

Development of an Experimental Model of Metabolic Syndrome in Sprague Dawley Rat

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ABSTRACT

The study was designed to establish an experimental models of metabolic syndrome that adequately mimic metabolic syndrome criteria as determined by NCEP ATP III.

Eighteen Male Sprague dawley rats, 2 - 3 months old were used in the study. Combination of high fat and high sucrose (HFHS) diet for eight weeks and streptozotocin (STZ) injection in the second week was administered to induce metabolic syndrome. The body weight and biochemical parameters (blood glucose, triglyceride, HDL-cholesterol) were measured every 2 weeks. The rats with blood glucose (>126mg/dL), triglyceride (TG) (>150mg/dL), high systolic blood pressure (≥ 140 mmHg), and reduced HDL levels (<40 mg/dL) confirmed presence NCEP-ATP III criteria of metabolic syndrome. The adiponectin level was analyzed by ELISA methods.

Fasting blood glucose, triglyceride, and systolic blood pressure increased significantly ($p < 0.05$) in HFHS group compared to that of NC group. Moreover, after 8 week a significant lower HDL level was observed in HFHS group

compared to that of NC group. In addition, HFHS group showed a significantly lower adiponectin level compared to that of NC group.

The combination of low doses of STZ (30mg/kg) and HFHS administration for 8 weeks could induce metabolic syndrome mimicking human criteria of metabolic syndrome.

Keywords: Metabolic syndrome animal model; streptozotocin; high fat and high sucrose diet.

INTRODUCTION

The increasing prevalence of metabolic syndrome poses a public healthcare problem worldwide. Metabolic syndrome prevalence was around 10 to 84 % based on age, gender, race, ethnicity and metabolic syndrome criteria (Cameron, 2004). Metabolic Syndrome represents the association of common pathologies such as obesity, diabetes, insulin resistance, dyslipidemia, fatty liver and associated cardiovascular complications Metabolic (Alberti, 2005). World Health Organization (WHO), International Diabetes Federation (IDF), and National Cholesterol of

Adult Treatment Panel III (NCEP ATP III) have determined specific criteria of metabolic syndrome which included central obesity, high blood pressure, high triglyceride levels, low high-density lipoprotein (HDL), and high glucose levels (Grundy, 2004).

Experimental animal models become essential for testing the efficacy of new agents, understanding the molecular basis, pathogenesis, and mechanism of actions of the therapeutic agents. Moreover, these models should represent all the characteristics of this human disease, establishing all major signs of metabolic syndrome, especially hyperglycemia, dyslipidemia, hypertension, and possibly fatty liver disease. Present study reported that combination of low dose streptozotocin (STZ) and high-fat diet developed type II diabetes more closely to humans. High-Fat Diet will induce insulin resistance in peripheral tissues due to lipotoxicity; meanwhile, low dose of streptozotocin will induce slight defect in insulin secretion (Atanasovska et al., 2014). These Combinations have successfully represented natural progress of metabolic features change in human type II diabetes (Wang et al., 2007). Moreover, several studies used combination of carbohydrate (fructose, sucrose) and fat-rich dietary components to establish metabolic syndrome characteristics in rodents that mimic those of human characteristics (Bantle, 2009).

This study aimed to establish an experimental models of metabolic syndrome that adequately mimic metabolic syndrome criteria as determined by NCEP ATP III. Our study revealed that the combination of low dose streptozotocin and consumption of high fat diet from hydrogenated vegetable fat and high sucrose diet could induce type II diabetes,

hypertriglyceridemia and low HDL level in Sprague Dawley rat. This model can be used for investigating the efficacy of some new agents and understanding the pathogenesis of metabolic syndrome.

MATERIALS AND METHODS

Animals and diet.

Eighteen Male Sprague dawley rats, 2-3 months old, weighed 230 to 340 gram, were used in the study. The rats were housed in the Central Animal Facility of Brawijaya University, Malang, Indonesia. They were maintained under standard laboratory conditions in the animal house. The study protocol was approved by Faculty of Medicine Ethics Committee and conforms to the Committee for the Purpose of Control and Supervision of Experiments on Animals. Rats were kept in polyacrylic cages (40×30×14cm) single animals per cage, housed in an air-conditioned room, and kept under natural light-dark cycles. The animals were allowed free access to Normal Pellet diet or High-Fat and High-Sucrose Diet and water *ad libitum*.

