Bioscience of Microbiota, Food and Health Vol. 41 (2), 54–65, 2022

Full Paper

Local free fatty acids trigger the expression of lipopolysaccharide-binding protein in murine white adipose tissue

Manami SEKI[1], Akiho MIWA[1], Fumina OHSAKA[2], Yugo KARATSU[2], Takeshi TSURUTA[3], Shingo HINO[4], Tatsuya MORITA[4] and Kei SONOYAMA[5*]

[1]*Graduate School of Life Science, Hokkaido University, Kita-9, Nishi-9, Kita-ku, Sapporo 060-8589, Japan*
[2]*Graduate School of Agriculture, Hokkaido University, Kita-9, Nishi-9, Kita-ku, Sapporo 060-8589, Japan*
[3]*Graduate School of Environmental and Life Science, Okayama University, 2-1-1 Tsushima-Naka, Kita-ku, Okayama-shi, Okayama 700-8530, Japan*
[4]*College of Agriculture, Academic Institute, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka-shi, Shizuoka 422-8529, Japan*
[5]*Research Faculty of Agriculture, Hokkaido University, Kita-9, Nishi-9, Kita-ku, Sapporo 060-8589, Japan*

Received September 8, 2021; Accepted December 26, 2021; Published online in J-STAGE January 28, 2022

Although lipopolysaccharide (LPS)-binding protein (LBP) is an acute-phase protein mainly produced by hepatocytes, it has also been proposed to be a pro-inflammatory adipokine. Obesity and the consumption of a high-fat diet (HFD) are reportedly associated with elevated levels of LPS in plasma and free fatty acids (FFAs) in white adipose tissue (WAT). We examined whether circulating LPS or local FFAs are responsible for the HFD-induced increase of LBP in WAT. Male C57BL/6J mice were fed either a normal-fat diet (NFD) or an HFD. The mRNA levels in the liver and mesenteric WAT (mWAT), total FFA content in mWAT, and LBP and LPS concentrations in plasma were determined. The *Lbp* mRNA level in mWAT was higher in mice fed the HFD than in those fed the NFD for 3, 7, or 28 days or 14 weeks, whereas the hepatic *Lbp* mRNA level did not differ between the groups. The *Lbp* mRNA level in mWAT was also increased by the HFD in germ-free mice, which do not have gut microbiota, the source of LPS. The plasma LPS level did not show a significant correlation with the mWAT *Lbp* mRNA level. The total FFA content in mWAT was higher in mice fed the HFD than in those fed the NFD and positively correlated with the *Lbp* mRNA level. Supplementation with palmitic acid increased the *Lbp* mRNA level in 3T3-L1 adipocytes. We propose that local FFAs, but not circulating LPS, are the trigger for increased *Lbp* expression in mWAT of mice fed the HFD.

Key words: lipopolysaccharide-binding protein, high-fat diet, white adipose tissue, inflammation

INTRODUCTION

Lipopolysaccharide (LPS)-binding protein (LBP) is an acute-phase circulating protein that is mainly produced by hepatocytes [1]. Moreno-Navarrete et al. found that the circulating LBP concentration is associated with obesity-related insulin resistance and systemic inflammatory markers in humans and mice [2]. They also demonstrated in humans and mice that LBP is produced by adipocytes and that the expression of the *Lbp* gene, which encodes LBP, is promoted by excessive fat accretion and associated with inflammation in white adipose tissue (WAT) [3]. Furthermore, they showed that knockdown of the *Lbp* gene in murine 3T3-L1 adipocytes reduces inflammatory phenotypes, such as the expression of interleukin (IL)-6 and monocyte chemoattractant protein-1 (MCP-1) [4]; thus, they proposed that LBP is a novel pro-inflammatory adipokine.

It has been reported that an elevated circulating LPS level is associated with the consumption of a high-fat diet (HFD) and obesity-related metabolic disorders [5]; therefore, it is natural to consider that elevated levels of circulating LPS, a ligand of LBP, may be responsible for the HFD- and obesity-related increase of *Lbp* expression in WAT. Indeed, the administration of LPS has been reported to increase *Lbp* expression in mesenteric WAT (mWAT) of mice [3]. In addition, free fatty acids (FFAs) may be another candidate trigger for the increased *Lbp* expression in WAT. Lee et al. showed that the total FFA levels within WAT

*Corresponding author. Kei Sonoyama (E-mail: ksnym@chem.agr.hokudai.ac.jp)
(Supplementary materials: refer to PMC https://www.ncbi.nlm.nih.gov/pmc/journals/2480/)

doi: 10.12938/bmfh.2021-061

were elevated at 3 days, 7 days, and 15 weeks of HFD feeding in mice [6]. Similar to LPS, FFAs induce adipocyte inflammation through the toll-like receptor 4 (TLR4)-nuclear factor κB (NF-κB) pathway [7]. In addition, local FFAs contribute to WAT inflammation by inducing WAT hypoxia, which occurs early (at around 3 days) in the course of HFD feeding in mice [6]. The present study thus examined whether circulating LPS or local FFAs are the trigger for the HFD- and obesity-related increase of *Lbp* expression in WAT.

