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Alterations of Liver Functions and Morphology in a Rat Model of Prediabetes After a Short-term Treatment of a High-fat High-glucose and Low-dose Streptozotocin

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Abstract

BACKGROUND: The administration of high-fat and high-glucose in diet followed by a low-dose streptozotocin injection in rats could mimic hyperglycemia, prediabetic, or diabetic conditions in humans. However, whether the rat model may lead to early liver impairment was still unclear.

AIM: This study was aimed to investigate the possible changes in liver functions and morphology in the rat model of prediabetes after a short-term administration of a high-fat and high-glucose diet followed by low-dose streptozotocin injection.

METHODS: Eighteen male Wistar rats were divided into nine rats in the control group and nine in the prediabetic group. To induce prediabetic rats, high-fat high-glucose in daily diets for 3 weeks continued with once to twice low-dose streptozotocin was given. Rats in control groups were fed with a standard diet for 2 months. Afterward, we analyzed glucose control parameters, liver functions, and liver histology of the rats.

RESULTS: High-fat, high-glucose diet combined with a low dose of streptozotocin successfully caused prediabetics in the rats. There was a significant increase in several liver enzymes, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT). However, no significant changes were found in the serum lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) levels. The histological changes in the liver confirmed the increase in liver enzymes.

CONCLUSION: Short-term administration of high-fat high-glucose in combination with low-dose streptozotocin triggers alterations in liver functions marker and liver morphology.

Introduction

Prediabetes is characterized by elevated blood sugar but not high enough to meet the criteria of diabetes mellitus. Prediabetes is a significant risk factor for the development of diabetes mellitus [1]. Combining a high-fat diet with a low dose of streptozotocin (STZ) injection can induce initial β-cell dysfunction [2]. High-fat diet administration leads to obesity, hyperinsulinemia, hyperglycemia, and diabetes [3]. STZ administration after high-fat diet induction can reduce the functional capacity of the pancreatic β-cells without ultimately impairing insulin secretion [4]. Calories from fat (40−60%) can lead to metabolic disorder, hypertension, and the production of pro-inflammatory cytokines [3]. The previous studies showed that high-fat diet administration for 2 months could induce metabolic syndrome associated with oxidative conditions [5]. Another study also showed that a high-fat diet combined with high-glucose induction has a greater risk of causing diabetes prevalence [6].

The liver is one of the primary organs which susceptible to glucose homeostasis. Several hormones are involved in the process of glucose homeostasis. Hepatocytes in the liver are the leading site of hepatic glucose metabolism. In diabetes, hepatocytes cannot respond to insulin-induced dyslipidemia and insulin resistance. This condition triggers diabetic liver complications [7]. The previous studies showed that diabetes is associated with several liver abnormalities, such as non-alcoholic fatty liver disease (NAFLD), abnormal glycogen deposition, abnormal elevated hepatic enzymes, acute liver disease, and liver fibrosis [8]. A high-fat diet induces fat accumulation in the liver, leading to insulin resistance. Hyperglycemia, fatty liver, and insulin resistance can destroy hepatocytes and increase patient morbidity. Hyperglycemia also

disrupts lipid metabolism then triggers inflammation cascade [9]. Inflammatory responses and oxidative stress worsen the condition of liver damage (NAFLD) in diabetes disease. NAFLD induces necrosis, hepatic inflammation, and fibrosis, which are symptoms of non-alcoholic steatohepatitis (NASH) [10]. STZ can cause diabetes and is associated with significant liver enzymes and morphology alterations [11]. The study shows that STZ 45 mg/kg body weight results in pathologic lesions in the liver and liver enzyme levels changes [12].

Many studies showed an association between diabetic conditions or complications in various organs, including the liver, but only limited data about the association between prediabetic and liver function. The present study was aimed to evaluate the changes in liver functions and morphology in the prediabetes rat model after a short period of high-fat and high glucose administration in diets followed by low-dose streptozotocin injection.

Methods

Animals and experimental design

We utilized 18 4-week-old male Wistar rats weighing 80–100 g. Standard laboratory settings included sufficient ventilation, temperature, relative humidity control, and standard light/dark cycle for all experimental animals. One week before the experiment began, all experimental rats were maintained acclimatized. The Faculty of Medicine Universitas Indonesia's Ethics Committee approved all of the experimental procedures.

