan Malaysia (Kuala Lumpur, Malaysia) under standard ambient temperature ($25 \pm 2°C$) with an alternate 12-hour light-dark cycle. Upon acclimatization for one week, the animals were randomized into two experimental arms: the normal and HCHF groups. The normal rats were given standard rat chow (Gold coin, Port Klang, Malaysia)

and tap water. The MetS-induced group was fed with a modified HCHF diet (consisting of 39.5% sweetened condensed milk, 20% ghee, 17.5% fructose, 15.5% powdered rat food, 2.5% Hubble Mendel and Wakeman salt mixture, and 5% water) plus 25% fructose (w/v) in drinking water (Wong et al. 2018b, 2017a). This MetS animal model has been successfully established using HCHF diet, whereby the components of MetS were partially developed at week 8 and fully developed at week 12 (Wong et al. 2017a). All food and drinks were provided *ad libitum*. The animals were euthanized after 16 weeks of feeding with assigned diet.

EVALUATION OF BODY COMPOSITION

The rats were positioned in ventral recumbency on the scan table. Whole body scans were performed at week 0, 8, and 16 to measure body composition (fat mass, lean mass, and percentage of fat) of the rats using the dual-energy X-ray absorptiometer (DXA) (Hologic QDR-1000 System, Hologic Inc., Waltham, USA) under general anaesthesia using the ketamine/xylazine/zoletil cocktail. All scans were analysed using the manufacturer's recommended software calibrated for small animals.

BIOCHEMICAL ASSAYS

Blood was collected via tail vein at week 0, 8, 12, and 16. Serum was extracted by centrifugation at 3000 rpm for 10 min and stored at -70°C until analysis. The postprandial glucose level in serum was quantified using a colourimetric assay kit (Catalog number: DIGL-100, BioAssay Systems, USA). Serum levels of leptin (Catalog number: JP27295; IBL International GmbH, Hamburg, Germany), adiponectin (Catalog number: QY-E10886, Qayee Bio-Technology, Shanghai, China), and insulin (Catalog number: A05105, SPI-Bio, Bertin Pharma, Saclay, France) were evaluated using commercial enzyme-linked immunosorbent assay (ELISA) kits as per the manufacturer's instructions.

STATISTICAL ANALYSIS

The statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) version 20 software (IBM, Armonk, NY, USA). The percentage change of body composition was calculated as ((reading at final – reading at baseline)/ reading at baseline × 100%). General linear (repeated) measure was used to analyse the significant difference for all the parameters between the study groups. All data were expressed as mean ± standard error of the mean (SEM). Value of $p<0.05$ was considered as statistical significance.

RESULTS

Male rats fed with HCHF diet showed significant increases in fat mass (3.8-fold change) and percentage of fat (6.3-fold change) but significant decrease in lean mass (0.2-fold change) at week 8 as compared to the rats fed with

standard diet (p<0.05). Similar trend was observed in these parameters at week 16, whereby fat mass (4.7-fold change) and percentage of fat (5.3-fold change) were increased but lean mass was reduced (0.3-fold change) in the HCHF animals relative to the normal animals (Figure 1). The postprandial glucose concentration in serum of the HCHF rats was significantly higher than the normal rats at week 12 (1.7-fold change) and week 16 (1.5-fold change) (p<0.05). For the hormone levels, as compared to the normal control, HCHF diet caused significant elevation of leptin (2.6- to 5.1-fold change) and insulin (2.2- to 10.6-fold change) starting from week 8 until week 16 (p<0.05). Whereas HCHF diet caused significant reduction in adiponectin level in the serum of animals at week 12 (0.5-fold change) and week 16 (0.7-fold change) (p<0.05). The leptin/adiponectin ratio of the HCHF animals was also significantly higher compared to the normal controls from week 8 to week 16 (p<0.05) (Figure 2).