MATERIALS AND METHODS

Animals and diets

All study protocols were pre-approved by the Animal Use Committee of Hokkaido University (approval no. 14-0028 and 19-0017), and all mice were maintained in accordance with the guidelines for the care and use of laboratory animals of Hokkaido University. Male specific-pathogen-free and germ-free (GF) C57BL/6J mice (age, 5 weeks) were purchased from Japan SLC (Hamamatsu, Japan) and housed in standard plastic cages in a temperature-controlled (21 to 25°C) room under a 12-hr light-dark cycle. They were allowed free access to food and water. The mice were acclimatized for 1 week with a normal-fat diet (NFD; D12450B, Research Diets, New Brunswick, NJ, USA). The specific-pathogen-free mice were kept under conventional (CV) conditions, whereas GF mice were maintained under GF conditions in a plastic isolator and fed irradiated diet and autoclaved water.

Experimental design

In experiment 1, mice were allocated to two groups (n=18 in each group) such that the average body weights of the two groups were similar and fed either the NFD or HFD (D12492, Research Diets) *ad libitum*. The first day of feeding of the test diets was referred to as day 0. On days 1, 7, and 28, six mice from each group were anesthetized by inhalation of sevoflurane without prior fasting and then euthanized by cardiac puncture. Plasma was separated from the blood samples and used for the measurement of LPS and LBP as described below. After a laparotomy, the liver, mWAT, and inguinal WAT (iWAT) were excised, weighed, and stored at −80°C for the measurement of LBP and total FFA and mRNA expression analysis as described below.

In experiment 2, mice were allocated to two groups (n=6 in each group) such that the average body weights of the two groups were similar and fed either the NFD or HFD *ad libitum*. After 13 weeks of feeding, the oral glucose tolerance test (OGTT) was performed as described below. One week later, the mice were euthanized without prior fasting, and plasma and tissue samples were obtained as described in experiment 1.

In experiment 3, mice kept under CV conditions (CV mice, n=12) and GF mice (n=12) were allocated to two groups (n=6 in each group) such that the average body weights of the two groups were similar and fed either the NFD or HFD *ad libitum*. On day 3, the mice were euthanized without prior fasting, and plasma and tissue samples were obtained as described in experiment 1.

In experiment 4, mice were allocated to two groups (n=12 in each group) such that the average body weights of the two groups were similar and fed either the NFD or HFD *ad libitum*. The mice in each group were further divided into two groups (n=6 in each group) and intraperitoneally administered daily either carboxyatractyloside potassium salt (CATr; 1 mg/kg body weight; Cayman Chemical, Ann Arbor, MI, USA), which is an inhibitor of adenine nucleotide translocase (ANT), or vehicle (phosphate-buffered saline; PBS). On day 7, without prior fasting, mice were intraperitoneally administered pimonidazole hydrochloride (60 mg/kg body weight; Hypoxyprobe-1 Omni Kit, Hypoxyprobe, Burlington, MA, USA) and then euthanized 60 min later, and plasma and tissue samples were obtained as described in experiment 1. In addition, mWAT was also subjected to immunohistochemistry to detect pimonidazole adducts as described below.

OGTT

Following a 16-hr fast, mice were intragastrically administered 150 mg/mL glucose in PBS at a dose of 1.5 g/kg body weight. Retro-orbital blood sampling was performed just before and 30, 60, 90, and 120 min after glucose administration. The plasma was separated by centrifugation, and the glucose concentration was measured with a Glucose CII-Test (Wako Pure Chemical, Osaka, Japan). Plasma samples were also used for the measurements of insulin, total cholesterol (TC), triacylglycerol (TG), total FFAs, and glycerol using an LBIS Mouse Insulin ELISA Kit (Fujifilm Wako Pure Chemical, Osaka, Japan), Cholesterol E-Test (Wako Pure Chemical), Triglyceride E-Test (Wako Pure Chemical), NEFA C-Test (Wako Pure Chemical), and Glycerol Assay Kit (BioAssay Systems, Hayward, CA, USA), respectively, according to the manufacturers' instructions.

