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Mild obesity reduces survival and adiponectin sensitivity in endotoxemic rats

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Running Title: Diet-induced mild obesity deteriorates septic injury

Subject Category: Shock/Sepsis/Trauma/Critical Care

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Abstract

Background—Recent meta-analyses have reported that critically ill patients with morbid obesity (body mass index > 40 kg/m²) have worse outcomes, but the effects of mild obesity and their mechanisms are still controversial. The purpose of this study was to evaluate the effect of mild obesity with lard-based high-fat diet (HFD) feeding on pathological conditions and the mechanisms focused on adiponectin in endotoxemic rats.

Materials and Methods—Male Wistar rats underwent HFD feeding for 4 weeks and then were killed at 0, 1.5, and 6 h after lipopolysaccharide (LPS) injection. Analysis included plasma levels of adiponectin, nitric oxide, and interleukin-6; messenger RNA (mRNA) expression of adiponectin receptors (adipoR1 and adipoR2) in the liver and the skeletal muscle; blood biochemical test results; and histology of the liver.

Results—HFD-fed rats had a lower survival rate (12.8% vs. 85.2%) and lower plasma adiponectin levels after LPS injection ($p < 0.01$). mRNA expression of adiponectin receptors in the liver, but not in the skeletal muscle, was also decreased in HFD-fed rats ($p < 0.05$). Tissue injury and oxidative stress in the liver, lipid metabolism abnormalities, and plasma inflammatory mediator levels were increased in HFD-fed rats. The findings indicated that HFD decreased the sensitivity of adiponectin and was related to increased oxidative stress and inflammation, which finally resulted in increased liver injury and a worse survival rate after injection of LPS.

Conclusions—Short-term HFD-induced mild obesity is harmful to the septic host, reduces adiponectin sensitivity, and could be the cause of worse pathological conditions.

Keywords: Adiponectin; obesity; sepsis; high-fat diet; lipopolysaccharide

INTRODUCTION

It is estimated that more than 1 billion adults worldwide are overweight, and recent large meta-analyses have reported that critical ill patients with morbid obesity (body mass index $>40 \text{ kg/m}^2$) have worse outcomes than nonobese patients when treated in an intensive care unit [1, 2]. However, the effects of mild or moderate obesity on outcomes in patients with sepsis are controversial, and the way in which obesity and a Western diet affect septic injury remains poorly understood.

The number of people with obesity is increasing, and excess intake of animal fats such as lard contributes to this phenomenon [3]. Lard is rich in saturated fatty acids [4], and saturated fatty acids are deeply related to formations of chronic inflammation and metabolic syndrome [5, 6]. Although many *in vivo* studies of obesity involve long-term high-fat diet (HFD) feeding, such as for 12 or 16 weeks, it has been reported that the effect of HFD becomes apparent after 4 weeks [7]. Indeed, Xu et al. reported that increased body weight and liver steatosis progressed after 4 weeks of HFD feeding that included lard [8].

Adipose tissue produces and secretes a variety of bioactive molecules called adipocytokines [9, 10]. Adiponectin plays an important role in the regulation of tissue inflammation, oxidative stress, and lipid metabolism [11]. It is well established that circulating adiponectin levels are reduced in obese patients with insulin resistance, and there is a strong negative correlation between plasma

adiponectin levels and body mass index [12]. Circulating adiponectin acts predominantly in the liver and the muscle via 2 specific receptors: AdipoR1 and AdipoR2. AdipoR1 is ubiquitously expressed with the highest expression found in the skeletal muscle, whereas AdipoR2 is mainly expressed in the liver [13]. Expression of AdipoR1 and AdipoR2 in the liver and the skeletal muscle is decreased in a diabetic model, such as in db/db mice and Zucker rats [14, 15], and these receptors play important roles in the regulation of inflammation, oxidative stress, and lipid metabolism *in vivo*. Matsunami et al. reported that hepatic expression of adipoR2 was decreased in rats in a model of nonalcoholic steatohepatitis (NASH), suggesting that reduced adipoR2 expression contributed to hepatic inflammation and oxidative stress and finally developed into NASH [16]. In addition, Goto et al. reported that treatment of KK-A^y mice with tiliroside, a glycosidic flavonoid, improved obesity-induced elevation of plasma triglyceride (TG) and free fatty acid (FFA) levels by increasing plasma adiponectin levels and expression of adiponectin receptors in the liver and skeletal muscle [17]. Alterations of adiponectin receptors are important for formation of hepatic inflammation, oxidative stress, and abnormalities of lipid metabolism as well as alteration of circulating adiponectin levels [16, 17].

Recently, the importance of adiponectin not only in chronic inflammation but also in cases of acute inflammation such as sepsis has been reported. Venkatesh et al. showed that serum adiponectin concentrations in critical ill patients were lower than those in healthy individuals and

indicated an inverse correlation between serum adiponectin levels and serum C-reactive protein levels [18]. Leuwer et al. reported that lipopolysaccharide (LPS) injection in mice decreased adiponectin expression and increased expression of tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and monocyte chemotactic protein-1 in adipose tissue [19]. Other studies have reported that adiponectin knockout (KO) mice had a profound reduction in survival rate and that plasma TNF- α and IL-6 levels were higher than those in wild-type mice in cecal ligation and puncture (CLP)-induced sepsis [20, 21]. Kaplan et al. reported that short-term HFD-induced obesity reduces plasma adiponectin levels and increases organ injury and mortality in CLP-induced sepsis [22]. However, changes in adiponectin receptor expression in sepsis and the precise effects of decreased adiponectin levels on LPS-induced septic injury have not yet been reported.

Based on these studies, we hypothesized that HFD feeding decreases not only plasma adiponectin levels but also expression of its receptors and then relates to worse obesity-induced septic injury. Therefore, the objective of this study was to examine whether short-term HFD-induced obesity contributes to worse outcomes in sepsis and the mechanisms focused on adiponectin in endotoxemic rats.

MATERIALS AND METHODS

Animals

Male Wistar rats (CLEA Japan, Tokyo, Japan) aged 4 weeks and weighing 70 to 90 g were used in all experiments. Rats were fed either a control diet (CD) (5% energy derived from fat, 25% from protein, and 70% from carbohydrates; 3.4 kcal/g) (CLEA Japan) or a HFD (60% energy derived from fat, 20% from protein, and 20% from carbohydrates; 5.2 kcal/g) (Research Diets, Inc., New Brunswick, NJ) for 4 weeks. Body weight and food intake were recorded every day for each animal. After 4 weeks of feeding, blood glucose levels were measured by self-monitoring (Arkray, Kyoto, Japan). All rats were kept at 22°C under a 12-h light/dark cycle and provided with food and water *ad libitum*. The day before the LPS injection, rats were provided with water only until they were killed. Rats were injected intraperitoneally with 10 mg/kg body weight of *Escherichia coli* O111:B4 LPS (Sigma-Aldrich, St. Louis, MO). Survival rates were monitored for 24 h, and blood samples and tissues were collected at 0, 1.5, and 6 h after LPS injection. All procedures for LPS injection, sampling, and sacrifice were performed under diethyl ether anesthesia. Blood samples were taken from the inferior vena cava with heparin-coated tubes. The liver, soleus muscle, epididymal fat, perirenal fat, and mesenterium fat were weighted and harvested for examination. All samples were stored at –80°C until analysis. This study was approved by the Institutional Animal Care and Use Committee and performed

according to the Kobe University Animal Experimentation Regulations.

