

High Fat High Fructose Diet Elevates Plasma Level of HMGB1 Due to Non-alcoholic Fatty Liver Disease (NAFLD)

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ABSTRACT

High fat and high fructose diet (HFHFD) gave a special impact on lipid and carbohydrate metabolism on liver. We investigated the impact of consumption of fructose, in combination fatty food, on the onset of Non-Alcoholic Fatty Liver Disease (NAFLD) and plasma HMGB1 level as a biomarker of necrotic cells. High fat high fructose diet (HFHFD) was compared to rats fed with normal diet for 17 weeks to induce macrovesicular hepatosteatosis attributable to NAFLD. Weights were measured 10 times over a 4-month period. Plasma HMGB1 level as necrotic parameter was studied by ELISA. At the end of the experiment, HFHFD group gained significantly more weight than the control group. Contrary to fat intake, fructose did not lead to increased weight. However, addition of fructose to the diet increases the number of hepatosteatosis and relates with plasma HMGB1 level elevation. We conclude that fat and high fructose diet induced non-alcoholic fatty liver. We also demonstrated that histologic findings on liver strongly related with the elevation of plasma HMGB1 level as biomarker of necrotic.

Keywords

HFHFD, NAFLD, HMGB1, hepatosteatosis

Introduction

The high consumption of sugary beverages rich in fructose is directly related to the development of obesity and its consequences, such as metabolic syndrome. High fat and high fructose diet (HFHFD) gave a special impact on lipid and carbohydrate metabolism, almost all fructose is metabolized in the liver through the process of fructolysis [1]. Fructose bypasses the glucose metabolic pathway in the liver, elevates production of acetyl coA, and de novo lipogenesis [2]. Increased lipogenesis and impaired fat oxidation due to high fructose diet caused hepatic triglyceride deposition [3], and contribute to the pathogenesis of NAFLD [4]

NAFLD is associated with necrosis of hepatocytes [5]. Fructose metabolism requires a lot of energy by its rapid phosphorylation within the cell and causes ATP depletion that increases the susceptibility of hepatocytes to various injuries [6]. However, lipotoxic hepatocytes cells that have necrosis can cause systemic disorders by releasing danger signals, such as High Mobility Group Box 1 (HMGB1) [7]. However, HMGB1 also play a role in the pathogenesis of acute liver injury such and potential to act as biomarker in assessing the presence and severity of liver damage [8].

HMGB1 was identified as a nonhistone chromatin-binding protein that functions as a pro-inflammatory cytokine and a Damage-Associated Molecular Pattern (DAMP) molecules when released from necrotic cells or activated leukocytes [9]. HMGB1 interacts with multiple pattern

recognition receptors (PRRs), many of its effects in injury models occur through an interaction with toll-like receptor 4 (TLR4) to alert the host of damage by triggering immune responses [10]. Recent studies demonstrated that the HMGB1 levels in plasma and mediates hippocampal inflammation in HFHFD-induced rats [11]. HMGB1 plays a pivotal role during NAFLD progression by mediating TLR4 activation which is involved in the hepatocyte free-cholesterol lipotoxicity [12]. However, it is not clear yet the effect of HFHFD on plasma HMGB1 levels and its relationship with the progression of NAFLD.

The objective of this study was to determine whether HMGB1 plays a role on NAFLD pathogenesis in a rat high-fat high-fructose diet (HFHFD) model. We hypothesized there is association between NAFLD progression and plasma HMGB1 level caused by high fat high fructose diet. These studies provide the discourse of HMGB1 as biomarker and target therapy of NAFLD.

Methods

All experimental procedures were approved by the research ethics committee from Medical Faculty University of Brawijaya in Indonesia(368/EC/KEPK/10/2017).

Animal Treatment

Twenty-four male Sprague Dawley rats at 8 weeks of age were given four weeks acclimatization period. They were housed one per cage and fed a normal diet during the acclimatization period. Rats were divided randomly into two groups [n=12] which the control group was fed normal diet that contains of 26% fat, 31% protein and 43% carbohydrate. Another group fed HFFD that contains of 52 % fat, 22 % protein and 29% carbohydrate pellets with an addition of 30% fructose solution. The energy density are 4,21 Kcal and 5,08 Kcal for control group and HFFD group respectively. The dietary intervention was carried out for 17 weeks. Food consumption were measured daily to calculate the amount of intake of fructose and fat.