Measurement of plasma LPS and LBP

Plasma LPS levels were determined by the limulus amebocyte lysate test, which was performed according to the Japanese Pharmacopoeia 17th Edition (https://www.mhlw.go.jp/stf/seisakunitsuite/bunya/0000066597.html); it involved a turbidimetric time assay at 450 nm with an ET-2000 Toxinometer (Wako Pure Chemical) [8]. The plasma sample was diluted ten-fold with sterile water for injection (Otsuka Pharmaceutical Factory, Tokushima, Japan) and heated at 80°C for 5 min to deactivate the LBP and CD14. The sample was then mixed with limulus reagent (Wako Pure Chemical) and subjected to the Toxinometer analysis. Endotoxin prepared from *Escherichia coli* O113:H10 (Wako Pure Chemical) was used as the standard. Plasma LBP levels were measured using a Mouse LBP PicoKine ELISA Kit (Boster Biological Technology, Wuhan, China) according to the manufacturer's instructions.

Measurement of WAT FFAs

Total lipids were extracted from mWAT using a Lipid Extraction Kit (Cell Biolabs, San Diego, CA, USA) and suspended in isopropanol according to the manufacturer's instructions. The total FFA concentration in the preparation was then determined using a NEFA C-Test according to the manufacturer's instructions.

Measurement of tissue LBP

Liver tissue and mWAT samples were homogenized in a buffer composed of 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 1% (w/v) Triton X-100, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) sodium dodecyl sulfate, and cOmplete protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany). After incubation on ice for 60 min, the homogenate was centrifuged at 10,000 × g for 15 min, and the

doi: 10.12938/bmfh.2021-061

LBP and total protein concentrations in the supernatant were measured using a Mouse LBP PicoKine ELISA Kit and Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Indianapolis, IN, USA), respectively, according to the manufacturers' instructions. The LBP concentration in the culture supernatant of 3T3-L1 adipocytes cultured as described below was also measured using the same kit.

Messenger RNA expression analysis

Messenger RNA expression was analyzed as previously described [9]. Total RNA was isolated from liver tissue, mWAT samples, and cultured cells using a ReliaPrep RNA Tissue Miniprep System (Promega Japan, Tokyo, Japan) according to the manufacturer's instructions. The RNA concentration was monitored with a spectrophotometer (NanoDrop Lite, Thermo Fisher Scientific) by absorbance at 260 nm (A260). An A260 of 1.0 was considered 40 μg/mL of the extracted RNA. The ratio of A260 to A280 nm was measured, and the samples with a ratio of 1.8 to 2.0 were used for reverse transcription. Total RNA (1 μg) was reverse transcribed to generate first-strand cDNA using ReverTra Ace qPCR RT Master Mix (Toyobo, Osaka, Japan) according to the manufacturer's instructions. The reaction was run in a thermal cycler (Life ECO, Bioer Technology, Hangzhou, China) with a thermal profile of 37°C for 15 min followed by 5 min at 95°C, and the obtained cDNA was stored at −20°C for subsequent polymerase chain reaction (PCR) reactions. Murine LBP, hormone-sensitive lipase (HSL), adipose TG lipase (ATGL), MCP-1, C-X-C motif chemokine ligand 8 (CXCL8), IL-1β, IL-6, tumor necrosis factor α (TNF-α), F4/80, hypoxia-inducible factor-1α (HIF-1α), glyceraldehyde-3-phosphate dehydrogenase, and hypoxanthine phosphoribosyltransferase (HPRT) are encoded by the *Lbp*, *Lipe, Pnpla2, Ccl2, Cxcl8, Il1b, Il6, Tnf, Adgre1, Hif1a, Gapdh*, and *Hprt* genes, respectively. To compare the steady-state levels of these mRNAs, quantitative real-time PCR (qRT-PCR) was performed using GeneAce SYBR qPCR Mix α No ROX (Nippon Gene, Toyama, Japan) with a Thermal Cycler Dice TP800 (Takara Bio, Otsu, Japan). The thermal profile was adjusted to denaturation at 95°C for 10 min, followed by 45 cycles of denaturation at 95°C for 30 sec, and then annealing and extension at 60°C for 60 sec. A melting curve analysis was performed after amplification to assess the specificity of qRT-PCR. The data were calculated by the $2^{-\Delta\Delta}$Ct method with the geometric mean of two endogenous reference genes, i.e., *Gapdh* and *Hprt*. RT-qPCR was carried out in duplicate. The method recommended in Minimum Information for Publication of Quantitative Real-Time PCR Experiments was strictly followed [10]. The sequences of primers used for RT-qPCR are described in Supplementary Table 1.