The Wistar rats were split into two groups, and each group included nine rats. The Ethics Committee approved the number of rodents used in the study. A standard rodent diet (TestDiet, 5012 rat diet from Richmond, USA) was provided to the first group for 3 weeks, followed by a saline injection at the end of the experiment. A single injection of streptozotocin (STZ; 30 mg/kg BW intravenously) at a low dose was used to produce the prediabetic state in rats in the second group, which was administered at the end of three-week administration of high-fat diet (HFD) once daily ad libitum, together with high glucose (20% glucose) in drinking water. The second STZ injection would only be repeated three days after the first dose, at 15 mg/kg BW, if the parameters had not achieved the prediabetic threshold. On the occasion that after two doses of STZ, the rats did not reach prediabetic parameters, they were then excluded. Prediabetes was considered successful if the glucose control parameters met both criteria of fasting blood glucose (FBG) levels in the range of 100 and 125 mg/dL, or blood glucose levels between 140 and 199 mg/dL 2 h after glucose induction (OGTT).

Blood collection

After an overnight fast of 12 h, all rats were sedated with ketamine-xylazine, and blood samples were drawn for biochemical analysis from the retro-orbital sinus. After centrifugation at 3000 rpm for 10 min, all blood samples were placed into test tubes and stored. Until the time of analysis, the supernatants were kept at −80°C. The same steps were performed to collect blood samples for OGTT.

Biochemical analysis

The lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) were determined in the rat serum using spectrophotometric kits provided by Diasys® Diagnostic System (Holzheim, Germany). Serum gamma-glutamyltransferase (GGT) levels were determined by applying a gamma-GT FS kit using the Szasz-Persijn method. The AST and ALT were measured using the Diasys® kit with a spectrophotometer at a wavelength of 340 nm at 37°C.

Histological studies

After 3 weeks of intervention, the animals were sacrificed, and liver tissues were quickly removed then fixed in 10% formal saline. The liver tissues were stained with Hematoxylin and Eosin, and then examined for liver histopathological conditions such as hemorrhage, necrosis, fat infiltration, sinusoid dilatation, and cloudy swelling using a ×400 magnification microscope.

Statistical analysis

The data were reported as mean SEM. An independent sample t-test was used to compare the two groups when the data distribution was normality and homogenous. Otherwise, Mann–Whitney U tests were used. A level of significance at 0.05 and a confidence level of 95% were used to determine statistical differences between the two groups. GraphPad Prism 9.1.2 software was used to analyze the data and generate bar graphs.

Results

Blood glucose control

After 3 weeks of high-fat and high glucose diet administration followed with STZ injection (30 mg/kg BW), prediabetic variables were determined by FBG and result from OGTT (Figure 1). The result showed that the fasting blood glucose and glucose level after OGTT from the prediabetic group were significantly higher ($p < 0.001$, t-test analysis) compared to the control group.

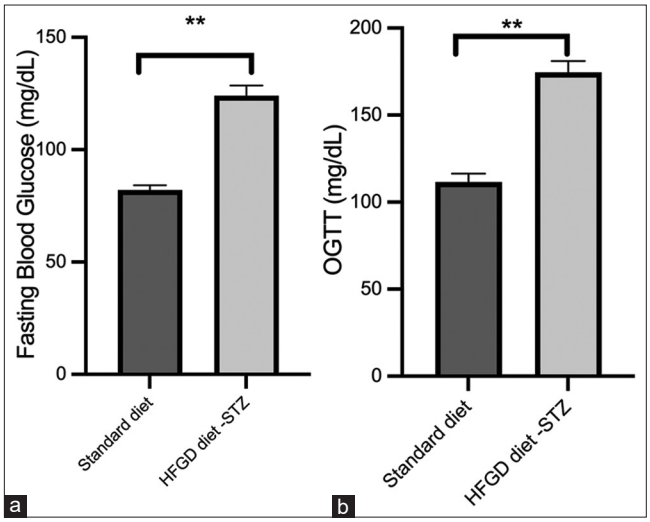

*Figure 1: The effect of high-fat, high-glucose, and low-dose streptozotocin or standard diet on (a) fasting blood glucose (FBG); (b) oral glucose tolerance test (OGTT). Data are presented as mean ± SEM. **Significant difference ($p < 0.001$ vs. control, after t-tests analysis). OGTT, oral glucose tolerance test; HGFD diet – STZ, high-fat and high glucose diet followed with STZ injection*