DISCUSSION

Other data obtained as part of this experiment was reported earlier (Wong et al. 2018a, 2017a, 2017b). Those papers reported on the successful induction of MetS in animals using HCHF diet. Elevations of systolic blood pressure, triglyceride, total cholesterol, and LDL cholesterol were observed after 8 weeks of HCHF diet. Other components of MetS including the increase in abdominal circumference, diastolic blood pressure, fasting blood glucose, glucose intolerance, and the decrease in HDL cholesterol were detected after 12 weeks of HCHF diet (Wong et al. 2018a, 2017a, 2017b). In this paper, we reported data

on the effects of HCHF diet on hormonal changes which potentially modulated the lipid and glucose metabolism in animals with MetS. Results from this study indicated that HCHF diet significantly raised leptin and insulin levels, while reduced adiponectin level in male rats. The animals fed with HCHF diet also had higher postprandial glucose level and fat content with concomitantly lower lean mass as compared to the normal controls.

Leptin is the protein encoded by the obese gene. The level of leptin is directly proportional to body fat mass. Leptin receptor is abundantly expressed in many organs including hypothalamus, heart, liver, kidney, pancreas, muscle, and adipose tissue, indicating the wide range of targets and widespread effects for leptin (Amitani et al. 2013). Under normal physiological conditions, leptin binds to its receptor and decreases food intake, increases energy expenditure, facilitates glucose utilization, and improves insulin sensitivity in the maintenance of metabolic balance (Srikanthan et al. 2016). In contrast, chronic carbohydrate and fat consumption evokes a decrease in leptin sensitivity, known as leptin resistance. Numerous *in vivo* studies showed that animals maintained on high-fat diet exhibited marked hyperleptinemia (Handjieva-Darlenska & Boyadjieva 2009; Jiang et al. 2009; Lin et al. 2000). The consumption of HCHF diet in this study resulted in the increase in fat mass and subsequently a positive trigger for more leptin production by fat tissues. Mechanistically, the impairment in leptin transportation, attenuation in leptin signalling, endoplasmic reticulum (ER) stress, deficiency in autophagy, and chronic inflammatory state are the common causes of obesity-associated leptin resistance
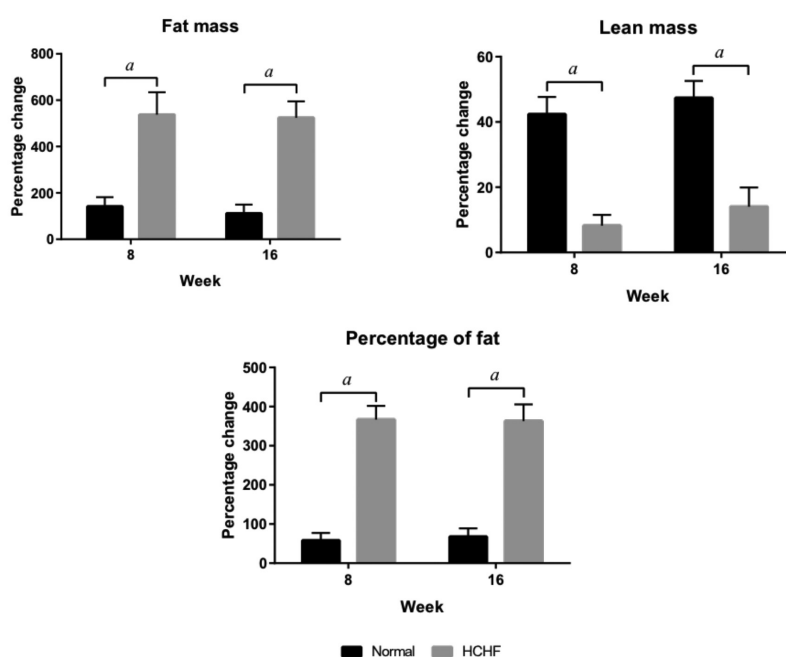