Immunohistochemistry

Immunohistochemistry to detect pimonidazole adducts in mWAT was performed as previously described [11]. Pimonidazole adducts were detected using anti-pimonidazole rabbit antibody (Hypoxyprobe-1 Omni Kit) according to the manufacturer's instructions. Slides were counterstained with ProLong Gold Antifade Reagent with DAPI (Life Technologies, Carlsbad, CA, USA) and viewed under a fluorescence microscope (BX40, Olympus, Tokyo, Japan).

Cell culture experiments

The murine preadipocyte cell line 3T3-L1 was obtained from the RIKEN BioResource Center (Tsukuba, Japan). The culturing and differentiation of 3T3-L1 preadipocytes into adipocytes were performed according to the method of Frost and Lane [12]. The cells were used as differentiated adipocytes 10 days after the induction of differentiation. Adipocytic differentiation was confirmed by monitoring cells for lipid droplet formation under phase-contrast microscopy (CKX41N-31PHP, Olympus; data not shown). Cultured differentiated 3T3-L1 adipocytes were supplemented with either a graded concentration (0, 0.1, 1.0, and 10 ng/mL) of LPS from *Salmonella enterica* serovar Abortusequi (L-5886, Sigma, St Louis, MO, USA) or a graded concentration (0, 250, 500, 1,000, and 2,000 μM) of palmitic acid (Sigma) conjugated with fatty acid-free bovine serum albumin (Wako Pure Chemical) for 24 hr. Additionally, cultured differentiated 3T3-L1 adipocytes were supplemented with a graded concentration (0, 250, 500, and 1,000 ng/mL) of recombinant murine LBP (RPB406Mu01, Cloud-Clone, Wuhan, China) for 24 hr. Cells were then harvested, and total RNA was isolated and analyzed as described above. Cell culture supernatant was subjected to LBP measurement as described above. The LPS concentration in the medium was measured as described above.

Statistical analyses

The sample size was calculated based on the experimental design (two-way analysis of variance [ANOVA] in experiments 1, 3, and 4 and Welch's t-test in experiment 2), and the mWAT *Lbp* mRNA level was determined as the primary outcome measure. We used the G*Power software (version 3.1.9.4) [13] for the power analysis with an α probability of 0.05 and a power of 0.80, and the effect size was estimated based on the results from preliminary experiments (unpublished results). Hence, the required sample size was six per group in all experiments.

Results are presented as standard box plots showing the median and interquartile range with the minimum and maximum or as the mean and standard error. The mean values of two groups were compared using Welch's t-test. To compare the mean values of three or more groups, one-way or two-way ANOVA was used. Dunnett's multiple comparison test was applied when a significant influence was found by one-way ANOVA. The Tukey-Kramer *post hoc* test was applied when a significant interaction was found by two-way ANOVA. The correlations between the mWAT *Lbp* mRNA level and mWAT total FFA content or plasma LPS concentration and between the plasma LBP level and mWAT *Lbp* mRNA level or plasma LPS level were evaluated using Spearman's correlation coefficient (r). Data were analyzed using the GraphPad Prism software for Macintosh (version 8, GraphPad Software, San Diego, CA, USA). P values of <0.05 were considered to be statistically significant.

Results

The HFD-induced increase of Lbp gene expression in mWAT was associated with the local FFA content (experiment 1)

Experiment 1 was performed to investigate the time-course changes in *Lbp* gene expression in the mice fed the NFD or HFD. Although no difference in final body weight was observed between the two groups at 1 day, final body weight was