Liver Histology

Twenty-four male Sprague Dawley rats at 8 weeks of age were given four weeks acclimatization period. They were housed one per cage and fed a normal diet during the acclimatization period. Rats were divided randomly into two groups [n=12] which the control group was fed normal diet that contains of 26% fat, 31% protein and 43% carbohydrate. Another group fed HFFD that contains of 52 % fat, 22 % protein and 29% carbohydrate pellets with an addition of 30% fructose solution. The energy density are 4,21 Kcal and 5,08 Kcal for control group and HFFD group respectively. The dietary intervention was carried out for 17 weeks. Food consumption were measured daily to calculate the amount of intake of fructose and fat.

Plasma HMGB1 Level

Levels of HMGB1 in plasma samples were measured with a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) using commercially available human HMGB1 (Bioassay Technology Laboratory, Shanghai, China).

Statistical Analysis

All statistical tests were conducted using Statistical Package for the social Sciences (SPSS, version 20.0, IBM Corp., Armonk, NY, USA) software. Paired data were analyzed with statistical significance, *t*-tests, with significance being $P < 0.05$, and non-parametric Kolmogorov-Smirnov test to compares the cumulative distributions of two data sets. All data were presented as mean $\pm$ SEM. The respective effects of operation and diet were analyzed by two-way ANOVA. The differences were considered to be significant when the *p* value was less than 0.05.

Results

Food intake and body weight gain

The first set of analyses examined the impact of varian food intake to weight gain. Fat and fructose intake of HFHFD group was significantly more than the control group ($p < 0.05$), whereas carbohydrate and protein intake actually decreased ($p = 0.00$).

Table 1. Food Intake and Body Weight Gain

	Group		Independent t-test
	Control (n=12)	HFHFD (n=11)	
Energy intake (kcal/day)	49.5 $\pm$ 1.9	67.5 $\pm$ 1.8	0.000
Fat intake (g/day)	1.4 $\pm$ 0.1	1.8 $\pm$ 0.1	0.003
Protein intake (g/day)	3.9 $\pm$ 0.3	1.8 $\pm$ 0.1	0.000
Fructose intake (g/day)	0.0 $\pm$ 0.0	8.8 $\pm$ 0.3	0.000
Carbohydrate intake (g/day)	5.3 $\pm$ 0.2	2.4 $\pm$ 0.2	0.000
Total carbohydrate intake (g/day)	5.4 $\pm$ 0.7	11.2 $\pm$ 0.3	0.000
Body weight gain (g)	10.3 $\pm$ 5.9	36.9 $\pm$ 8.5	0.017

Values are means $\pm$ SEM, n=23, HFHFD= High Fat High Fructose Diet

After 17 weeks of diet, HFHFD induced a significant increase in body weight ($p = 0.017$). Further statistical tests revealed that fat intake is the most influence on weight gain ($p = 0.00$).

Hepatic pathologic findings

HFHFD was highly related with hepatosteatosis, simple statistical analysis was used to differ the number of hepatosteatosis cells between two groups. Excessive intrahepatic triglyceride (IHTG), or hepatosteatosis, has been histologically defined when 5% or more of hepatocytes contain visible intracellular triglycerides (TG), with necro-inflammatory foci are scattered in the hepatic lobule of liver.

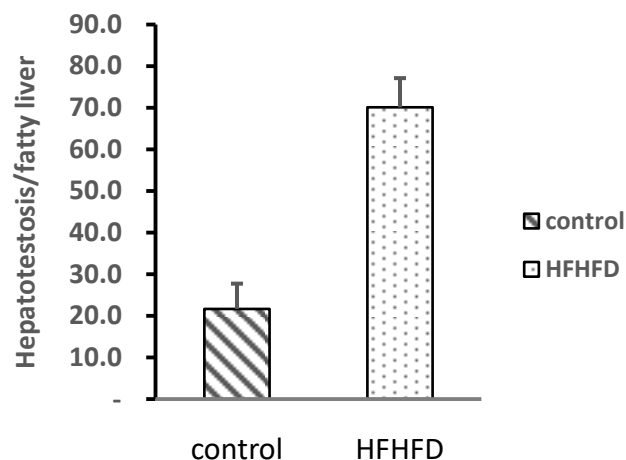


Figure 1. The number cells which defines as hepatosteatosis/ fatty liver

The histogram in Fig 1 indicates that high fat high fructose diet induce macrovesicularsteatosis of liver ($p = 0.00$). Interestingly, further analyses showed that fructose plays a role in the increase of hepatosteatosis ($p = 0.00$), whereas fat intake had no effect ($p = 0.05$).