Preparation of High-Fat Diet and High Sucrose Diet.

The High-Fat Diet and High-Sucrose (HFHS) diet was prepared indigenously in our laboratory by using normal pellet diet, sucrose, methionine, hydrogenated vegetable fat, salt, monosodium glutamate and yolk eggs. Normal rat pellet diet was powdered by grinding and mixed with 20% sucrose, 0.5% methionine, 2.5% salt, 2% monosodium glutamate and 15% egg yolks and 20% white fat. The mixture was made into pellet form and put into freezer to solidify.

Table 1. High-Fat Diet and High-Sucrose Diet composition.

Ingredient	Amount	
	Normal Control (NC) Diet (g/kg)	High – Fat & High-Sucrose (HFHS) Diet (g/kg)
Normal Pellet Diet	877	250
DL methionine	3	3
Hydrogenated vegetable fat	-	200
Salt	10	25
Monosodium glutamate	10	22
Sucrose	100	300
Egg yolks	-	200

Experimental Model of Metabolic Syndrome.

After 2 weeks of dietary manipulation, rats were injected intraperitoneally with STZ (30mg/kg). The body weight and biochemical parameters (blood glucose, triglyceride, HDL-cholesterol) were measured 7 days after the vehicle or STZ injection; the HFHS diet was continued for the rest of 6 weeks of dietary manipulation in rats. The rats with blood glucose (>126mg/dL), triglyceride (>150mg/dL), high systolic blood pressure (\geq 140 mmHg), and reduced HDL levels (<40 mg/dL) confirmed presence NCEP-ATP III criteria of metabolic syndrome (Grundy, 2004). Thereafter the rats were either fed normal diet or HFHS diet as per the protocol for 8 weeks. Blood samples were collected from the tail veins under light anesthesia at 2, 4, 6, and 8 weeks for estimation of biochemical parameters.

Experimental Groups.

Group 1: Normal Control (NC). In Normal Control group, rats were administered distilled water orally using a feeding bottle during study period of 8 weeks. At the end of 2 weeks, 0.01M citrate buffer, pH 4.5, was

injected intraperitoneally to mimic the STZ injections.

Group 2: High Fat and High Sucrose Diet (HFHS). The high fat diet and high sucrose diet were fed to rats for 8 weeks to produce metabolic syndrome. At the end of second week rats was induced by a single STZ injection (30 mg/kg body wt, i.p., dissolved in 0.01M citrate buffer, pH 4.5).

Physiological Measurement.

Daily food intake, fluid intake, and calorie intake were measured every day and Body weight was measured every week for eight weeks. The food and fluid intake for each rat were measured by subtracting the measured amount provided to the remaining amounts in the cage [11].

Blood Biochemistry.

The rat blood samples of all experimental groups were collected from the tail veins under light anesthesia at 2, 4, 6, and 8 weeks for estimation of fasting blood glucose, Triglyceride, and HDL level. In addition, after the completion of the experimental duration (8 weeks), serum was used for the determination of insulin level.

Blood Pressure Measurements.

Blood pressure was measured using the tail-cuff method with sphygmomanometer technique at the baseline and at the end of the experiment. Three readings were taken consecutively and the average was then calculated and taken as a final reading for SBP.

Statistical Analysis.

All data was analyzed with Statistical Package for Social Sciences (SPSS, version 22) and were presented as mean values with their standard error of means (SEM) and subjected to Independent T- Test and pair T- Test with significant *P* value as <0.05.

RESULTS

Physiological Parameter.

Table 1 showed the effect on physiological parameters following consumption control diet and High fat diet and high sucrose diet with low STZ. The food intake and fluid intake were increased significantly with the consumption high fat diet and high sucrose diet with low STZ after eight weeks and there was statistical difference compared to control group. On the other hand, the body weight was significantly

increased in the intervention group after eight weeks period followed by control group and no significantly difference between groups.

Blood Pressure.

Intervention groups showed significant blood pressure increase after eight weeks compared to that of the baseline. The final systolic blood pressure also revealed a significant higher level of systolic blood pressure compared to that of control group (Table 2).

