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A lard-rich high-fat diet increases hepatic peroxisome proliferator–activated receptors in endotoxemic rats

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ABSTRACT

Background: Diets high in saturated fatty acids activate chronic inflammation. We previously reported that, in even acute inflammation caused by lipopolysaccharide (LPS), liver injury was exacerbated in rats fed a lard-rich diet. Peroxisome proliferator–activated receptors (PPARs) are related to inflammation and are also key regulators of lipid metabolism. In this study, we examined effects of high-fat diet on liver injury and hepatic lipid metabolism during endotoxemia, measuring hepatic PPARs and other markers.

Materials and methods: Male Wistar rats were fed a high-fat diet (HFD, 60 kcal% fat) or control diet (CD, 10 kcal% fat) for 4 or 12 wk, injected with LPS and sacrificed at 0, 1.5, or 6 h. Analyses included plasma aspartate transaminase (AST) and alanine transaminase (ALT) levels, messenger RNA (mRNA) and protein levels of hepatic PPARα and PPARγ, and mRNA levels of enzymes related to fatty acid oxidation and synthesis.

Results: Endotoxemic rats on HFD for 12 wk, but not 4 wk, had higher mRNA and protein levels for hepatic PPARs, than did those on CD ($P < 0.01$-0.05). Similarly, these rats had increased mRNA expression of hepatic fatty acid oxidation- and synthesis-related enzymes ($P < 0.01$-0.05). Rats injected with LPS had more severe liver injury, indicated by plasma AST/ALT, if on the HFD for 12 wk, compared with for 4 wk.

Conclusions: Consumption of a lard-rich diet for 12 wk worsened liver injury and increased hepatic PPARα and PPARγ expression in endotoxemic rats.

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Introduction

The incidence of obesity is increasing and excessive intake of foods high in saturated fatty acids (SFA), such as lard, is one cause of obesity.[1,2] SFA are intimately involved in the development of chronic inflammation and metabolic syndrome.[3] Lard consumption itself, in the absence of severe obesity, was reported to increase liver injury and exacerbate deleterious effects of sepsis, as indicated by lower survival rate, increased oxidative stress, increased inflammatory cytokines, and proinflammatory lipid mediators.[4-6] A clinical study indicated that morbid obesity (body mass index > 40 kg/m^2) was a risk factor for mortality in intensive care unit patients with sepsis.[7] However, a detailed analysis of metabolic disturbances in morbidly obese sepsis patients is difficult to perform because of the multiple and complicated reasons for obesity.[8] Therefore, a rat model consuming a lard-rich high-fat diet (HFD) was selected for our study.

Peroxisome proliferator–activated receptors (PPAR)s are subfamily members of nuclear receptors. PPARs can sense and interpret fatty acid signals derived from dietary fat and transduce these signals to influence immune regulation.[9] Various fatty acids and their derivatives have been identified as PPAR ligands. SFA is known as an endogenous ligand for PPARα.[9] Lipid mediators such as 15-deoxy-$\Delta^{10,11}$-prostaglandin J_2 (15-deoxy-PGJ_2) and 15-hydroxyeicosatetraenoic acid are recognized as endogenous PPARγ ligands, and their levels were increased in this experimental model.[6,9] As transcription factors regulated by fatty acids, PPARα plays a central role in fatty acid oxidation, and PPARγ is involved in fatty acid synthesis in the liver.[3,12] In particular, PPARα controls transcription of fatty acid oxidation-related enzymes (fatty acid transporter protein [FATP], fatty acid–binding protein [FABP], and carnitine palmitoyltransferase [CPT]-1a), and PPARγ regulates that of fatty acid synthesis-related enzymes (sterol regulatory element-binding protein [SREBP]-1c, acetyl-CoA carboxylase [ACC], fatty acid synthase [FAS]).[13]