Hepatosteatosis is related to Plasma HMGB1 level

Subjects fed high fat high fructose diet exhibited significantly higher level of plasma HMGB1 than those fed a normal diet ($p = 0.00$).

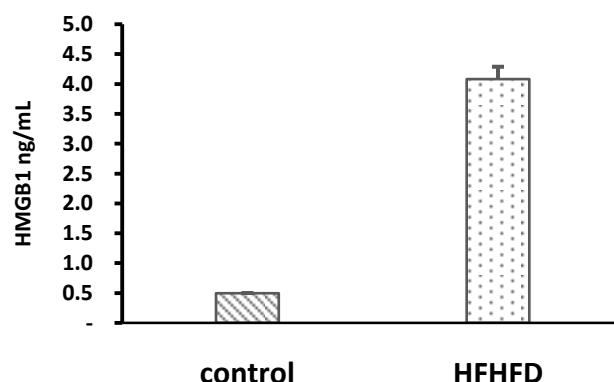


Figure2. Plasma HMGB1 level

Further analysis showed that that histologic appearance of fatty liver strongly related to plasma HMGB1 level ($p = 0.00$).

Discussion

A strong relationship between high fat high fructose intake as a potential risk factor for metabolic syndrome and diabetes has been reported in the prior study (Baena et al, 2016). Excessive consumption of fructose may affect the liver, in the present study we tested the hypothesis whether fructose, in its metabolic effects, particularly as it relates to the development of fatty

liver in rats and plasma HMGB1 level [4]. This protein is passively released by cells undergoing necrosis, and serve as a biomarker for non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) due to its correlation with degree of liver fibrosis [14].

The results of this study that highfat high fructose consumption causes liver deposition of triglycerides compare with no fructose supplementation. It would most likely be noted in the liver because this is the primary site of metabolism of fructose. The most interesting finding was that a number of hepatosteatosis cells were associated with elevation of plasma HMGB1 levels in HFHFD group. Emerging evidence has demonstrated that HMGB1, a protein that is highly conserved nonhistone nucleons which serves as damage associated with a molecular pattern (DAMP) molecule, associated with the pathogenesis of T2D. HMGB1 can signal through the receptor for advanced glycation end products (RAGE) and a Toll like receptor (TLR) signaling pathways [15]. TLR4 is activated by bacteria-derived lipopolysaccharide (LPS) and by saturated free fatty acids (FFA) and expressed on insulin-target, such as myocytes, hepatocytes, adipocytes, and inflammatory cells [16].

Fructose has a selective hepatic metabolism, and trigger activation of c-Jun N-terminal kinases resulting in a hepatic stress response and some degree of fatty liver [6]. Moreover, includes fructose and fat diet would increase lipid accumulation in the liver, followed by proinflammatory mediators induce inflammation, hepatocellular injury, fibrosis [17], and necrosis [18]. Despite the lack of an inflammatory cytokine response, we nevertheless assume that elevation of plasma HMGB1 level is caused by hepatocellular injury leading to cell death and passively secreted HMGB1 as necrotic biomarker.

Conclusion

We can conclude that fructose-induced insulin resistance and rather than being directly related to the amount of hepatosteatosis and elevation of plasma HMGB1, impairment of insulin sensitivity was dependently related to fructose intake.

One of the strengths of our study is similarity dietary treatment with modern food consumption, i.e. sugar-sweetened beverages, high in calories and saturated fats and poor in complex carbohydrates and micronutrients, allowing us to demonstrate that the effects of high fructose to metabolic syndrome.

Authors Contribution : RR performs the concept of experiments and performs HMGB1 assay analyses. DH constructs the design of experiment and perform hepatic pathology measurement. YY, EW, and MAW improved the concepts and design of experiments. ATE do analyses statistical analyses. RR, YY, EW, DH, and ATE wrote and revised the manuscript. All author read and approved the final manuscript.

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Limitation and Future Studies : This research has methodological limitations a set of areas with open questions have remained, and need further analysis to determine the pathway of HMGB1

induce hepatosteatosis. In the future, discovering the pathogenic molecules that mediate organ/cell crosstalk and contribute to NAFLD development will provide much needed insights into disease management.

Ethical Approval : All experiments were conducted according to the principles of Guide for the Care and approved by The Research Ethics Committee from Medical Faculty University of Brawijaya in Indonesia (368/EC/KEPK/10/2017).

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