Blood Biochemistry.

Fasting blood glucose and TG levels of intervention group showed significant increase during eight weeks and differed significantly compared to that of control group (Table 2). HDL levels of intervention group showed significant decrease during eight weeks and differed significantly compared to that of control group (Table 2). On the other hand, no significant difference was observed in the fasting glucose, TG and HDL level among control group. Insulin level and HOMA IR index showed significantly different between these groups.

Table 2. Effect of high fat and high sucrose diet with low dose STZ on body weight, food intake, fluid intake in experimental group for eight weeks

Variabels	Normal Control		HFHS group (High fat and High sucrose with low dose STZ)	
	Baseline	After 8 weeks	Baseline	After 8 weeks
Body weight	289.67±16.02	400.22±25.11*#	276.89±42.44	329.22±41.85*
Food intake	21.48±0.43	22.06±0.62	21.59±1.1	23.20±0.97*#
Fluid intake	29.74±5.06	22.06±0.62	30.37±6.5	60.53±6.79*#

*: significant between baseline and after 8 weeks (p<0.05).

#: significant between group (p<0.05).

Table 3. Effect of high fat and high sucrose diet with low dose STZ on metabolic variable in experimental group for eight weeks

Variabels	Normal Control		HFHS group (High fat and High sucrose with low dose STZ)	
	Baseline	After 8 weeks	Baseline	After 8 weeks
Fasting glucose	92.22±14.86	86.56±16.05	103.33±17.88	232.00±25.09*#
Triglyceride level	105.56±35.15	115.22±28.38	109.56±18.13	265.00±43.38*#
HDL level	49.78±7.67	44.33±2.18	55.67±7.38	33.89±4.01*#
Insulin	-	2.52±0.38	-	3.32±0.47#
Homa-IR Index	-	1.46±0.40	-	5.19±1.13#
Adiponectin	-	125.57±25.14	-	97.8±18.58#
SBP (systolic Blood Pressure)	124±4.2	126.89±5.4	128.78±6.24	155.89±8.93*#

*: significant between baseline and after 8 weeks (p<0.05).

#: significant between group (p<0.05).

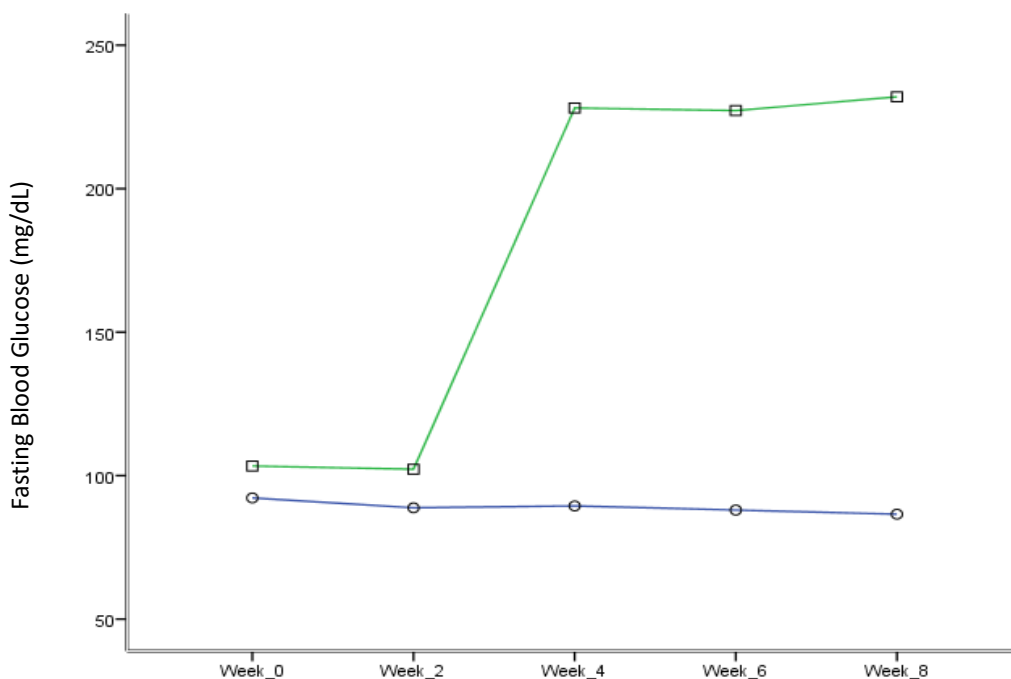


Figure 1. Time course changes of blood glucose level of NC (n = 9), HFHS group (n = 9). Values are expressed as mean ± SD, *: significant between baseline and after 8 weeks (p<0.05), #: significant between group (p<0.05), NC versus HFHS.