significantly higher in the HFD-fed mice than in the NFD-fed mice at 7 and 28 days (Table 1). The HFD-fed mice at 28 days showed a significantly higher mWAT weight than the NFD-fed mice. No difference in liver weight was observed. The results of two-way ANOVA for tissue mRNA and plasma LBP levels are summarized in Supplementary Table 2. The *Lbp* mRNA level in the liver was the same in the NFD- and HFD-fed mice throughout the experimental period (Fig. 1A). In contrast, the *Lbp* mRNA level in mWAT was significantly higher in the HFD-fed mice than in the NFD-fed mice at 7 and 28 days (Fig. 1B). Because a significant difference in the *Lbp* mRNA level in mWAT was observed between the NFD- and HFD-fed mice at 7 days, the LBP and FFA concentrations were measured in the mWAT and liver at 7 days. Although the hepatic LBP concentration was the same in the NFD- and HFD-fed mice (Fig. 1C), the HFD-fed mice showed a significantly higher mWAT LBP concentration than the NFD-fed mice (Fig. 1D). The total FFA content in mWAT was significantly higher in the HFD-fed mice than in the NFD-fed mice at 7 days (Fig. 1E). We observed no detectable LPS in the plasma. The plasma LBP concentration did not differ between the two groups throughout the experimental period (Fig. 1F). The HFD-fed mice showed a significantly higher *Lipe* mRNA level than the NFD-fed mice in mWAT (Fig. 1G), whereas HFD feeding had no significant influence on the *Pnpla2* mRNA level (Fig. 1H). The HFD-fed mice showed significantly higher *Ccl2* and *Cxcl8* mRNA levels (Fig. 1I and 1J, respectively) than the

Table 1. Mouse body and tissue weights in experiments 1–4

	Body weight (g)		Liver weight (mg/g BW)		mWAT weight (mg/g BW)	
	Mean	SE	Mean	SE	Mean	SE
Experiment 1						
1 day						
NFD	21.1	0.2	47.5	1.9	4.6	0.3
HFD	21.4	0.4	45.4	0.9	3.4*	0.2
7 days						
NFD	21.0	0.4	47.8	3.8	5.0	0.5
HFD	22.7*	0.3	45.7	1.0	6.1	0.4
28 days						
NFD	22.7	0.5	41.1	3.9	3.3	0.5
HFD	28.8*	1.0	37.4	1.1	7.4*	0.6
Experiment 2						
NFD	30.8	0.8	40.4	1.2	9.6	0.3
HFD	50.6*	1.1	49.7*	2.9	32.6*	1.6
Experiment 3						
CV mice						
NFD	19.4	0.3	61.2	1.1	2.3^{c}	0.2
HFD	21.8	0.5	56.3	1.5	6.2^{a}	0.3
GF mice						
NFD	18.9	1.2	54.5	1.7	1.7^{c}	0.2
HFD	23.0	0.6	53.3	1.3	3.3^{b}	0.2
Two-way ANOVA						
Microbiota	p=0.7082		p=0.0055		p<0.0001	
Diet	p=0.0006		p=0.0627		p<0.0001	
Interaction	p=0.3068		p=0.2601		p=0.0002	
Experiment 4						
PBS-treated mice						
NFD	20.3	0.5			5.2	0.5
HFD	21.4	0.6			6.6	0.9
CATr-treated mice						
NFD	20.1	0.5			3.8	0.3
HFD	21.3	0.4			6.0	0.5
Two-way ANOVA						
Diet	p=0.0339				p=0.0064	
CATr	p=0.7695				p=0.1065	
Interaction	p=0.9221				p=0.5067	

NFD: normal-fat diet; HFD: high-fat diet; CV: conventional; GF: germ-free; PBS: phosphate-buffered saline; CATr: carboxyatractyloside potassium salt. *p<0.05 vs. NFD using the Welch's t-test. The Tukey–Kramer *post hoc* test was applied when a significant interaction was found by two-way ANOVA. Mean values with different superscript letters are significantly different by Tukey–Kramer *post hoc* test (p<0.05).

doi: 10.12938/bmfh.2021-061

NFD-fed mice. HFD feeding had no significant influence on the *Il1b*, *Il6*, *Tnf,* and *Adgre1* mRNA levels (Fig. 1K, 1L, 1M, and 1N, respectively). A significant positive correlation was observed between the total FFA content and the *Lbp* mRNA level in mWAT (Fig. 1O). Similar to mWAT at 7 days, the *Lbp* mRNA level and total FFA content in iWAT were significantly higher in the HFD-fed mice than in the NFD-fed mice at 7 days (Supplementary Fig. 1A and 1B, respectively), and a significant positive correlation was observed between the total FFA content and the *Lbp* mRNA level in iWAT (Supplementary Fig. 1C).

