LOX-1 is required for the adipose tissue expression of proinflammatory cytokines in high-fat diet-induced obese mice

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ABSTRACT

Lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) is a receptor for oxidized LDL, and is strongly expressed in endothelial cells at an early stage of atherosclerosis. LOX-1 expression in adipocytes is induced by PPAR(ligands and appears to be involved in adipocyte cholesterol metabolism. However, the role of adipose tissue LOX-1 in high-fat diet-induced obesity is unknown. We found that mRNA levels of adipose tissue LOX-1 were markedly increased in obese mice fed a high-fat diet (HFD) compared with those fed normal chow. The levels were closely correlated with those of a proinflammatory cytokine, monocyte chemoattractant protein-1 (MCP-1). Then, LOX-1 knockout (LOX-1-KO) and wild-type (WT) mice were fed HFD for 16 weeks. HFD feeding increased the body and mesenteric fat weights similarly in WT and LOX-1-KO mice. HFD-induced expressions of proinflammatory cytokines such as MCP-1, MIP-1α, and IL-6 were significantly less in LOX-1-KO than WT mice. Thus, LOX-1 is required for the HFD-induced expression of proinflammatory cytokines in the adipose tissue of obese mice.

Keywords:

oxidized LDL

obesity

inflammation

mesenteric fat

Obesity is one of the most common lifestyle-related diseases, and is closely associated with insulin resistance and glucose intolerance. Being accompanied by hypertension and dyslipidemia, it is also a component of metabolic syndrome and is involved in the development of atherosclerosis and cardiovascular events such as cerebral and myocardial infarction [1]. The chronic inflammation of adipose tissue caused by obesity is attracting attention as a possible mechanism of inducing such vascular diseases [2]. While some agents such as thiazolidinediones (TZDs) and statins have been reported to reduce this inflammation, a treatment directly targeting the adipose inflammation has yet been established [3, 4].

LOX-1 is a scavenger receptor [5] and is predominantly expressed by endothelial cells in early foci of atherosclerosis [6]. The expression of LOX-1 is known to be enhanced by not only oxidized LDL [7, 8], one of its ligands, but also angiotensin II [9], proinflammatory cytokines [10], and sheer stress [11]. Expressed LOX-1 takes up oxidized LDL and is thought to induce an inflammatory reaction of the vascular wall [6, 12]. Recently, relationship of LOX-1 with obesity has attracted attention. A high LOX-1 level in the urine of obese rats [13] and an increase in soluble LOX-1 in the plasma of obese women [14] have been reported. Also, LOX-1 expression in human adipose tissue has been reported to be positively correlated with the BMI and negatively with insulin sensitivity [15]. *In vitro* experiments have suggested that LOX-1 expression in adipocytes is induced by stimulation with the PPARγ ligand and is involved in cholesterol and fatty acid uptake by adipocytes [16]. To the present, however, there has been no detailed analysis on the relationship between adipose tissue inflammation and LOX-1 as a mechanism leading to the progression of obesity into cardiovascular diseases. In this study, we evaluated the expression of proinflammatory cytokines in the adipose tissue and glucose tolerance using obese and LOX-1 knockout (KO) mice fed a high-fat diet (HFD).

Materials and methods

Mice. Mice were maintained in a specific pathogen-free facility. All animal procedures were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee. Mice were given free access to water and normal chow (NC) or HFD under controlled light and temperature conditions. Our study protocol was approved by an ethical committee on animal experiments at Kyoto Medical Center, National Hospital Organization and at the Faculty of Medicine, Kyoto University.

Eight-week-old male C57BL/6 mice were placed on NC (4.8% of the total calories from fat) or HFD (45% of the total calories from fat; Research Diets, N.J., USA) for 8 weeks. To measure the plasma leptin level, blood samples were collected from the inferior vena cava using an Na-heparinized syringe under pentobarbital anesthesia. Blood was collected into a centrifuge tube and centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant was transferred to a new centrifuge tube and immediately stored at −80°C. Plasma leptin concentrations were determined using leptin ELISA kits (LINCO Research, Mo., USA). After the mice were sacrificed by exsanguination, mesenteric fat tissue samples were immediately frozen in liquid nitrogen and stored at −80°C.