RNA extraction, complementary DNA synthesis, and reverse-transcription polymerase chain reaction assay

Total RNA was extracted from the liver with TRIzol reagent (Invitrogen, Carlsbad, CA). The RNA concentration and purity were determined by absorbance at 260 and 280 nm. One microgram of total RNA extracted from each tissue was reverse transcribed to yield single-stranded complementary DNA using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA) according to the manufacturer's protocols. Real-time quantitative polymerase chain reaction (PCR) analysis (SYBR Green PCR Master Mix; Toyobo, Osaka, Japan) was performed with MyiQ (Bio-Rad). The real-time PCR conditions and primer sequences are listed in Table 1. All measurements were performed in duplicate. All specific quantities were corrected for the amplification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). For each sample, the threshold cycle (Ct) was calculated based on the cycle at which the fluorescence increased above a threshold level. The ΔCt values were calculated in every sample for the target genes as follows: $\text{Ct (target gene)} - \text{Ct (internal control gene)}$, with GAPDH as the internal control gene. The relative expression level for one target gene ($\Delta\Delta\text{Ct}$) was calculated by subtraction of the mean ΔCt of the control group from the ΔCt of each sample of the treated groups. Finally, the relative

expression value, normalized to an endogenous reference, was given by $2^{-\Delta\Delta Ct}$.

Determination of plasma adiponectin, IL-6, and nitric oxide levels

Plasma adiponectin levels were evaluated using the Mouse/Rat Adiponectin ELISA Kit (Otsuka Pharmaceuticals, Tokyo, Japan). Plasma IL-6 levels were evaluated using the Rat IL-6 Quantikine ELISA Kit (R&D Systems, Inc., Minneapolis, MN). Plasma nitric oxide (NO_x) levels were evaluated by measuring the nitrite (NO₂⁻) and nitrate (NO₃⁻) content using a colorimetric assay kit (BioVision Research Products, Mountain View, CA). All procedures were performed according to the manufacturer's protocols.

Lipid analysis

Plasma levels of aspartate transaminase (AST) and alanine aminotransferase (ALT) were measured using Japanese Society of Clinical Chemistry standardized procedures. Plasma levels of TG, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and FFA were measured by an enzymatic method.

Hepatic lipids were extracted by the method of Folch et al. [23]. The extract was dissolved in 10% Triton X-100 (Sigma-Aldrich) in 2-propanol. Hepatic TG and total cholesterol levels were determined using commercial kits (Triglycerides E-test Wako and Cholesterol CII-test Wako,

respectively; Wako Pure Chemical Industries, Osaka, Japan), according to the manufacturer's protocols.

Immunohistochemistry

To examine the development of DNA oxidative injury in the liver at 6 h after LPS injection, the tissue was stained immunohistochemically with monoclonal antibody 8-hydroxy-2'-deoxyguanosine (8-OHdG) (N45.1, 10 mg/mL; Japan Institute for the Control of Aging, Shizuoka, Japan). Briefly, after deparaffinization, the sections were treated with 3% H₂O₂ in distilled water for 5 minutes at room temperature and with citrate buffer solution (pH 6.0) for 10 minutes by pressure. Then the sections were reacted with N45.1 monoclonal antibody (diluted 1:2000) overnight at room temperature in a humidity chamber, followed by incubation with Dako EnVision HRP System (Dako, Tokyo, Japan) for 30 minutes at room temperature. Sections were then treated with 3,3'-diaminobenzidine for 5 min, and counterstaining was performed with hematoxylin and eosin. The number of cells was counted in 5 randomly chosen areas per slide under a bright-field microscope. The results are expressed as the mean percentage of immunoreactive-positive cells/total cells.

Histopathological analysis

The livers were fixed in 4% phosphate-buffered paraformaldehyde and embedded in paraffin, cut into 4- μ m-thick sections, and stained with hematoxylin and eosin. An expert pathologist evaluated fat deposition and tissue damage in the liver. Tissue damage was evaluated using the modified histological activity index (HAI) grading score [24]. The HAI grading score also includes assessment of periportal or periseptal interface hepatitis, confluent necrosis, focal lytic necrosis, apoptosis, and focal and portal inflammation.

Statistical analysis

All data are expressed as means \pm standard error of the mean (SEM). Comparison with each basal level was analyzed using the Tukey-Kramer test. For the identification of differences between CD- and HFD-fed rats at each time point after LPS injection, *t*-test was performed. To estimate the difference in survival rate between the CD- and HFD-fed rats, log-rank test was performed. Differences were considered significant when the *p* value was <0.05 .

RESULTS

Body weight, caloric intake, organ weights, hepatic lipid levels, and blood glucose level

The effects of short-term HFD feeding on the body weight, caloric intake, organ weights, hepatic lipid levels, and blood glucose level of the rats are shown in Table 2. Final body weight was increased by 7% in HFD-fed rats compared with CD-fed rats ($p < 0.01$), and HFD-fed rats consumed significantly more calories on a “per day” basis than CD-fed rats ($p < 0.01$). The increase in body weight was associated with the development of liver, epididymal fat, perirenal fat, and mesenterium fat weight ($p < 0.01$). Hepatic TG, total cholesterol, and blood glucose levels were also higher in HFD-fed rats ($p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively).

Survival rate after LPS injection

The effects of HFD on survival rate after LPS injection are shown in Figure 1. The survival rate in CD- and HFD-fed rats was 97.3% and 77.0% at 12 h and 85.2% and 12.8% at 24 h, respectively. Thus, the survival rate in HFD-fed rats was significantly lower than that in CD-fed rats after LPS injection ($p < 0.01$).

Plasma adiponectin concentration and its expression in adipose tissue

To evaluate adiponectin secretion, plasma adiponectin levels were measured (Figure 2A). The

basal level of adiponectin was significantly decreased by HFD feeding ($p < 0.05$). In endotoxemia, the level was decreased by LPS injection both in CD and HFD-fed rats and the level in HFD-fed rats was significantly lower than that in CD-fed rats at 1.5 and 6 h after LPS injection ($p < 0.01$). In particular, a 58% reduction was observed at 6 h after LPS injection.