Liver function test

The effect of 3 weeks of administration of high-fat and high glucose diet followed with STZ injection on the serum level of liver injury markers among the experimental group is shown in Figure 2a-e. The liver functions of the rats in the control group were considered the normal value. The result showed that Gamma-glutamyl transferase (GGT), AST, and ALT were significantly higher ($p < 0.05$, t-test analysis) in the prediabetic group compared to the control group. ALP level in the prediabetic group was increased compared to the control group, although there is no statistically significant difference. Similarly, we found no significant difference in LDH levels in the two groups.

Histological observation

The comparison of the histological structure of the liver between control and prediabetic groups is shown in Figure 3. The section liver from the control group showed a healthy histological structure around the central vein, standard form of the sinusoid, and nuclei. The control group has no necrosis, fat infiltration, sinusoid dilatation, and cloudy swelling (Figure 3a). Slight hemorrhage (H) in the liver of the control rats was shown compared to the prediabetic group. After 3 weeks of administration of high-fat and high glucose diet on the prediabetic group, exhibited different hepatic necrosis (N), fat infiltration (F), and sinusoid dilatation (S) on hepatocytes (Figure 3b). The portal veins (PV) of the prediabetic group with high-fat and high glucose diet were dilated compared to the control group.

Discussion

Many studies have confirmed that diabetes can cause liver impairment [8], [9], [10], [11]. [12]. However, whether the injuries had already started in prediabetic conditions was unclear. In prediabetes, inflammation and oxidative stress in the liver are exacerbated by hyperglycemia and insulin resistance [10], [11]. Therefore, our study investigated whether liver injuries had been shown in a prediabetic rat model.

In diabetes or prediabetes rat model, glucose intolerance, and insulin resistance are affected by the duration of high-fat and high glucose [13]. In this study, combining a 3-week high-fat high-glucose diet with a low dose streptozotocin was efficient in causing the development of a prediabetes state associated with FBG and OGTT impairment. To confirm hyperglycemias, the FBG of the rats was re-measured two days after STZ injections. Once the FBG was steadily increased, then the rats were considered prediabetes. The short duration of high-fat diet induction induces insulin resistance and glucose intolerance [14]. Short periods of high-fat diet feeding have been reported to stimulate insulin resistance in non-obese patients [15]. A study from Guo *et al*. showed that a combination of a high-fat diet for 8 weeks followed by low dose streptozotocin could induce type 2 diabetes [16]. In this study, we showed that a shorter duration of a high-fat high-glucose diet successfully obtained a prediabetes model.

Excessive intake of a high-fat diet can induce fat accumulation in the body, which leads to obesity.[17] This condition can cause insulin resistance, leading to prediabetes or type 2 diabetes mellitus. High-fat diet induction is also associated with a spectrum of liver abnormalities [18]. Accumulated fat in the liver increases the risk of non-alcoholic fatty liver disease (NAFLD) [19]. In addition, a high-fat and high-glucose diet can decrease glucose uptake and cannot suppress insulin-stimulated hepatic glucose production [20]. The liver plays multiple functional roles, including hormone production, metabolism of carbohydrates and lipids, detoxification, and synthesis of clotting factors [21]. Liver morphology changes and is directly correlated to its function. In this study, high-fat and high-glucose can increase alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. In the liver function test abnormality, AST and ALT levels increased 2–3 times. The leakage of ALT and AST can reflect liver damage into the plasma [22]. Increased ALT levels related to obesity and hyperlipidemia, whereas increased AST was notably related to diabetes mellitus [23]. A study showed that high-fat diet induction in mice resulted in increased inflammation, liver fibrosis, and high plasma activity of liver enzymes [24], [25]. High-fat diets can induce diabetes mellitus complications by increased oxidative stress in the animal. An excessive amount of fat consumption results in an energy overload then

