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Original article

Changes of hepatic lipid mediators associated with intake of high-fat diet for 12 weeks in endotoxemic rats using LC-ESI-MS/MS

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SUMMARY

Background & aims: It has recently been reported that anti-inflammatory lipid mediators are increased in the late phase of acute inflammation, whereas proinflammatory lipid mediators are regulated at the initiation of inflammation. The purpose of this study was to evaluate changes of hepatic lipid mediators due to high-fat diet (HFD) feeding in endotoxemic rats.
Methods: Male Wistar rats were fed either HFD or control diet for 12 weeks, and were then killed 0, 1.5, and 6 h after lipopolysaccharide (LPS) injection. Analyses included lipidomics assessment of mediators using liquid chromatography-electrospray ionization/multi-stage mass spectrometry; measuring expression of hepatic polyunsaturated fatty acid (PUFA)-oxidizing enzyme, tumor necrosis factor (TNF)-α, interleukin (IL)-6, and inducible nitric oxide synthase mRNA levels; blood biochemical tests; and liver histology.
Results: HFD feeding worsened liver injury, increased expression of TNF-α and IL-6 mRNA, and increased oxidative stress after LPS injection. PUFA-oxidizing enzymes were higher in HFD-fed rats after LPS injection. The proinflammatory prostaglandin (PG)E_2 and thromboxane B_2 were increased 1.5 h after LPS injection, and had decreased by 6 h in HFD-fed rats. In contrast, potent pro-resolving resolvins derived from eicosapentaenoic acid and docosahexaenoic acid were not detected, but anti-inflammatory epoxyeicosatrienoic acids, lipoxin A_4, and 15-deoxy-PGJ_2 were increased after LPS injection in HFD-fed rats.
Conclusions: HFD feeding for 12 weeks enhanced proinflammatory lipid mediators 1.5 h after LPS injection suggesting relation to liver injury.

1. Introduction

More than 1 billion adults worldwide are regarded as overweight and approximately 500 million are estimated to be obese, which represents one of the most important global health problems [1]. Overweight and obesity are increasing worldwide, in part because of increasing adoption of a Western diet high in saturated fat [2]. It has recently been reported that fatty acid (FA)-derived lipid mediators are altered under pathological conditions; this has been demonstrated via comprehensive analyses of lipid mediators using a liquid chromatography-electrospray ionization/multi-stage mass spectrometry (LC-ESI-MS/MS)-based lipidomics system [3]. This approach enables simultaneous and quantitative measurements of a large number of lipid mediators. Polyunsaturated fatty

Abbreviations: CD, control diet; HFD, high-fat diet; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; iNOS, inducible nitric oxide synthase; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acids; GC, gas chromatography; LC-ESI-MS/MS, liquid chromatography-electrospray ionization/multi-stage mass spectrometry; COX, cyclooxygenases; LOX, lipoxygenases; CYP, cytochrome P450; sEH, soluble epoxide hydrolase; PG, prostaglandin; Tx, thromboxane; HHT, hydroxyheptadecatrienoic acid; LT, leukotriene; HETE, hydroxyeicosatetraenoic acid; ETE, eicosatetraenoic acid; LX, lipoxin; EET, epoxyeicosatrienoic acid; DHT, dihydroxyeicosatrienoic acid; HEPE, hydroxyeicosapentaenoic acid; EpETE, epoxyeicosatetraenoic acid; Rv, resolvin; HDoHE, hydroxydocosahexaenoic acid; EpDPE, epoxydocosapentaenoic acid; HDoPE, hydroxydocosapentaenoic acid.

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acids (PUFA) including arachidonic acid (AA, 20:4*n*-6), eicosapentaenoic acid (EPA, 20:5*n*-3), and docosahexaenoic acid (DHA, 22:6*n*-3) are metabolized by PUFA-oxidizing enzymes such as cyclooxygenases (COX), lipoxygenases (LOX), and cytochrome P450 (CYP). PUFA-derived lipid mediators such as prostaglandins (PG), leukotrienes (LT), lipoxins (LX), resolvins (Rv), and protectins are potent endogenous regulators of inflammation. In addition, it has been reported that lipid mediators and PUFA-oxidizing enzymes are affected by high-fat diet (HFD) containing lard or corn oil [4,5]; however, no report has yet been published describing a comprehensive analysis of lipid mediators in HFD-fed animal models.