The expression of adiponectin in the adipose tissue was analyzed (Figure 2B), and LPS injection was shown to decrease the expression of plasma adiponectin in both CD- and HFD-fed rats; expression in HFD-fed rats was significantly lower than that in CD-fed rats at 6 h after LPS injection ($p < 0.05$). Collectively, these results indicated that HFD feeding decreases both secretion and expression of adiponectin in endotoxemia.

Plasma IL-6 and NOx concentrations and their expression in adipose tissue

To assess the grade of systemic inflammation and systemic oxidative stress, plasma IL-6 and NOx concentrations, respectively, were analyzed (Figure 3A and 3B). At 1.5 h after LPS injection, their levels were not increased in both CD- and HFD-fed rats. At 6 h, the level of plasma IL-6 was increased by LPS injection in CD-fed rats. In HFD-fed rats, the level was further increased by LPS injection, but there was not a statistical difference. On the other hand, the level of plasma NOx in HFD-fed rats demonstrated a greater increase than that in CD-fed rats at 6 h after LPS injection ($p < 0.05$). These results indicated that HFD resulted in worse systemic

oxidative stress in endotoxemia.

Similarly, expression of IL-6 and NOx in the adipose tissue was analyzed (Figure 3C and 3D); expression of IL-6 in HFD-fed rats was further increased by LPS injection as compared with CD-fed rats at 6 h, but there were no statistical differences. In contrast, expression of inducible nitric oxide synthases (iNOS) in HFD-fed rats was significantly increased in CD-fed rats in endotoxemia at 6 h after LPS injection ($p < 0.05$).

Expression of adiponectin receptors in the liver and skeletal muscle

To evaluate the sensitivity of adiponectin, expression of adipoR1 and adipoR2 in the liver and of adipoR1 in the skeletal muscle was assayed (Figure 4). Basal expression of adipoR2 in the liver was significantly decreased by HFD feeding ($p < 0.05$), but the effect of HFD on the expression of adipoR1 in the skeletal muscle was not observed.

At 6 h after LPS injection, expression of adipoR1 in the liver was slightly decreased in CD-fed rats, but the degree of reduction in HFD-fed rats was more remarkable ($p < 0.05$). Hepatic expression of adipoR2 was decreased by LPS injection both in CD- and HFD-fed rats, and the expression in HFD-fed rats was significantly lower than that in CD-fed rats ($p < 0.05$). On the other hand, expression of adipoR1 in the skeletal muscle was not changed by LPS injection both in CD- and HFD-fed rats.

Liver injury

Figure 5 shows plasma AST and ALT levels. The basal level of ALT was significantly increased by HFD feeding ($p < 0.01$). At 6 h after LPS injection, AST and ALT levels were increased both in CD- and HFD-fed rats, and their levels in HFD-fed rats were significantly higher than those in CD-fed rats ($p < 0.01$).

For gross histological examination, hematoxylin and eosin-stained liver sections obtained at 0 and 6 h after LPS injection was studied by light microscopy (Figure 6). At baseline, microvesicular fat was seen in approximately 40% of hepatocytes and infiltration of inflammatory cells was rarely observed in HFD-fed rats. At 6 h after LPS injection, ~~extensive necrosis, hemorrhagic changes indicated by the arrow and inflammatory cell infiltration were prominent in the portal or periportal area in~~ HFD-fed rats compared with CD-fed rats. Additionally, HFD significantly increased the modified HAI grading score to 5.25 ± 0.41 , compared with 3.5 ± 0.35 in CD-fed rats ($p < 0.01$). Collectively, these results indicated that HFD exacerbates liver injury in endotoxemia.

8-OHdG immunostaining in the liver

To investigate the effect of HFD on LPS-induced oxidative stress in the liver, 8-OHdG immunostaining was performed (Figure 7) ~~and shows the DNA affected by oxidative damage.~~ This staining shows the DNA affected by oxidative damage turns brown and staining intensity reflects oxidative damage. At baseline, liver tissues in HFD-fed rats were damaged by oxidative stress whereas those in CD-fed rats were not. The rate of positive cells was significantly higher in HFD-fed rats than in CD-fed rats ($p < 0.01$). Thus, it was indicated that 4 weeks of HFD feeding induced oxidative stress in the liver without LPS stimulation. At 6 h after LPS injection, liver tissues in CD-fed rats were damaged by oxidative stress by LPS injection. In particular, sites around Glisson's capsule were well stained. In HFD-fed rats, the liver tissues were totally and strongly stained, indicating that HFD deteriorated hepatic oxidative stress in endotoxemia. ~~The rate of positive cells in HFD-fed rats was significantly higher than that in CD-fed rats ($p < 0.01$).~~ And HFD significantly increased the rate of positive cells to 82.3%, compared with 49.5% in CD fed rats ($p < 0.01$).

Mitochondrial contents in the liver

To assess mitochondrial contents in the liver, expression of D-loop, the region of mitochondrial DNA, was measured (Figure 8). The contents of hepatic mitochondrial DNA were

decreased by LPS injection both in CD- and HFD-fed rats, and its expression in HFD-fed rats was significantly lower than that in CD-fed rats after LPS injection ($p < 0.05$).

Plasma lipid levels

Table 3 lists the plasma lipid contents. At baseline, TG levels in HFD-fed rats were significantly higher than those in CD-fed rats ($p < 0.01$) and HDL-C levels in HFD-fed rats were significantly lower than those in CD-fed rats ($p < 0.01$). At 6 h after LPS injection, TG levels were increased both in CD- and HFD-fed rats. On the other hand, LDL-C levels in HFD-fed rats were further increased by LPS injection compared with CD-fed rats ($p < 0.05$) and HDL-C levels in HFD-fed rats were significantly lower than those in CD-fed rats ($p < 0.01$). Although there were no statistical differences, plasma FFA levels in HFD-fed rats were increased compared with those in CD-fed rats.

DISCUSSION

This study revealed the effect of short-term HFD feeding on LPS-induced septic injury. Four weeks of HFD feeding slightly increased final body weight, and the detailed condition of obesity is discussed later. Although the increase in body weight was mild, HFD-fed rats had worse survival rates (85.2% vs. 12.8%) at 24 h after LPS injection, with higher plasma levels of IL-6 and NOx. Hepatic expression of both adiponectin receptors (adipoR1 < adipoR2) and plasma adiponectin levels were decreased in endotoxemia, and HFD feeding facilitated this reduction. LPS-induced liver injury and oxidative stress were increased in HFD-fed rats, as indicated by increased HAI grading score and 8-OHdG immunostaining. Lipid metabolism abnormality was also deteriorated, as indicated by elevated plasma LDL-C and FFA levels and reduced plasma HDL-C levels. The results of this study indicate that even a mild obese condition is harmful to a septic host and reduced adiponectin sensitivity is one of the causes of worse pathological conditions in sepsis.