FIGURE 1. Percentage change of body composition (fat mass, lean mass, and percentage of fat) in the normal and HCHF rats at week 8 and 16 compared to baseline (week 0). The data are expressed as mean ± SEM. Letter 'a' represents significant difference (p<0.05) compared to the normal group
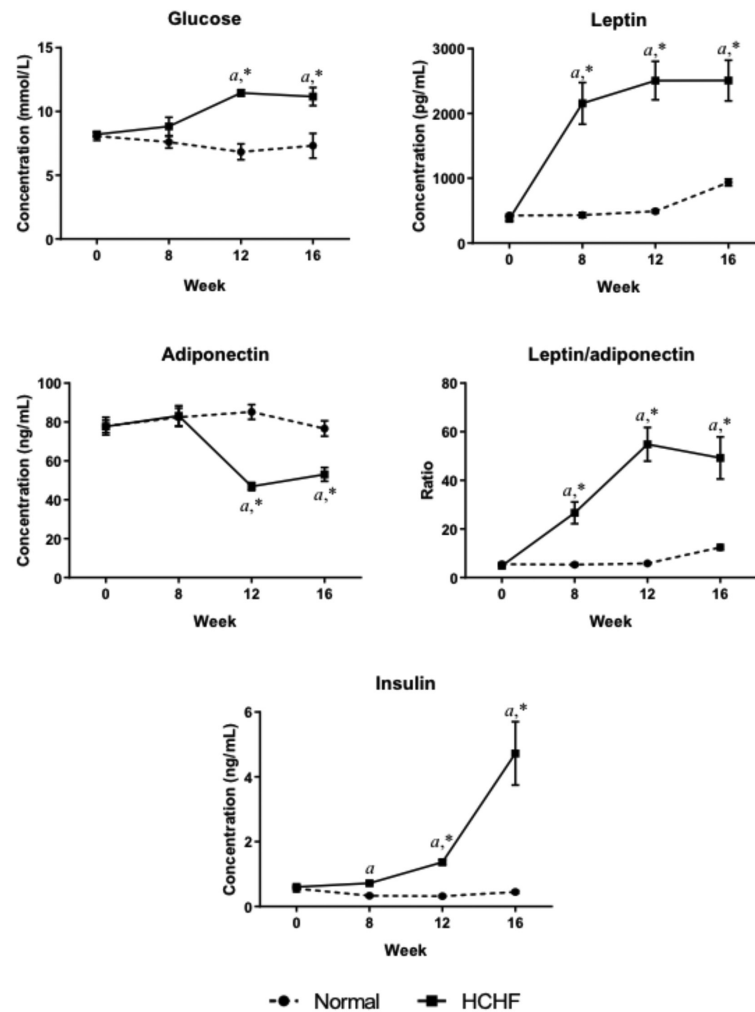
FIGURE 2. Levels of glucose, leptin, adiponectin, and insulin in the normal and HCHF rats at week 0, 8, 12, and 16. The data are expressed as mean ± SEM. Letter 'a' represents significant difference (p<0.05) compared to the normal group and symbol '*' represents significant difference (p<0.05) compared to week 0

(Li & Li 2016). Therefore, leptin resistance disturbs the normal leptin signalling and function by causing: the imbalance of energy homeostasis and body weight by the central nervous system, the inhibition of fatty acid oxidation and glucose uptake in the skeletal muscle, the induction of insulin and glucagon secretion by the pancreas, the elevation of ectopic lipid accumulation in the liver, and the increase of free fatty acids (FFA) release from adipose tissue via lipolysis (Harris 2014; Jung & Choi 2014; Li & Li 2016).

Adiponectin is an adipocytokine chiefly produced by fat cells. The circulating adiponectin level is paradoxically related to fat mass. Similar to leptin, adiponectin receptors such as AdipoR1 and AdipoR2 are predominantly found in skeletal muscle and liver to regulate lipid and glucose metabolism. Previous studies reported that low levels of adiponectin in serum can be a strong predictor for MetS (Kim et al. 2013), insulin resistance (Awazawa et al. 2011) and type 2 diabetes (Yamamoto et al. 2014). Previous studies reported the reductions in adiponectin gene and protein expression