Sepsis is a fatal whole-body inflammation caused by severe bacterial infection and recent large meta-analyses report that critically ill patients with obesity have a worse outcome than non-obese patients [6]. However, how obesity as a result of a Western diet affects septic injury is still poorly understood. Lipopolysaccharide (LPS)-injected animals are used as typical and reproducible sepsis model. Approximately 80% of intraperitoneally injected LPS accumulates in the liver, leading to liver injury [7], Because of this we have focused on the liver. Our previous study showed that short-term HFD-induced obesity aggravates liver injury in endotoxemic rats [8]. Moreover, Xu et al. have reported that in the absence of LPS stimulation, liver injury was worse after 12 weeks administration of HFD containing lard and cholesterol which is rich in saturated fatty acid, but different from HFD we used, because of increased inflammatory cell infiltration [9]. However, analyses of lipid mediators have often been performed in experiments using isolated cells rather than whole tissues [3,10]. and the importance of CYP-AA metabolites in liver physiology and pathology has been reviewed recently [11]. Previous studies have described the relationships between lipid mediators, proinflammatory cytokine, and oxidative stress in the kidney, liver, and brain [12–14] and have reported an improvement of LPS-induced injury owing to administration of anti-inflammatory lipid mediators such as LXA_4, 15-deoxy-PGJ_2, and RvE1 [15–17]. However, comprehensive analyses of lipid mediators in sepsis after HFD feeding, particularly in the whole liver, have not yet been reported.

We hypothesized that both HFD feeding and LPS stimulation alter hepatic lipid mediators, and that proinflammatory lipid mediators affect LPS-induced liver injury. In this study, changes of lipid mediators and PUFA-oxidizing enzymes in septic liver injury associated with HFD feeding and/or LPS stimulation were evaluated.

2. Materials and methods

2.1. Animals and diets

Male Wistar rats (CLEA Japan, Tokyo, Japan) aged 4 weeks and weighing 70–90 g were used in all experiments. Rats were fed either 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) or 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.) for 12 weeks. The FA composition of the experimental diets is shown in Table 1.

Body weight and food intake were recorded every day for each animal. 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 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, USA). 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 collected from the inferior vena cava using heparin-coated tubes. The liver was harvested and weighed 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.

Table 1
Fatty acid composition (g of 100 g diet) of diets.

Fatty acid	CD	HFD
C 10:0	0	0.01
C 12:0	0	0.03
C 14:0	0.02	0.36
C 15:0	0	0.03
C 16:0	0.62	6.45
C 16:1*n*-7	0.03	0.44
C 17:0	0.01	0.12
C 18:0	0.29	3.48
C 18:1*n*-9	1.19	11.19
C 18:2*n*-6	1.73	9.45
C 18:3*n*-3	0.21	0.67
C 20:0	0	0.05
C 20:1*n*-9	0.01	0.19
C 20:2*n*-6	0.02	0.26
C 20:3*n*-6	0	0.04
C 20:4*n*-6	0.01	0.09
C 22:5*n*-6	0	0.03
n-6/*n*-3	8.36	14.19
SFA (%)	23.11	32.78
MUFA (%)	29.75	35.97
n-6 PUFA (%)	42.11	29.21
n-3 PUFA (%)	5.03	2.04

CD, control diet; HFD, high-fat diet; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

2.2. Blood biochemical tests

Plasma levels of aspartate transaminase (AST) and alanine aminotransferase (ALT) were measured using the standardized procedures of the Japanese Society of Clinical Chemistry.

2.3. RNA extraction, complementary DNA synthesis, and reverse-transcription polymerase chain reaction (PCR) assay

Total RNA was extracted from liver using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The RNA concentration and purity were determined by measuring the absorbance at 260 and 280 nm. One microgram of total RNA extracted from each tissue sample was reverse transcribed to yield single-stranded complementary DNA using the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's protocols. Real-time quantitative PCR analysis (SYBR Green PCR Master Mix; Toyobo, Osaka, Japan) was performed using the MyiQ Real-Time PCR system (Bio-Rad). The real-time PCR conditions and primer sequences are listed in Table 2. All specific quantities were corrected for 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 specified threshold level. The ΔCt values were calculated in every sample for the target genes as follows: Ct (target gene) − Ct (internal control gene); GAPDH was used as the internal control gene. The relative expression level for 1 target gene (ΔΔCt) 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 $2^{-\Delta\Delta Ct}$.