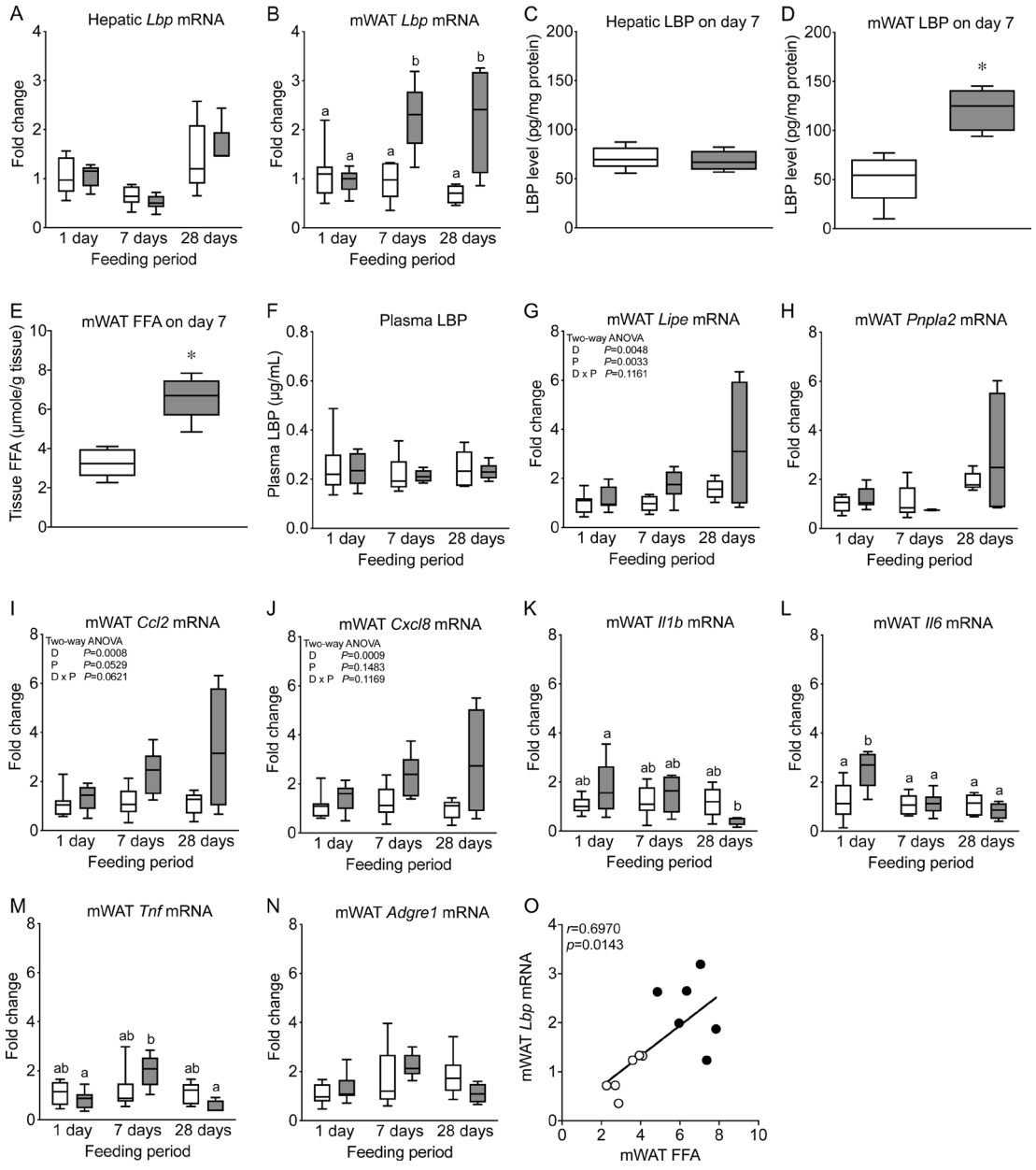

Fig. 1. The *Lbp* gene expression level in mesenteric white adipose tissue (mWAT) is associated with the local free-fatty acid (FFA) content in male C57BL/6J mice fed the normal-fat diet (NFD) or high-fat diet (HFD). (A and B) *Lbp* mRNA levels in the liver and mWAT, respectively. (C and D) Lipopolysaccharide-binding protein (LBP) concentrations in the liver and mWAT at 7 days, respectively. (E) Total FFA content in mWAT at 7 days. (F) Plasma LBP level. (G–N) Messenger RNA levels of lipolytic enzyme and adipokine genes in mWAT. (O) Relationship between the total FFA content and the *Lbp* mRNA level in mWAT at 7 days. In panels A, B, and G–N, data are shown relative to the levels in the NFD-fed mice at 1 day, which were set to 1. In panels A–N, results are presented as standard box plots showing the median and interquartile range with the minimum and maximum (n=6). White and gray boxes represent the NFD- and HFD-fed mice, respectively. The results of two-way ANOVA for panels A, B, and F–N are summarized in Supplementary Table 2, whereas p values for two-way ANOVA are shown in panels G, I, and J, with D, P, and D × P representing diet, period, and the interaction of diet and period, respectively. The Tukey–Kramer *post hoc* test was applied when a significant interaction was found by two-way ANOVA, and mean values with different superscript letters are a significantly different ($p<0.05$). In panels C–E, mean values were compared between the groups by Welch's t-test. *$p<0.05$ vs. NFD. In panel O, open and closed circles represent mice fed the NFD and HFD, respectively, and Spearman's correlation coefficient (r) was used to evaluate the relationships.