under high-fat dietary condition in animals (Barnea et al. 2006; Landrier et al. 2017). Congruent with these past findings, our results demonstrated that the animals with MetS induced by HCHF diet had a lower level of adiponectin compared to the normal control animals. Adiponectin helps in maintaining systemic energy balance by enhancing fatty acid oxidation, triglyceride catabolism, and fatty acid uptake (Lee & Shao 2012). The underlying mechanisms of action of adiponectin include the activation of peroxisome proliferator activated receptor (PPAR) and adenosine monophosphate-activated protein kinase (AMPK) (Ghadge et al. 2018). A study by Nawrocki et al. (2006) reported that mice lacking adiponectin showed decreased hepatic insulin sensitivity and reduced responsiveness to PPARγ agonists. Another study indicated that the transgenic mice with elevated circulating adiponectin displayed improved insulin sensitivity and increased lipid clearance in the liver through increased AMPK activity (Combs et al. 2004). Hence, the reduction of adiponectin in the animals fed with HCHF diet might be one of the contributing factors
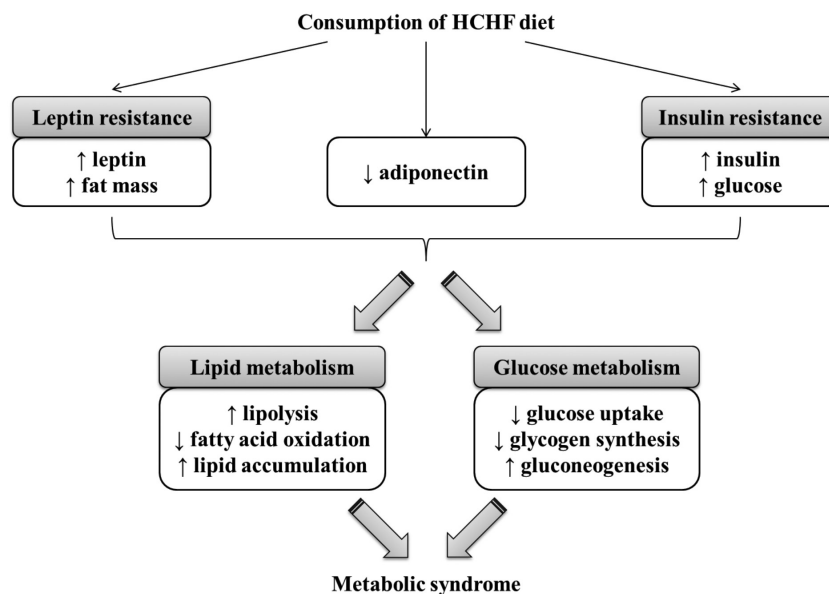
FIGURE 3. Summary of the effects of HCHF diet on hormonal changes in the regulation of lipid and glucose metabolism

for the imbalance in blood glucose and energy profile leading to MetS.

Insulin is a peptide hormone secreted by β-cells of the pancreatic islet to regulate metabolism of carbohydrates, fats, and proteins. Under normal condition, insulin is secreted to enhance glycolysis process and lower glucose level by increasing the transportation of glucose from the bloodstream to body cells when glucose concentration is high, such as after eating (Qaid & Abdelrahman 2016). In the state of over-nutrition and excessive intake of fat, accumulation of triglyceride in the organs causes insulin resistance thus glucose builds up in the bloodstream, leading to diabetes (Wong et al. 2016). A human study conducted by von Frankenberg et al. (2017) indicated that overweight adults fed on a diet high in fat especially saturated fat had decreased insulin sensitivity. Our results demonstrated comparable outcomes that HCHF diet increased insulin and postprandial glucose levels, suggesting the occurrence of insulin resistance during MetS. Apart from regulating glucose metabolism, insulin signalling is uniquely involved in lipid metabolism, which functions to maintain the capacity of adipose tissue in triglyceride synthesis and storage under healthy conditions. Paradoxically, chronic hyperinsulinemia increases cytokines within adipose tissue leading to the dysfunction in the control of lipogenesis (Czech et al. 2013). Taken together, insulin resistance during MetS is closely associated with the increase of triglyceride accumulation in liver and the decrease triglyceride storage in the adipose tissue.

Overall, findings from the present study validated the role of leptin, adiponectin, and insulin as the hormones that affect fuel metabolism, particularly in the regulation of lipid and glucose metabolism during MetS (Figure 3). However, some limitations of this study need to be addressed. Firstly, food intake was not recorded. Secondly, the hormones and adipokines measured were limited. Thirdly, the evaluation of lipoprotein lipase (an enzyme responsible for hydrolysing triglycerides and promoting cellular uptake of fatty acids) was not carried out.

CONCLUSION

Leptin, adiponectin, and insulin may act as a central rheostat that controls the energy balance tightly during MetS. These hormones respond to the nutrient flux and equilibrate the energy demands in maintaining systemic metabolic homeostasis. The uncoupling of hormonal signalling potentially leads to the development of various metabolic abnormalities associated with MetS. In this context, the evaluation of leptin, adiponectin, and insulin levels may be a valuable panel of biomarkers for early detection of MetS.

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