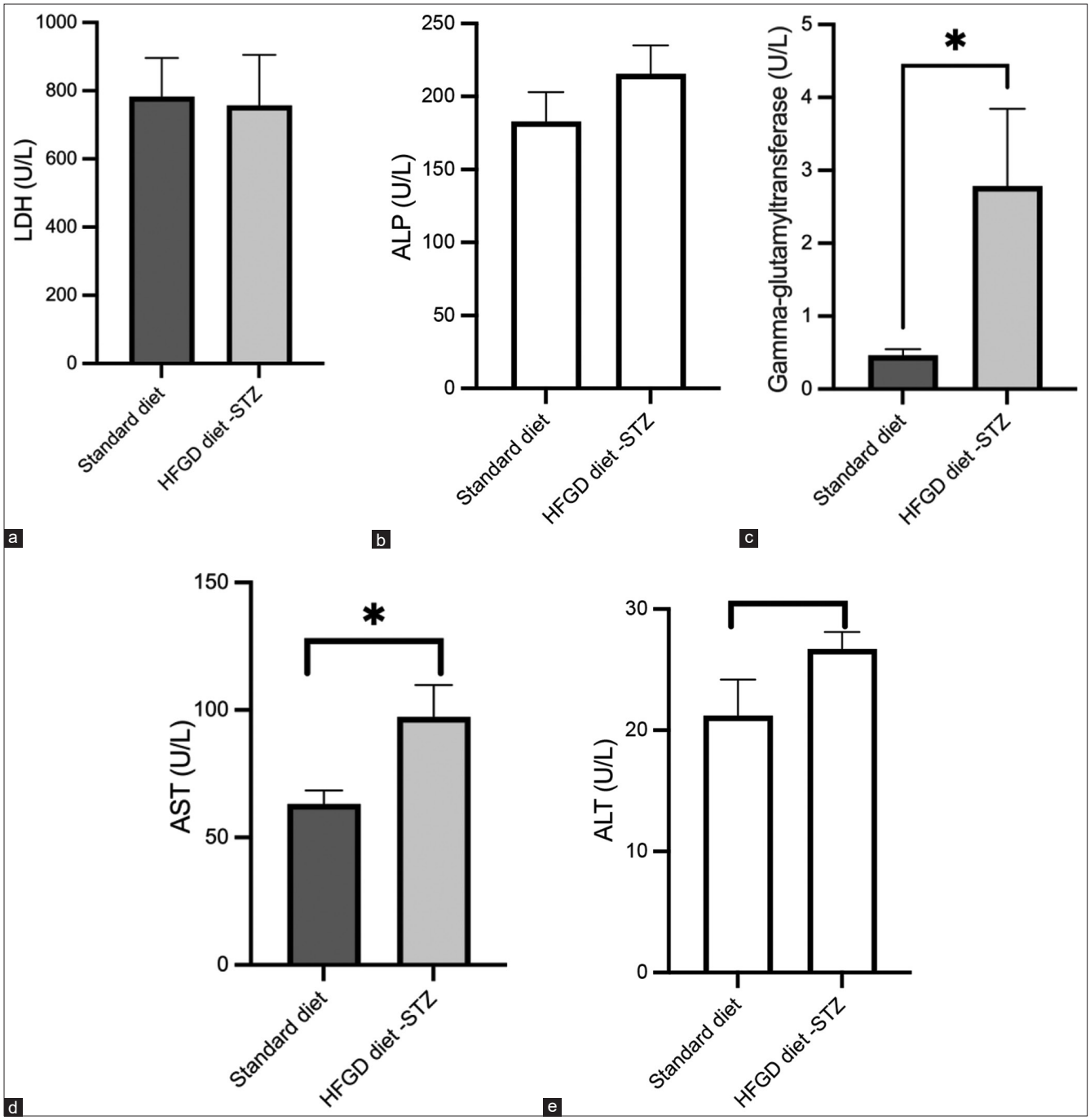

*Figure 2: The effect of high-fat and high glucose diet on (a) LDH, (b) ALP, (c) Gamma-glutamyltransferase, (d) AST, (e) ALT. Data are presented as mean ± SEM. LDH, lactate dehydrogenase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase. *Significant difference ($p < 0.05$ vs. control, after t-tests analysis)*

leads to the expansion of adipose tissue and metabolic inflammation. Circulating free fatty acids from fat accumulation can induce lipotoxicity to peripheral tissues such as the pancreas and liver [26]. Hepatic enzymes level of ALT and AST were also higher in STZ diabetic rats. STZ has been reported to significantly alter liver morphology and enzymes [12], [27]. In this study, a high-fat diet combined with low dose STZ (30 mg/kg BW), as desired that STZ was not inducing β-cell destruction predominantly in prediabetes animal model.