In most studies, the degree of obesity has been evaluated by comparing the body weight of the HFD group with that of the CD group [25, 26]. A 10% to 25% increase in body weight was defined as moderate obesity [27, 28] and an increase greater than 40% as severe obesity [29]. Kaplan et al. fed a lard-based HFD to mice for 3 weeks, and the body weight of the HFD-fed mice was 7% greater than that of the CD-fed mice [22]. They suggested that long-term HFD

feeding does not reflect the inflammatory changes that occur in the early stage of obesity in humans. In terms of increases in body weight, the 7% increase with HFD feeding in the present study reflects mild obese conditions. With regard to elevation of fat weight by HFD feeding, Woods et al. reported that 10 weeks of butter-based HFD feeding increased total fat weight 1.5-fold compared with a low-fat diet in rats [28]. Considering this report, an increase in each adipose tissue weight of more than 2-fold in this study might be appropriate for obesity. However, Fan et al. reported that rats in the NASH model, which was induced by lard-based HFD feeding during a 12-week period, had an HAI grading score of 3.4 [30]. The baseline score in the HFD group in this study was 0.5 point, and lipid droplets in the liver were not observed markedly. Therefore, 4 weeks of HFD feeding resulted in obese conditions and reflected mild obesity, but not morbid obesity or the NASH model. HFD-fed rats had worse outcomes in endotoxemia because of a large amount of lard. A previous study by Rivera et al. reported that a Western diet containing lard increased hepatic inflammation in a CLP-induced sepsis mouse model [31]. They suggested that saturated fatty acids worsen hepatic inflammation by activating toll-like receptor 4 signaling after CLP. The results of their report and our findings indicate that excess intake of saturated fatty acids, even in a mild obese condition, is quite harmful to the host in sepsis.

The decrease in plasma adiponectin level after LPS injection is in agreement with previous studies indicating that the circulating concentration of adiponectin is decreased in septic patients

and a septic animal model [18, 32]. And the reduction was seen at 1.5 h after LPS injection when plasma IL-6 and NOx levels were not yet increased by LPS. Thus, decreased plasma adiponectin level may be one of key factors of the onset of elevation in plasma inflammatory mediators after LPS injection. A 58% reduction of the plasma adiponectin level in HFD-fed rats indicated that HFD feeding decreases secretion of adiponectin in endotoxemia. Elevation of plasma NOx, adipose tissue iNOS messenger RNA (mRNA), and hepatic 8-OHdG staining with increased plasma IL-6 and adipose tissue IL-6 mRNA expression indicated that HFD feeding worsened systemic oxidative stress and inflammation in endotoxemia. Adiponectin has an anti-oxidative effect [33], and it has been reported that circulating adiponectin levels correlate inversely with systemic oxidative stress in humans and rodents [34]. Therefore, decreased adiponectin secretion by HFD feeding induced deterioration of systemic oxidative stress in endotoxemia.

To the best of our knowledge, this is the first study to show decreased expression of adiponectin receptors in the liver, but not in the skeletal muscle in endotoxemia, and that HFD feeding enhanced this reduction. Combined with the decreased plasma adiponectin levels, HFD feeding reduces the hepatic sensitivity of adiponectin in endotoxemia. The expression of the hepatic adiponectin receptor in the NASH model is reported to be decreased by half of the level in control rats [16]. Therefore, it is suggested that more than a 50% decrease of expression of adiponectin receptors in HFD-fed rats in the present study strongly affects hepatic injury in

endotoxemia. The finding of no changes of adiponR1 expression in the skeletal muscle by HFD feeding or LPS injection is consistent with previous studies. Barnea et al. reported that soybean oil-based HFD feeding for 4 months did not decrease the mRNA expression of adiponectin receptors in the skeletal muscle, whereas the expression in the liver was decreased [35]. In addition, several studies have reported that LPS-induced changes in the skeletal muscle appear at later than 12 h in a septic animal model [36, 37]. Thus, the effect of HFD on expression of adiponectin receptors is less effective in the skeletal muscle in comparison with the liver and it might be too early to observe LPS-induced changes of expression in the skeletal muscle. As for the relationship between adiponectin and liver injury in sepsis, Uji et al. reported that adiponectin KO mice experienced worse liver injury than wild-type mice after CLP [21], suggesting that adiponectin inhibited accumulation of macrophages into the liver and protected hepatic inflammation. In addition, Masaki et al. reported that intraperitoneal injection of adiponectin in mice improved survival rate and liver injury after D-galactosamine/LPS injection, suggesting that adiponectin might be a useful therapeutic tool for endotoxin-induced liver injury [38]. Therefore, the reduction of hepatic adiponectin sensitivity by HFD feeding greatly influenced deterioration of LPS-induced liver injury.

The decreased amount of mitochondrial DNA in HFD-fed rats after LPS injection might be related to peroxisome proliferator-activated receptor- γ coactivator (PGC)-1 α , which is activated

by the adiponectin signal [39]. PGC-1 α regulates mitochondrial biogenesis [40], and its expression in the liver is decreased in endotoxemia [41]. It is suggested that reduction of adiponectin sensitivity in HFD-fed rats led to a decreased amount of mitochondrial DNA via inactivation of PGC-1 α . Mitochondrial dysfunction is reported to be a cause of increased FFA levels [42]. Therefore, the reduction of mitochondrial DNA might influence elevated plasma FFA levels in HFD-fed rats. The changes in total, LDL-cholesterol, and HDL-cholesterol levels in HFD-fed rats after LPS injection might be partly explained by the effect of decreased adiponectin. Previous studies reported that adiponectin decreased LDL-C levels and increases HDL-C levels via secretion and synthesis of apolipoprotein [43, 44]. Therefore, reduction of adiponectin sensitivity might induce alterations of cholesterol levels in HFD-fed rats. Collectively, the present study revealed that HFD feeding worsens LPS-induced lipid metabolism abnormality.

In the current study, we focused on the changes in the liver and the skeletal muscle, but adiponectin receptors were expressed in various tissues such as the lung and the heart [45]. Konter et al. reported that adiponectin alleviates LPS-induced acute lung injury by suppressing endothelial cell activation using adiponectin KO mice [46]. Changes in adiponectin sensitivity and injury in these organs will be examined in a future study. In addition, lard-based HFD feeding alters fatty acid composition, especially polyunsaturated fatty acids in the liver [47], and

it has been reported that polyunsaturated fatty acid-derived lipid mediators such as prostaglandins, leukotrienes, and protectin regulate formation and resolution of the acute inflammation [48]. Therefore, whether lard-based HFD feeding alters these mediators and is involved with organ dysfunction is a subject of great interest.

In summary, the results of this study indicated that short-term HFD feeding with lard worsened mortality and liver injury after LPS injection. The reduction of adiponectin sensitivity in the liver was speculated to be the mechanism. Only 4 weeks of HFD-induced mild obesity is harmful in endotoxemia, and adiponectin sensitivity is important not only in chronic inflammation but also in acute inflammation. Enhancing adiponectin sensitivity is quite important to the host, and adiponectin may be a useful therapeutic target in sepsis.