doi: 10.12938/bmfh.2021-061

The increase of Lbp gene expression in mWAT was associated with the local FFA content, but not the circulating LPS, in mice with obesity-related metabolic disorders (experiment 2)

Experiment 2 investigated the *Lbp* gene expression in mice with obesity-related metabolic disorders induced by chronic feeding of the HFD. After consuming the test diets for 14 weeks, the final body weight, liver weight, and mWAT weight were significantly higher in the HFD-fed mice than in the NFD-fed mice (Table 1). In the OGTT performed after 13 weeks of feeding, the plasma glucose concentration was significantly higher in the HFD-fed mice than in the NFD-fed mice at 0, 60, and 120 min after glucose administration, and the area under the curve was also significantly higher in the HFD-fed mice than in the NFD-fed mice (Fig. 2A). The fasting plasma insulin concentration was significantly higher in the HFD-fed mice than in the NFD-fed mice after 13 weeks of feeding (Table 2). Under non-fasting conditions, the plasma

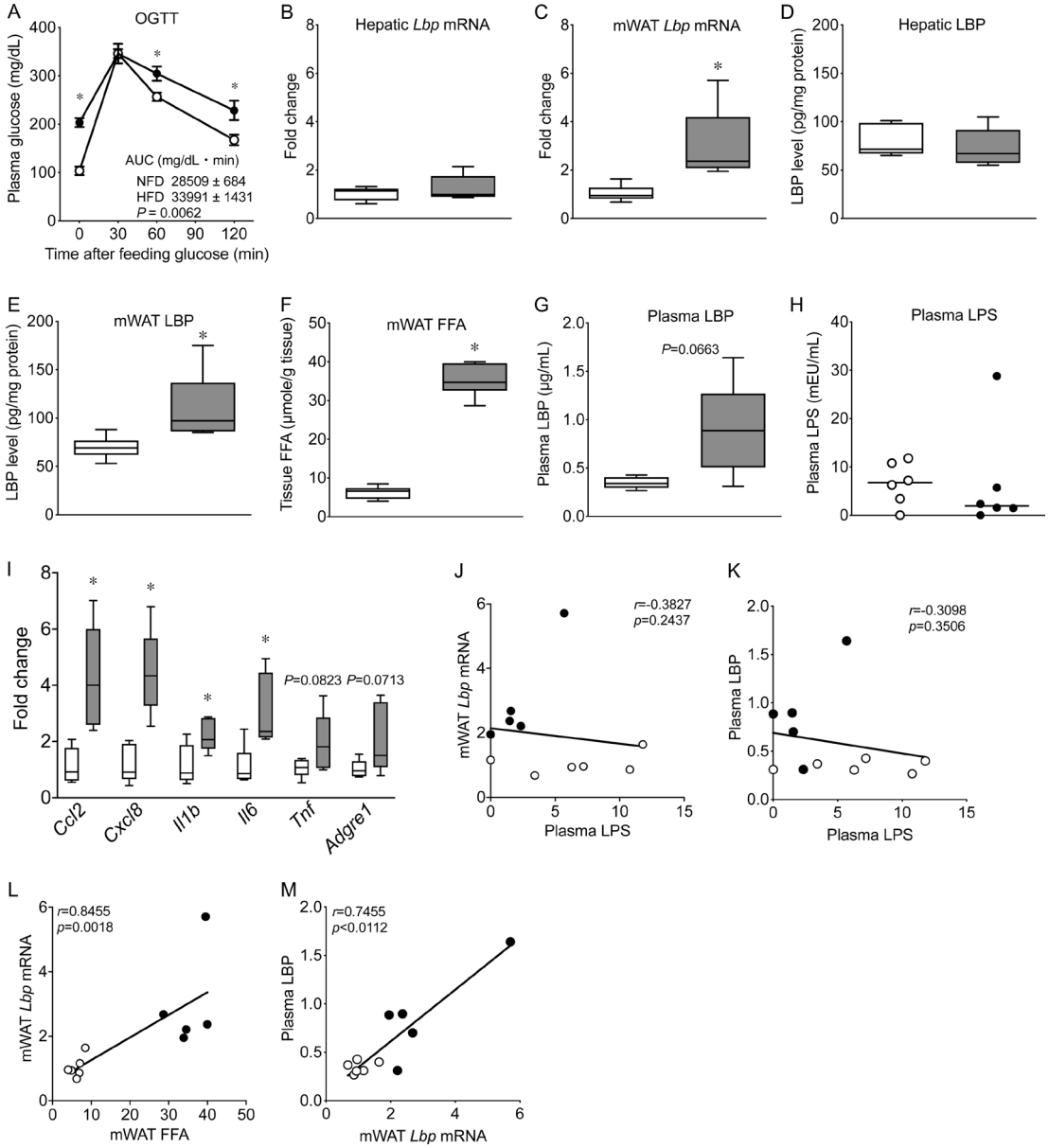

Fig. 2. The *Lbp* gene expression level in mesenteric white adipose tissue (mWAT) is associated with the local free-fatty acid (FFA) content, but not the circulating lipopolysaccharide (LPS), in male C57BL/6J mice fed a normal-fat diet (NFD) or high-fat diet (HFD) for 14 weeks. (A) Oral glucose tolerance test (OGTT). (B and C) *Lbp* mRNA levels in the liver and mWAT, respectively. (D and E) LBP concentrations in the liver and mWAT, respectively. (F) Total FFA content in mWAT. (G and H) Plasma LBP and LPS levels, respectively. (I) Messenger RNA levels of adipokine genes in mWAT. In panels B, C, and I, data are shown relative to the levels in the NFD-fed mice, which were set to 1. In panel A, results are presented as the mean ± SEM (n=6), and open and closed circles represent the mice fed the NFD and HFD, respectively. In panels B–G and I, results are presented as standard box plots showing the median and interquartile range with the minimum and maximum (n=6). White and gray boxes represent the NFD- and HFD-fed mice, respectively. In panel H, open