Generation of the LOX-1-KO mouse has previously been described in detail [17]. The 6th to 8th exons of the LOX-1 gene were replaced with a neomycin-resistant gene. LOX-1-KO mice with a 129/SV background were backcrossed with a C57BL/6 background 8 times. Then, 8-week-old male LOX-1-KO and wild-type C57BL/6 (WT) mice were placed on NC (10% of the total calories from fat; Research Diets, N.J., USA) or HFD (45% of the total calories from fat; Research Diets, N.J., USA) for 16 weeks. Then, mice were sacrificed by exsanguination.

Quantitative real-time PCR. The mesenteric fat samples were homogenized in Trizol

(Invitrogen, CA, USA), and the total RNA was isolated from each sample according to the manufacturer's instructions. Total RNA was reverse-transcribed into cDNA using the SuperScript™ III First-Strand Synthesis System for RT-PCR (Invitrogen, CA, USA). cDNA was treated with RNase H. The primer sequences were as follows:

LOX-1 (forward) 5'- TCTTTGGGTGGCCAGTTACTACA-3',

(reverse) 5'- AACGCCCCTGGTCTTAAAGAA-3'.

leptin (forward) 5'-ATGTTCAAGCAGTGCCTATCCA-3',

(reverse) 5'-GGTGAAGCCCAGGAATGAAG-3'.

IL-6 (forward) 5'- ACCACGGCCTTCCCTACTTC-3',

(reverse) 5'- AGATTGTTTTCTGCAAGTGCATCA-3'.

Primer sequences for PPARγ [18], aP2 [18], MCP-1 [19], MIP-1α [20] and GAPDH [18] were previously described. The level of mRNA for each gene was normalized to the level of GAPDH detected in each sample. SYBR® Green PCR Master Mix (Applied Biosystems) was used to amplify and monitor cDNA. Amplification was performed as previously described [18].

Statistical analysis. Data are expressed as means ± SE. Differences between the groups were analyzed by ANOVA, followed by Fisher's PLSD post-hoc test for group comparisons. Linear regression analysis with Pearson's coefficients was performed to investigate correlations. $p < 0.05$ was considered significant.

Results

The mesenteric fat levels of LOX-1 in mice fed HFD increased in association with obesity

C57BL/6 mice were fed either HFD or NC for 8 weeks. HFD induced an increase in the body weight by 32% (NC: 25.5 ± 0.7 g, HFD: 33.6 ± 1.0 g, $p < 0.0001$) and in the mesenteric fat weight by 194% (NC: 150.4 ± 19.7 mg, HFD: 441.8 ± 39.3 mg, $p < 0.0001$) compared with NC. LOX-1 mRNA levels in mesenteric fat were examined by real-time PCR. LOX-1 expression levels were significantly (3.6-fold) increased in HFD compared to NC mice (Fig. 1Aa). HFD mice showed a high plasma leptin concentration (Fig. 1Ab), suggesting increased leptin resistance. Next, we examined whether the LOX-1 levels were correlated with the extent of obesity and plasma leptin concentration. LOX-1 expression levels exhibited significant, positive correlations with the body weight, mesenteric fat weight, and plasma leptin concentration (Fig. 1Ba, b and c). Thus, feeding mice HFD induced LOX-1 expression in association with obesity.

Fat expression levels of LOX-1 are closely correlated with those of MCP-1

We examined the mRNA expression levels of two adipocyte differentiation markers, peroxisome proliferator-activated receptor γ (PPARγ) and adipocyte fatty acid-binding protein-2 (aP2). PPARγ and aP2 mRNA expression levels were increased in HFD mice 1.7- and 2.9-fold, respectively (Fig. 2Aa and b). Next, we examined mRNA levels of a proinflammatory cytokine, monocyte chemoattractant protein-1 (MCP-1), which plays a role in macrophage infiltration [21-23]. MCP-1 mRNA levels were increased 2.9-fold in HFD compared with NC mice (Fig. 2Ac). mRNA levels of LOX-1 showed no correlation with adipocyte differentiation markers, PPARγ and aP2 (Fig. 2Ba and b). Notably, LOX-1 mRNA levels were strongly correlated with the

MCP-1 levels (Fig. 2Bc). These findings suggest that HFD-induced LOX-1 expression in mouse adipose tissue is associated with inflammation rather than adipocyte differentiation.