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Figure Legends

Figure 1. Effect of HFD on survival rate after LPS injection. HFD-fed rats had a significantly lower survival rate than CD-fed rats. $**p < 0.01$ vs. CD-fed rats using log-rank test.

Figure 2. Effects of HFD on plasma adiponectin concentration and mRNA expression of adiponectin in the adipose tissue. At 0, 1.5, and 6 h after LPS injection, plasma and epididymal fat were harvested from CD- and HFD-fed rats. (A) The plasma concentration of adiponectin was measured by enzyme-linked immunosorbent assay. (B) The mRNA expression of adiponectin was measured using real-time PCR and was expressed as a ratio based on the amount of mRNA in the control group. Values represent the means \pm SEM of 4–8 animals per group. $^{\$}p < 0.05$ vs. CD at 0 h, $^{\#}p < 0.05$, $^{##}p < 0.01$ vs. HFD at 0 h, $^{*}p < 0.05$, $^{**}p < 0.01$ vs. CD at the same time point.

Figure 3. Effects of HFD on plasma IL-6 and NOx concentrations and their mRNA expression in adipose tissue. At 0, 1.5, and 6 h after LPS injection, plasma and epididymal fat were harvested from CD- and HFD-fed rats. The plasma concentration of (A) IL-6 and (B) NOx was measured by enzyme-linked immunosorbent assay. The mRNA expression of (C) IL-6 and (D) iNOS was measured using real-time PCR and was expressed as a ratio based on the amount of mRNA in the

control group. Values represent the means \pm SEM of 4–8 animals per group. $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ vs. CD at 0 h, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. HFD at 0 h, $^{*}p < 0.05$ vs. CD at the same time point.

Figure 4. Effects of HFD on adiponectin receptor mRNA expression in the liver and the skeletal muscle. At 0 and 6 h after LPS injection, liver and soleus muscle were harvested from CD- and HFD-fed rats. The mRNA expression of (A) adipoR1 and (B) adipoR2 in the liver as well as (C) adipoR1 in the skeletal muscle was measured using real-time PCR and was expressed as a ratio based on the amount of mRNA in the control group. Values represent the means \pm SEM of 7–9 animals per group. $^{\$\$}p < 0.01$ vs. CD at 0 h, $^{\#\#}p < 0.01$ vs. HFD at 0 h, $^{*}p < 0.05$ vs. CD at the same time point.

Figure 5. Effects of HFD on plasma AST and ALT levels. At 0 and 6 h after LPS injection, plasma (A) AST and (B) ALT levels were assessed. Values represent the means \pm SEM of 4–8 animals per group. $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ vs. CD at 0 h, $^{\#\#}p < 0.01$ vs. HFD at 0 h, $^{*}p < 0.05$, $^{**}p < 0.01$ vs. CD at the same time point.

Figure 6. Effect of HFD on pathological changes in the liver after LPS injection. (A) CD 0 h; (B) HFD 0 h; (C) CD 6 h; (D) HFD 6 h after LPS injection (hematoxylin sand eosin staining,

200× magnification). (E) Tissue damage was evaluated by the HAI grading score. Values represent the means \pm SEM of 4–8 animals per group at each time point. The arrow show hemorrhagic changes. $^{ss}p < 0.01$ vs. CD at 0 h, $^{##}p < 0.01$ vs. HFD at 0 h, $^{**}p < 0.01$ vs. CD at the same time point.

Figure 7. Effect of HFD on oxidative stress in the liver. (A) CD 0 h; (B) HFD 0 h; (C) CD 6 h; (D) HFD 6 h after LPS injection (8-OHdG staining, 200× magnification). (E) Positive ratio was expressed as the mean percentage of immunoreactive-positive cells/total cells. Values represent the means \pm SEM of 5 animals per group at each time point.

$^{ss}p < 0.01$ vs. CD at 0 h, $^{##}p < 0.01$ vs. HFD at 0 h, $^{**}p < 0.01$ vs. CD at the same time point.

Figure 8. Effects of HFD on mitochondria DNA expressions in the liver. At 0 and 6 h after LPS injection, the liver was harvested from CD- and HFD-fed rats. The mRNA expression of D-loop was measured using real-time PCR and was expressed as a ratio based on the amount of mRNA in control group. Values represent the means \pm SEM of 7–9 animals per group at each group. $^s p < 0.05$ vs. CD at 0 h, $^{\#}p < 0.05$ vs. HFD at 0 h, $^*p < 0.05$ vs. CD at the same time point.

Table 1. Primer sequences used for real-time PCR

Gene	Forward primer (5'–3')	Reverse primer (3'–5')	Annealing Temperature (°C)
Adiponectin	GGGAGACGCAGGTGTTCTTG	CGCTGAATGCTGAGTGATACATG	61
IL-6	GCCCTTCAGGAACACTATGA	TGTCAACAACATCAGTCCCAAGA	59
iNOS	AACCCAAGGTCTACGTTCAAG	AAAGTGGTAGCCACATCCCG	59
AdipoR1	CTTCTACTGCTCCCCACAGC	GACAAAGCCCTCAGCGATAG	59
AdipoR2	ATGTTTGCCACCCCTCAGTA	AGCCTATCTGCCCTATGGT	57
D-loop	TGGTAAAATTTCCCGACACA	ATAAGGCCAGGACCAAACCT	61
GAPDH	GGCACAGTCAAGGCTGAGAATG	ATGGTGGTGAAGACACCAGTA	59

Table 2. Effect of HFD on body weight, caloric intake, organ weights, hepatic lipid levels, and blood glucose level

Parameter	CD	HFD	
Final body weight (g)	289.0 ± 3.5	310.6 ± 1.9	**
Increasing rate of body weight (%)	205.0 ± 3.4	232.5 ± 2.5	**
Caloric intake (cal/day)	68.1 ± 1.2	74.3 ± 0.7	**
Liver weight (g/100 g BW)	3.3 ± 0.3	3.7 ± 0.5	**
Epididymal fat weight (g/100 g BW)	1.1 ± 0.1	2.2 ± 0.1	**
Perirenal fat weight (g/100 g BW)	0.8 ± 0.1	1.8 ± 0.1	**
Mesenterium fat weight (g/100 g BW)	0.7 ± 0.1	1.7 ± 0.1	**
Hepatic triglyceride levels (mg/g liver)	3.2 ± 0.5	17.7 ± 0.7	**
Hepatic total cholesterol levels (mg/g liver)	1.1 ± 0.1	2.8 ± 0.3	**
Blood glucose levels (mg/dL)	102.0 ± 2.2	116.0 ± 3.7	*

Note: Values represent the means ± SEM.

BW, body weight. *p < 0.05, **p < 0.01 vs. CD group.