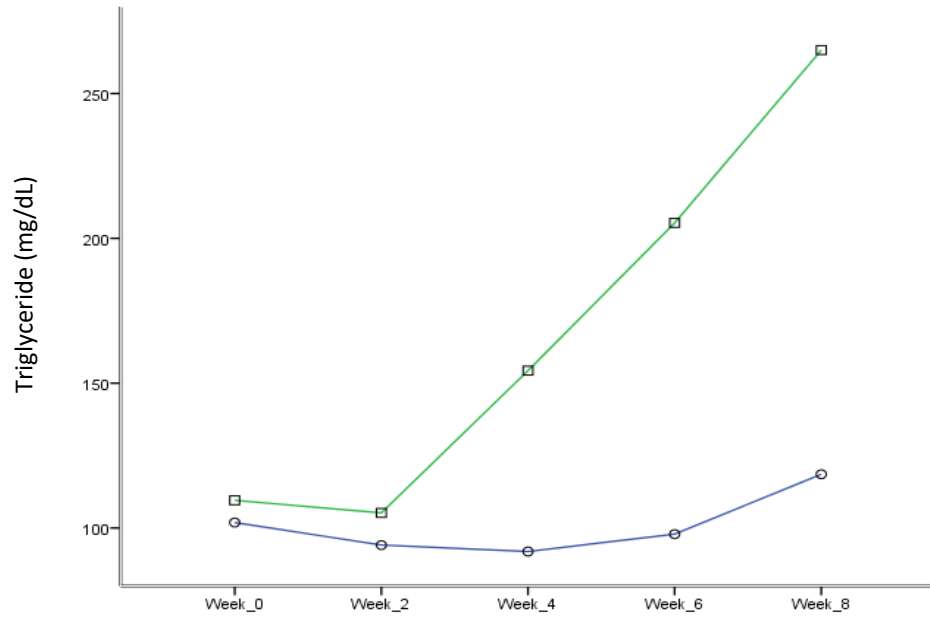


Figure 2. Time course changes of Triglyceride level of NC ($n = 9$), HFHS group ($n = 9$). Values are expressed as mean \pm SD, *: significant between baseline and after 8 weeks ($p < 0.05$), #: significant between group ($p < 0.05$), NC versus HFHS.

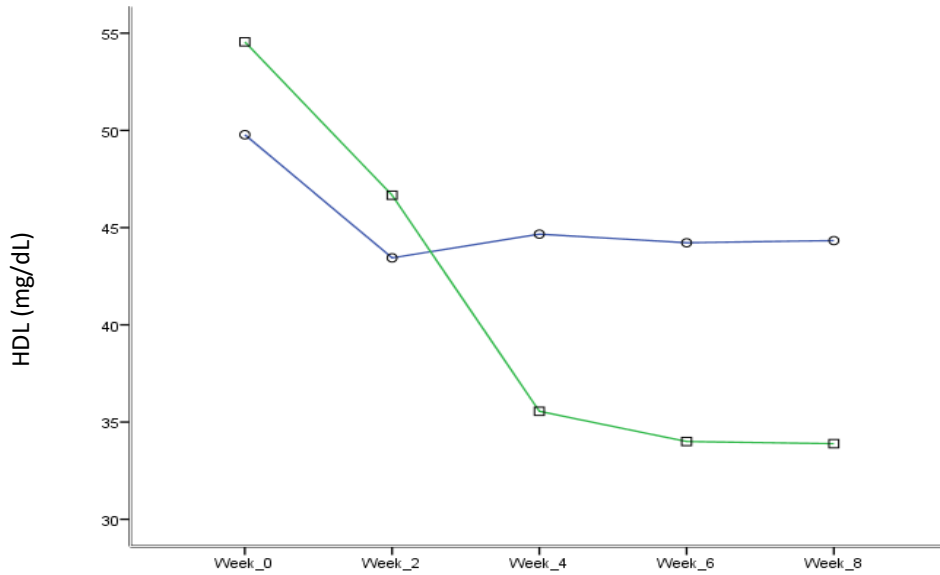


Figure 3. Time course changes of HDL level of NC ($n = 9$), HFHS group ($n = 9$). Values are expressed as mean \pm SD, *: significant between baseline and after 8 week ($p < 0.05$), #: significant between group ($p < 0.05$), NC versus HFHS.