Gamma-glutamyltransferase (GGT) is one of the liver enzymes and acts as a hepatobiliary disease biomarker [28]. Elevated GGT is related to the development of metabolic syndrome, cardiovascular diseases, and type 2 diabetes mellitus [29]. GGT is synthesized in the intrahepatic duct epithelial cells and is a clinical marker for inflammation and free-radical formation [30]. In this study, induction of high-fat and high-glucose significantly increases GGT levels. Our findings confirmed several other studies which presented significant correlations between GGT serum and insulin resistance [31], [32], [33]. An elevated level of GGT indicated hepatic steatosis, and fat deposition in the liver then may lead to diabetes [34].

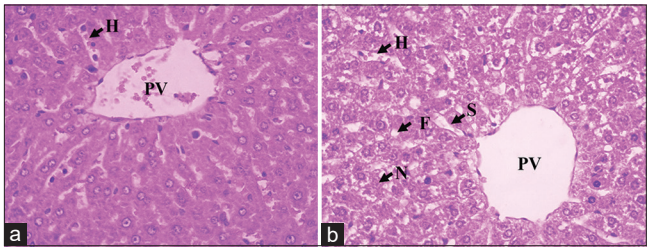

Figure 3: Histopathology of the rat liver using hematoxylin and Eosin staining. (a) Control group; (b) prediabetic group. H arrow indicates hemorrhage, N indicates necrosis, F indicates fat infiltration, S indicates sinusoid dilatation. PV=Portal vein, Magnification=×400

Lactate dehydrogenase (LDH) is one of the indicators of liver injury. In this study, no significant difference was found after high-fat diet induction. In contrast with this result, several previous studies showed that in diabetes conditions, LDH levels were significantly increased compared to the control group [35], [36], [37]. This difference was probably due to this study's short induction period of a high-fat diet. In addition, the animal model in this study was prediabetic, not diabetic. Alkaline phosphatase (ALP) is an enzyme in the liver and elevates in type 2 diabetes [38]. In this study, the ALP level in the prediabetic group was higher than in the control group, but there was no significant difference. Another study found no significant association between ALP level and diabetes [39]. Elevated ALP can indicate decreased survival in diabetic patients with myocardial infarction [40].

Studies have confirmed that diabetic conditions, not prediabetics, clearly cause morphological changes in the liver [23]. A high-fat diet induces fat accumulation in the visceral of the liver (liver steatosis) [41]. This condition can progress into steatohepatitis and liver cirrhosis [42]. A high-fat diet can cause abnormal mitochondria and mononuclear inflammation. In abnormal liver after induction of high-fat diet, found pericellular fibrosis, lobular inflammation, portal fibrosis, and hepatocellular ballooning [43]. In this study, a high-fat diet and high-glucose induce slight hemorrhage, hepatic necrosis, fat infiltration, sinusoid dilatation, and cloudy hepatocyte swelling. The portal veins of the prediabetic group with high-fat and high glucose diet were dilated compared to the control group. Another study found that a high-fat diet induces hepatocytes liver necrosis, hepatocellular steatosis, liver shrinkage, mononuclear cell infiltrations, additional acidophile, and nuclear density [44]. High-fat diet administration for 6 months induces liver damage associated with low-grade inflammation [24]. Another study showed that STZ injection also causes veins dilatation, liver fibrosis, and lipid droplets in hepatocytes [12].

There were some limitations to our study, one of which was the short-term duration of the study. However, we discovered that even after a short period of a high-fat, high-glucose diet followed by a low-dose STZ, there were significant changes in liver function markers and morphology. Our study might suggest that a longer duration of diet-induced prediabetes or diabetes conditions would lead to more severe liver impairment.

Conclusion

This research is a comprehensive study of the prediabetes model in animals. After 3-week administration of a high-fat and high-glucose diet followed by low-dosage of streptozotocin, it showed pathological changes in the liver. It also alters the various enzymatic function of the liver. It may also be concluded that a high-fat diet can induce prediabetic conditions and may be attributed to liver morphology and liver enzymes level alterations.

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