Table 3. Effect of HFD on plasma lipid levels

Parameter	CD 0 h	HFD 0 h	CD 6 h	HFD 6 h
TG (mg/dL)	33.9 ± 7.6	52.9 ± 4.0**	113.0 ± 8.4 ^{\$\$}	117.0 ± 12.7 ^{##}
LDL-C (mg/dL)	10.1 ± 0.7	8.6 ± 0.6	11.7 ± 0.4	14.0 ± 0.5 ^{##*}
HDL-C (mg/dL)	21.8 ± 0.7	17.8 ± 0.5**	20.3 ± 0.6	16.3 ± 0.7**
FFA (μEq/L)	1508 ± 113	1784 ± 120	1747 ± 55	2234 ± 179

Note: Values represent the means ± SEM.

^{\$\$}p < 0.01 vs. CD at 0 h, ^{##}p < 0.01 vs. HFD at 0 h, *p < 0.05, **p < 0.01 vs. CD at the same time point.

Figure 1.

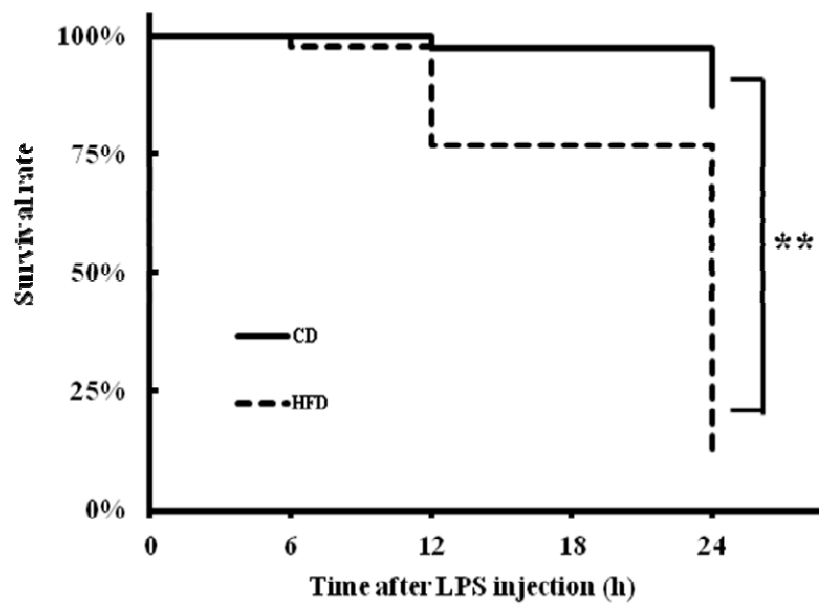


Figure 2.

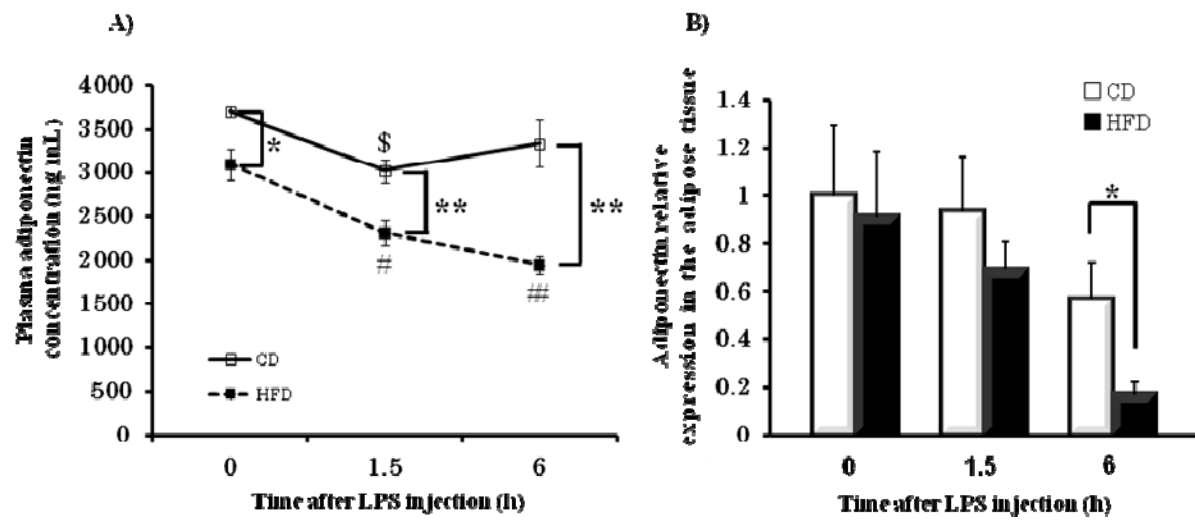


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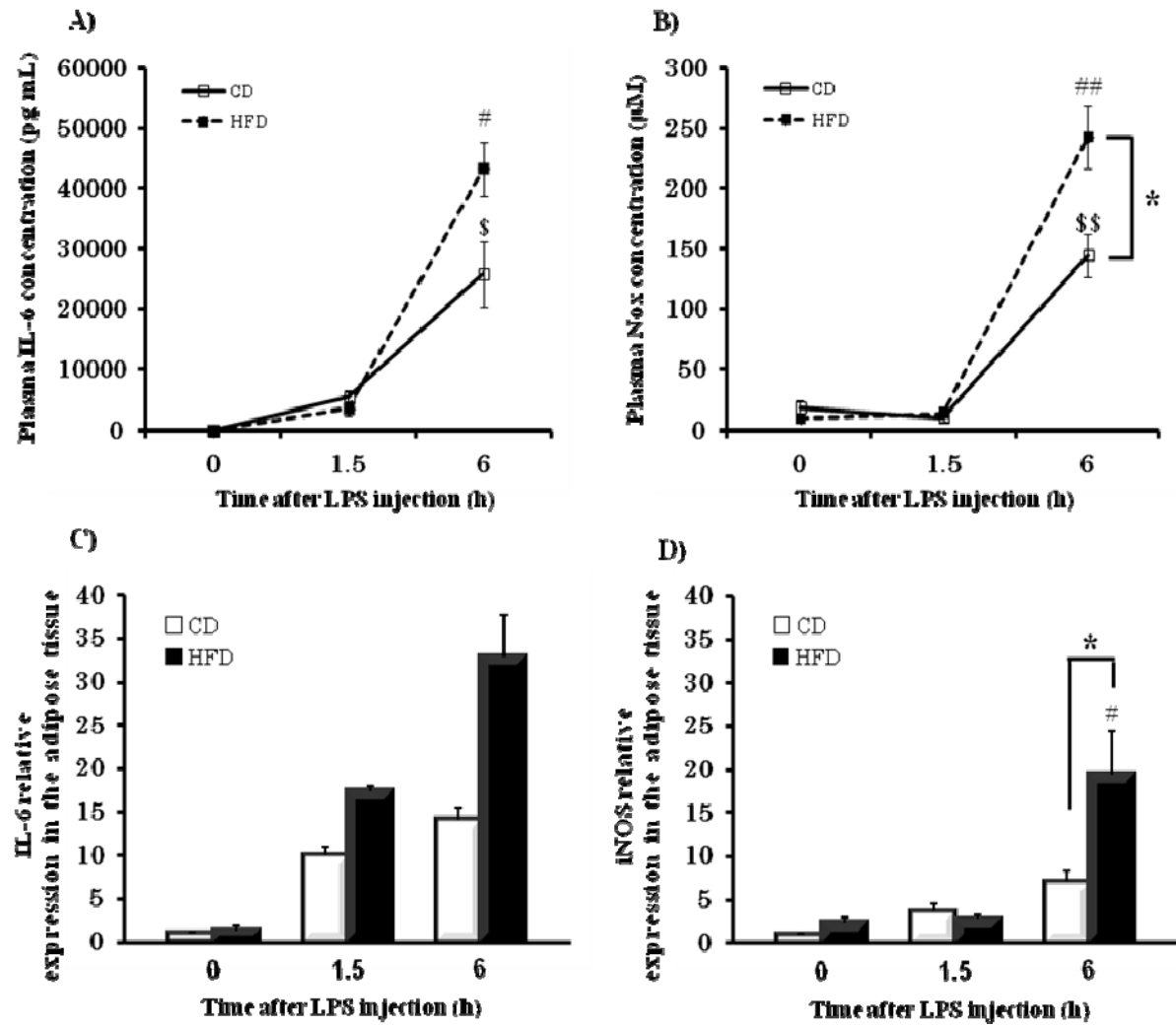