DISCUSSION

This study developed metabolic syndrome based on NCE-ATP III criteria that characterized by hyperglycemia, insulin resistance, elevated blood pressure, and dyslipidemia. There are several animal models of diabetes as well as metabolic syndrome. Some of them were genetically modified such leptin-deficient (*ob/ob*) mice, leptin receptor deficient (*db/db*) mice, Zucker fatty (ZF) rats, Zucker diabetic fatty (ZDF) rats, DahlS.Z-Leprfa/Leprfa (DS/obese) rats, Goto-Kakizaki (GK) rats, obese spontaneous hypertensive rat (Koletsy rat), and the POUND mice (Wong, Chin, Suhaimi, Fairus, & Ima-Nirwana, 2016). Moreover, there were some diet induced metabolic syndrome rat model such as high-fructose, high-sucrose, high-fat, high-fructose/ high-fat, or high-sucrose/high-fat diets (Gajda, Pellizzon, Ricci, & Ulman, 2007). Furthermore, some chemicals might induce metabolic syndrome such as alloxan, streptozotocin, glucocorticoid agent (Bertram & Hanson, 2001). Further, it was kept in mind while developing the model that it should be less expensive, easily available, taking relatively short periods for development, reproducible, and displaying the various components of metabolic syndrome and diabetes mellitus. This study comply those criteria and suggested a novel approach in inducing rat metabolic syndrome characteristics through combination of low dose streptozotocin and high fat diet derived from hydrogenated fat.

Physiological Parameters such as body weight, food intake, fluid intake were evaluated in the healthy normal control (NC) and High Fat and High Sucrose (HFHS) groups. At the end of study, the NC group body weight was higher than that of HFHS group. Similarly,

the Food intake and Fluid Intake of the HFHS group also increased significantly as compared to the NC rats at similar time points. This result was similar with (Novelli et al., 2007) and (Suman, Ray Mohanty, Borde, Maheshwari, & Deshmukh, 2016) that showed an increase in all physiology parameters until the end of the study. Moreover they showed that diabetic state induced by insulin resistance caused weight loss, increase food intake and fluid intake increase.

Development of Hyperglycemia.

The present study suggested the development of hyperglycemia after streptozotocin and HFHS diet administration. It was characterized by high fasting blood glucose and HOMA-IR index. The blood glucose level in the HFHS group rats was significantly increased as compared to that of the baseline. Moreover, the blood glucose of HFHS group was significantly higher than that of NC group at the end of study. As explained by (Kuate et al., 2015), high carbohydrate and fat diet only would not induce frank hyperglycemia. Moreover, (Wang et al., 2007) revealed that hyperglycemia appeared at 3-4 weeks subsequent to streptozotocin administration. We hypothesized that the metabolic changes were manifested after a longer duration.

Insulin resistance and pancreatic function decline were demonstrated by a significant higher HOMA IR index in HFHS group as compared to NC group rats. Some studies revealed that streptozotocin injection resulted in diabetes type 1 it is due to massive pancreatic injury. However multiple low dose streptozotocin induced mild to moderate pancreatic damage that resulted in diabetes type 2 animal model (Arshad, 2013). However, this study induced diabetes type 2 only