PPARs can also exert their antiinflammatory effects through transrepression of genes and, indirectly, by altering lipid metabolism.[9] Liganded PPARα modulates obesity-related inflammation, either through its metabolic activity or antiinflammatory effects. A study on HFD-induced obesity in mice suggested that PPARα protected against obesity-induced chronic inflammation in the liver.[10] Results obtained with PPARγ knockout compared with wild-type mice indicated that PPARγ activity improved not only metabolic syndrome but also insulin resistance, by inhibiting macrophage induction.[14] PPARs are believed to directly modify the pool of lipid molecules in the body, and changes in the lipid environment would induce secondary regulatory processes. It is also regarded that the antiinflammatory effects of PPARs are not exclusively mediated to change lipid metabolism, at least partly.[9] Hepatic PPARα and PPARγ expressions were increased in rats fed an HFD for 12 wk.[11] However, these mediators were, instead, downregulated after lipopolysaccharide (LPS) injection in rats fed a standard diet.[15] The alteration of PPARs in the liver is important, but there have been no reports of changes in hepatic PPARα and PPARγ expression in sepsis with morbid obesity.

Based on this background information, we had two aims for this study. The first was to test the hypothesis that HFD feeding would worsen liver injury after LPS injection and that the effect would be greater with 12 than with 4 wk on this diet. The second aim was to investigate the influence of an HFD, after either 4 or 12 wk feeding, on hepatic expression of PPARα and PPARγ during LPS-induced liver injury. Hepatic fat deposition, oxidative stress, PPARα and PPARγ, as well as fatty acid oxidation–related (FATP, FABP, CPT-1a) and fatty acid synthesis–related (SREBP-1c, ACC, FAS) enzymes were measured in this study.

Materials and methods

Animals and diets

Male Wistar rats (4-wk-old, 70-90 g) used for all experiments were obtained from CLEA Japan (Tokyo, Japan). All rats were kept under controlled conditions (22°C, 12 h day/night cycle) and provided with food and water ad libitum. These rats were randomly divided into two groups. One group was fed a control diet (CD) in which the fat component was made up of 45% lard and 55% soybean oil (D12450B, 10% energy derived from fat, 20% from protein and 70% from carbohydrates; 3.85 kcal/g, Research Diets, Inc, New Brunswick, NJ, USA) and the other group was fed an HFD in which the fat component was made up of 90% lard and 10% soybean oil (D12492, 60% energy derived from fat, 20% from protein and 20% from carbohydrates; 5.24 kcal/g, Research Diets, Inc). The fatty acid compositions of these diets were the same as in our previous report.[16] Rats fed an HFD showed body weight increases and advanced hepatic steatosis after 4 wk of feeding and then reached a maximum change after long-term feeding periods, such as at 14 wk.[17-20] Body weight and food intake were measured daily for each rat. Beginning on the day before LPS injection, rats were provided with only water, not food, until euthanized. *Escherichia coli* O111:B4 LPS (Sigma–Aldrich, St Louis, MO) was administered by intraperitoneal injection (10 mg/kg) to rats fed with each diet: CD + LPS group and HFD + LPS group. Blood samples and livers were collected at 0, 1.5, or 6 h after LPS injection under diethyl ether anesthesia ($n = 4$ at 0 h and $n = 6$-8 at 1.5, 6, and 24 h). A sample size of 4-8 rats per group was calculated by using Power and Sample Size Calculation (PS version 3.1.2 Power and sample size calculator). PS version 3.1.2 available at http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize based on our previous results.[4] For this, an error of 0.05 and power of 90% was defined to calculate the above sample size. Blood samples were collected from the inferior vena cava using heparin-coated tubes. The livers were weighed, and all samples were stored at −80°C until analysis. All animal experiments were approved by the Institutional Animal Care and Use Committee under the Kobe University Animal Experimentation Regulations (P101208).

Blood biochemical tests

Plasma samples for aspartate transaminase (AST), alanine transaminase (ALT), triglyceride (TG), free fatty acid (FFA),