HFD-induced expression of proinflammatory cytokines is significantly lower in LOX-1-KO than WT mice

LOX-1-KO and WT mice were fed NC or HFD for 16 weeks. On HFD feeding, the body and mesenteric fat weights were increased similarly in WT and LOX-1-KO mice (Fig. 3Aa and b). The mesenteric fat expression levels of leptin and proinflammatory cytokines such as MCP-1, macrophage inflammatory protein-1α (MIP-1α), and interleukin-6 (IL-6) were markedly increased in WT mice fed HFD compared with WT mice fed NC (Fig. 3Ba, b, c and d). In LOX-1-KO mice, however, HFD feeding did not induce the expression of MCP-1 and IL-6. HFD feeding increased the leptin levels in LOX-1-KO mice similarly to those in WT mice (Fig. 3Ba). Notably, in HFD-induced obese mice, the mRNA levels of proinflammatory cytokines such as MCP-1, MIP-1α, and IL-6 in LOX-1-KO mice were significantly lower compared with the levels in WT mice (Fig. 3Bb, c and d). These findings suggest that LOX-1 is required for the HFD-induced expression of proinflammatory cytokines in the mesenteric fat of mice.

Discussion

LOX-1 gene is predominantly expressed in early lesions of atherosclerosis [6], but is also known to be expressed in adipocytes [15, 16]. We found that LOX-1 expression in the mouse mesenteric fat was increased in the HFD group. We also demonstrated that the fat LOX-1 level was positively correlated with the body weight, mesenteric fat weight, and plasma leptin concentration. These observations are in agreement with the previous report that subcutaneous fat LOX-1 expression was positively correlated with the BMI in humans [15]. In adipose tissue of obese individuals, an increase in number of small adipocytes is accompanied by hypertrophy of adipocytes [24]. Moreover, hypertrophied adipocytes release proinflammatory cytokines and induce adipose tissue inflammation [25, 26]. MCP-1, a proinflammatory cytokine, induces monocyte migration to adipose tissue and, as a result, facilitates macrophage infiltration into the adipose tissue [21-23]. MCP-1 expression was increased in the HFD group, and showed a close positive correlation with LOX-1 expression. In contrast, the expression of PPARγ and aP2, markers of adipocyte differentiation, had no correlation with that of LOX-1. These observations suggest that LOX-1 plays a more important role in adipose inflammation rather than in adipocyte differentiation. In the adipose tissue of WT mice fed an HFD for 16 weeks, expression of the proinflammatory cytokines MCP-1, MIP-1α, and IL-6 was increased, but these increases were significantly suppressed in LOX-1-KO compared with WT mice. These results suggest that LOX-1 is required for HFD-induced increases in the expression of these cytokines in the adipose tissue. The activation of LOX-1 in human vascular endothelial cells is known to induce MCP-1 expression via mitogen-activated protein kinase [27]. Further studies are necessary to elucidate precise mechanisms that link LOX-1 with MCP-1 expression in adipocytes.

The activation of LOX-1 is known to enhance the uptake of cholesterol and fatty acid in adipocytes [16]. The scavenger receptor CD36 is also known to be present in adipocytes and to be involved in their uptake [28-30]. In LOX-1-KO mice, the increase in the adipose tissue weight due to an HFD was similar to that in WT. Such scavenger receptors as CD36 might complement LOX-1 function of cholesterol and fatty acid uptake in adipocytes of LOX-1-KO mice.

Conclusions

LOX-1 expression in the adipose tissue of obese mice was closely correlated with the expression of MCP-1. Also, the expression of proinflammatory cytokines such as MCP-1, IL-6, and MIP-1α was suppressed by knocking out LOX-1. Thus, LOX-1 is considered to be required for adipose tissue inflammation in obesity.

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Figure legends

Fig. 1. The mesenteric fat levels of LOX-1 in high-fat diet-induced obese mice. A: Using real-time PCR, we measured mRNA levels of LOX-1 relative to GAPDH in mesenteric fat (panel a) from C57BL/6 mice fed normal chow (NC, n=10) or a high-fat diet (HFD, n=10) for 8 weeks. The plasma leptin concentration in these mice was measured using a leptin ELISA kit (panel b). Data represent the means ± SE. B: Correlations of LOX-1 mRNA levels with the body weight (panel a), mesenteric fat weight (panel b), and plasma leptin levels (panel c).

Fig. 2. Correlation between the expression levels of LOX-1 and those of adipocyte differentiation markers and MCP-1. A: Using real-time PCR, we measured mRNA expression levels of adipocyte differentiation markers, PPARγ (panel a) and aP2 (panel b), and a proinflammatory cytokine, MCP-1 (panel c) in the mesenteric fat of C57BL/6 mice fed normal chow (NC, n=10) or a high-fat diet (HFD, n=10). Data represent the means ± SE. B: Linear regression analysis of the correlations of the LOX-1 levels with the PPARγ (panel a), aP2 (panel b), and MCP-1 (panel c) levels.