without any evidence of dyslipidemia and hypertension. Our study use the combination the streptozotocin and high fat diet (40%) fat to maintain the hyperglycemic state. Previous report suggested that HFD administration induce insulin resistance via PKC activation by high level free fatty acid that consequently interfered IRS activation (Jang, 2013). Moreover, our study used the combination of animal fat and hydrogenated fat to maintain insulin resistance. Previous study explained that *trans* fatty acid ingestion induced insulin resistance ingestions could be related to the innate immune cascade in adipose tissue that promote pro-inflammatory adipokines expression increase (Ibrahim et al., 2005). Furthermore, NF- κ B, a key transcription factor in inflammatory cascade coordinated pro-inflammatory adipokines in adipose tissue (Aldhahi, 2003). Previous study also showed TRAF-6 increase that contributes to inflammatory response leading to NF- κ B pathway activation (Ibrahim, 2005). Moreover, (Creely et al., 2007) revealed a positive correlation between insulin serum concentration and endotoxemia. Therefore, it is possible that the hyperinsulinemia presented in TFA group associated with the effect of fatty acids in the intestinal microbial composition(Cani et al., 2008). It modified the intestinal permeability elevating lipopolysaccharides the inflammatory cascade activation. Finally, it promoted increasing TRAF-6. Thus, the combination of low dose STZ and high fat diet could induce diabetes type 2 via insulin resistance.

Development of dyslipidemia.

Dyslipidemia is a hallmark of metabolic syndrome. Dyslipidemia was appeared after 8th week induction with HFHS. Abnormal lipid profile in metabolic syndrome was

characterized by high triglyceride (>150 mg/dL), and low HDL levels (<40 mg/dL). This study showed significant rises triglyceride level and decrease HDL level compared to that of the baseline in HFHS group. At the end of study, a significant higher triglyceride level and lower HDL level was observed in the HFHS group as compared to that of NC group. Some previous studies also showed the efficacy of high fat and high carbohydrate on raising the triglyceride level and reducing HDL level. Dyslipidemia occurred due to free fatty acid increased after high fat diet and high carbohydrate administration. Free fatty acid could induce inflammation and increase triglyceride and cholesterol metabolism. We hypothesized that different fat sources used in our study progressively induced dyslipidemia. Hydrogenated vegetable fat used in our study promoted an increase in serum triacylglycerol and total cholesterol levels. Long term intake of TFAs altered plasma lipoprotein profile that increased the risk of cardiovascular diseases, decreased insulin sensitivity, and higher risk of type 2 diabetes (Ibrahim et al., 2005; Lichtenstein et al., 2001). Based on our knowledge this study was the first study revealed metabolic syndrome features with the use of fat sources that mimic daily consumed fat in human.

Development of hypertension.

Present study showed systolic blood pressure elevation after low dose STZ and HFHS diet administration for 8th week. Previous studies also showed increase in systolic blood pressure in metabolic syndrome induced by high carbohydrate and fructose diet (Bantle, 2009). This condition explained by peripheral resistance increase and renin-angiotensin-aldosterone system (RAAS) activation. RAAS activation plays a key role in

the development and pathophysiology of hypertension by several mechanisms such as vasoconstriction, cell growth, oxidative stress production and inflammation via angiotensin II production (Beevers, 2001). Moreover, free fatty acid induced hypertrophy and hyperplasia of adipocyte. This condition led to proinflammatory mediator release such as angiotensin 2 and suppression of anti inflammatory mediator such as the adiponectin (Aldhahi, 2003). Our study showed low adiponectin level as important biomarker of metabolic syndrome. Finally, we assumed that the development of hypertension in our study was induced by high grade inflammation.

The distinctiveness Animal Model of Metabolic Syndrome.

The present study has formulated a unique formulation of HFD which can be prepared indigenously in laboratory and is therefore feasible and cost effective. Thus, the present study has attempted to develop a unique rodent model of metabolic syndrome in the setting of diabetes mellitus. Although presently there is no perfect animal model of these comorbidities, the present study for the first time has successfully developed an experimental model with specific attributes (dyslipidemia, hypertension, and diabetes) that makes them useful for studying the mechanisms and potential therapies of metabolic syndrome in the setting of diabetes.

CONCLUSION

The combination of low doses of STZ (30mg/kg) and HFHS administration for 8 weeks could induce metabolic syndrome mimicking human criteria of metabolic syndrome based on NCE ATP III criteria such as hyperglycemia, hypertriglyceridemia, low HDL level, and hypertension. Moreover, low

adiponectin level as an important biomarker of metabolic syndrome was observed in HFHS group. The developed model will be helpful in screening of different pharmacological compounds.

Conflict of Interests.

The authors declare that there is no conflict of interests regarding the publication of this paper.

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