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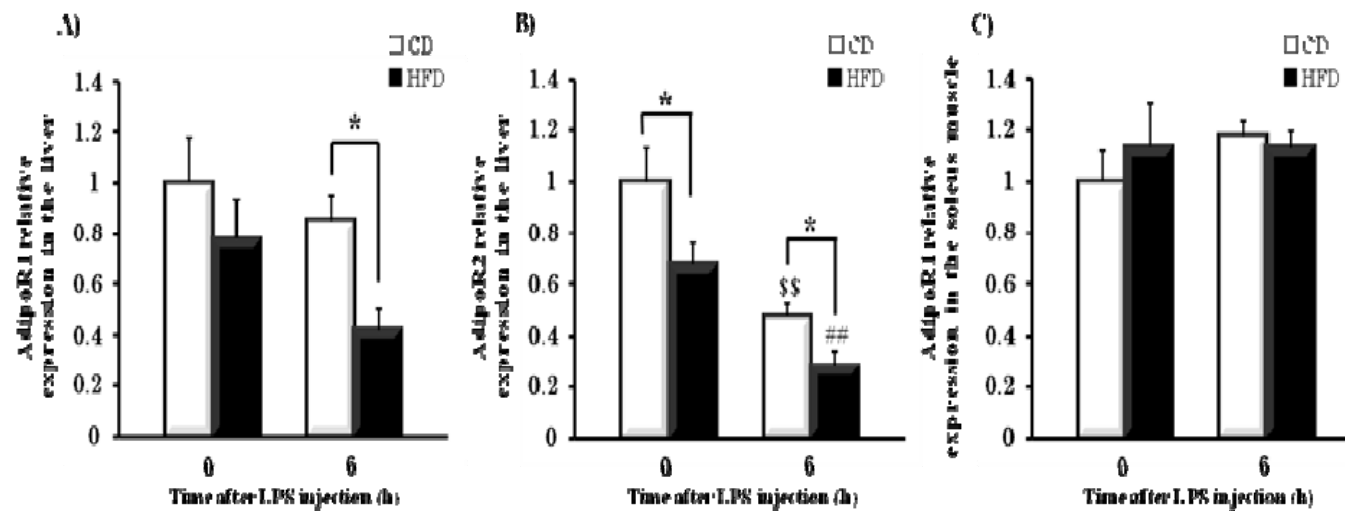


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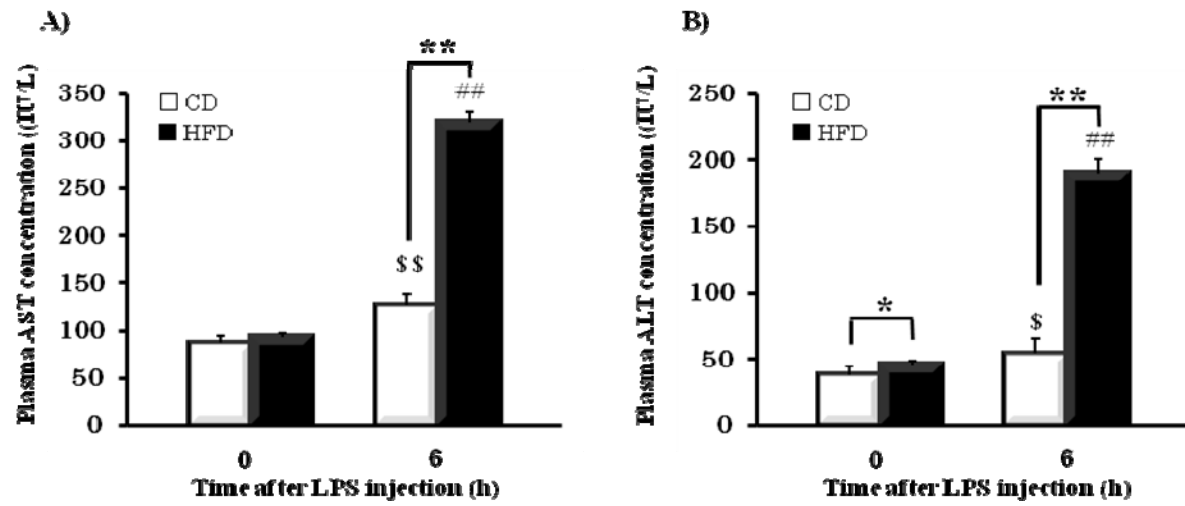