Fig. 3. Expression levels of inflammation markers in wild-type and LOX-1 knockout mice fed normal chow or a high-fat diet. A: Body (panel a) and mesenteric fat (panel b) weights of wild-type (WT, C57BL/6) and LOX-1 knockout (KO) mice fed normal chow (NC, n=8) or a high-fat diet (HFD, n=10) for 16 weeks. B: Using real-time PCR, we measured mRNA expression levels of leptin (panel a) and proinflammatory genes, MCP-1 (panel b), MIP-1α (panel c), and IL-6 (panel d), in the mesenteric fat of WT and LOX-1 KO mice fed NC (n=8) or HFD (n=10) for 16 weeks. Data represent the means ± SE.

Fig. 1

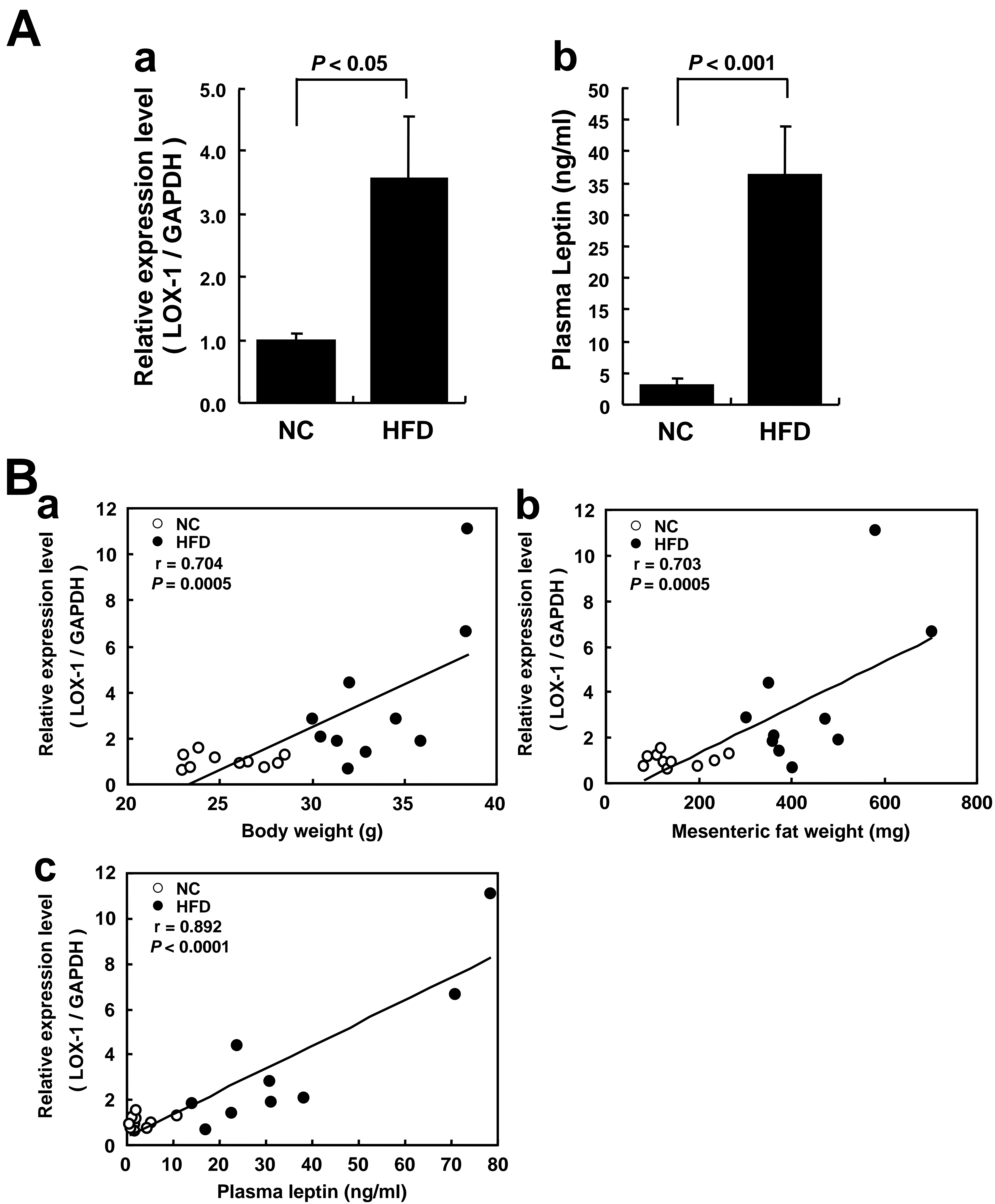
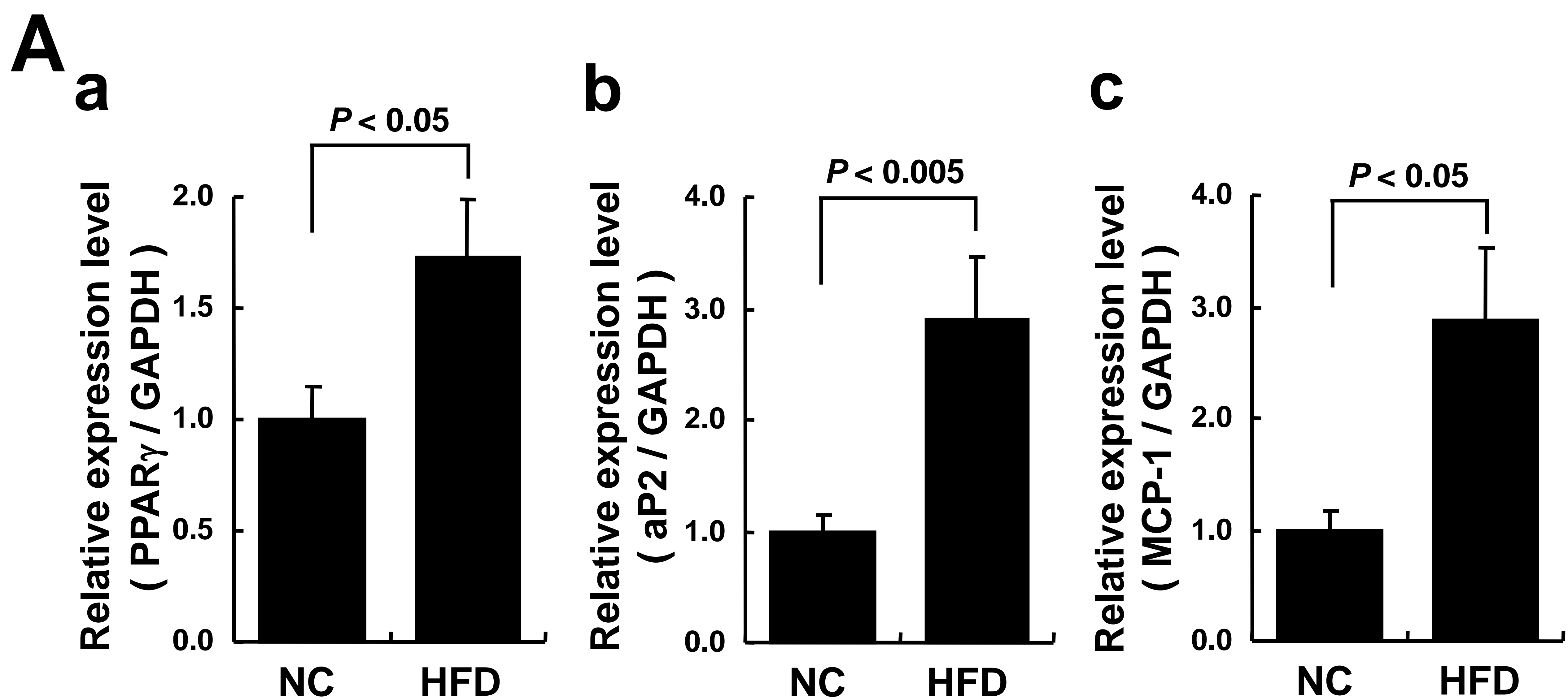


Fig. 2



B
a
Relative expression level
(LOX-1 / GAPDH)
NC
HFD
r = 0.170
P = 0.4739
Relative expression level
(PPARγ / GAPDH)

b
Relative expression level
(LOX-1 / GAPDH)
NC
HFD
r = 0.072
P = 0.7627
Relative expression level
(aP2 / GAPDH)

c
Relative expression level
(LOX-1 / GAPDH)
NC
HFD
r = 0.909
P = 0.0001
Relative expression level
(MCP-1 / GAPDH)

Fig. 3