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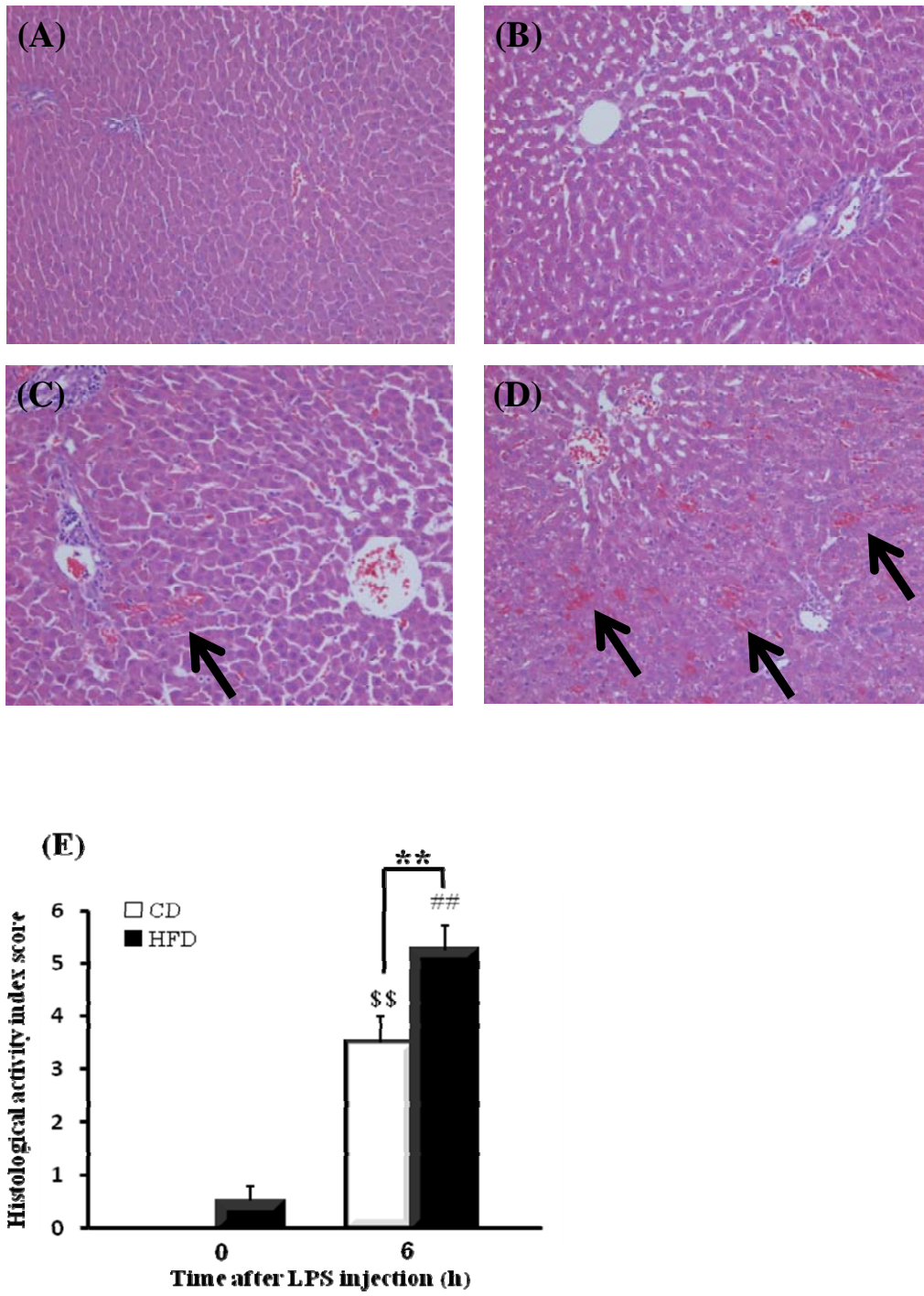


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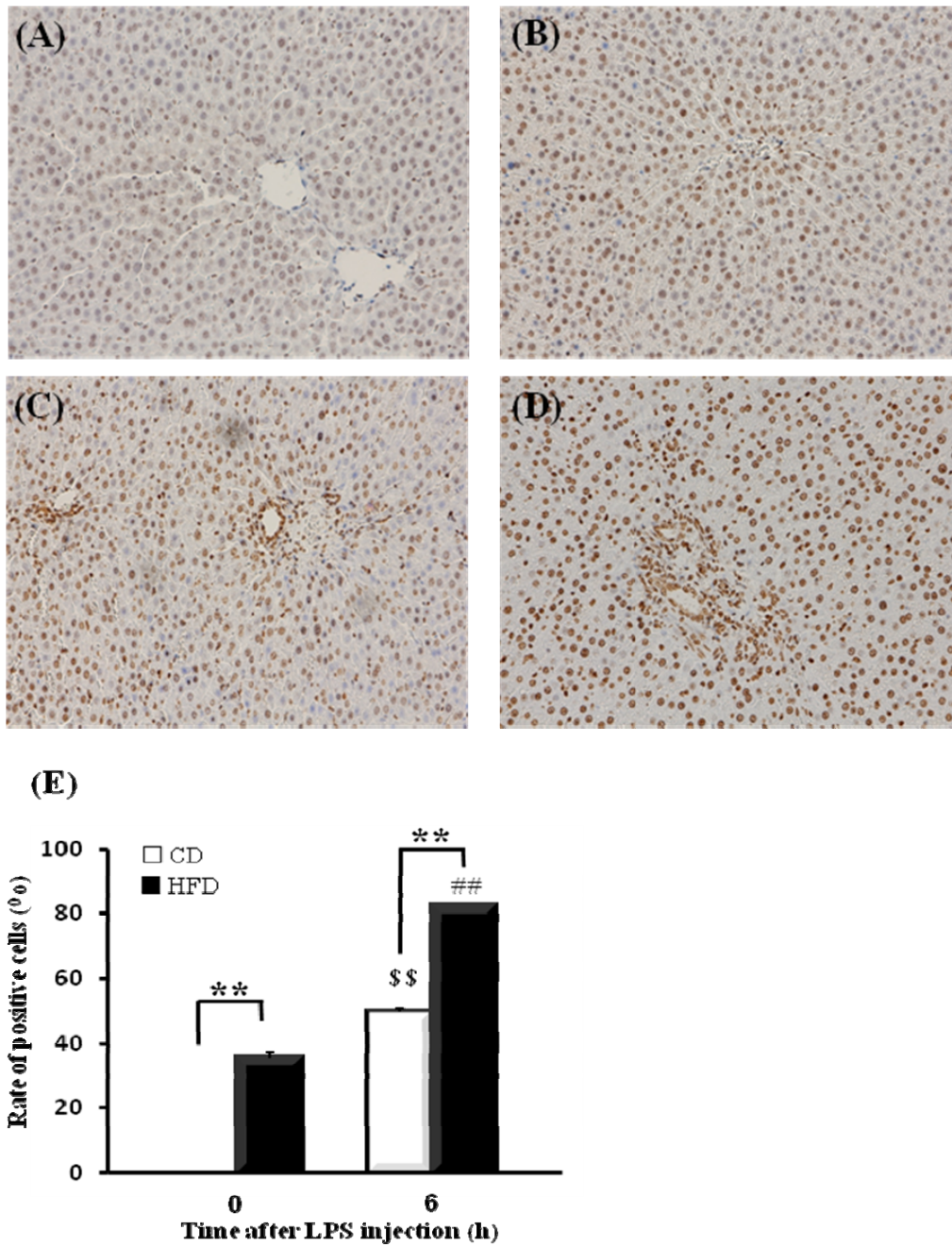


Figure 8.