and closed circles represent individual mice fed the NFD and HFD, respectively, and horizontal bars represent the mean values. Mean values were compared between groups by Welch's t test, and the Mann-Whitney test was used in panel H. *p<0.05 vs. NFD. In panels J–M, open and closed circles represent mice fed the NFD and HFD, respectively, and Spearman's correlation coefficient (*r*) was used to evaluate the relationships.

doi: 10.12938/bmfh.2021-061

Table 2. Biochemical parameters of the plasma in mice fed a normal-fat diet (NFD) or high-fat diet (HFD) in experiment 2

	NFD		HFD	
	Mean	SE	Mean	SE
Insulin (ng/mL)	0.25	0.09	2.55*	0.33
TC (mg/dL)	86	7	151*	11
TG (mg/dL)	87	11	74	7
FFA (mEq/L)	0.94	0.09	0.82	0.07
Glycerol (μmole/L)	324	24	379	28

NFD: normal-fat diet; HFD: high-fat diet; TC: total cholesterol; TG: triacylglycerol; FFA: free fatty acid. Fasting plasma insulin was measured at 13 weeks, whereas TC, TG, FFA, and glycerol were measured at 14 weeks without prior fasting. *p<0.05 vs. NFD by Welch's t-test.

TC concentration was significantly higher in the HFD-fed mice than in the NFD-fed mice after 14 weeks of feeding, whereas no difference was observed in the plasma TG, total FFA, and glycerol concentrations between the groups. After 14 weeks of feeding, the hepatic *Lbp* mRNA level was the same in the two groups (Fig. 2B), whereas the *Lbp* mRNA level was significantly higher in the WAT of the HFD-fed mice than in the WAT of the NFD-fed mice (Fig. 2C). Although the hepatic LBP concentration was the same in the NFD- and HFD-fed mice (Fig. 2D), the HFD-fed mice showed a significantly higher mWAT LBP concentration than the NFD-fed mice (Fig. 2E). The total FFA content in mWAT was significantly higher in the HFD-fed mice than in the NFD-fed mice (Fig. 2F). The LBP concentration in plasma tended to be higher in the HFD-fed mice than in the NFD-fed mice (Fig. 2G), whereas no difference in the plasma LPS level was observed between the groups (Fig. 2H). The *Ccl2*, *Cxcl8*, *Il1b,* and *Il6* mRNA levels in mWAT were significantly higher in the HFD-fed mice than in the NFD-fed mice, and the *Tnf* and *Adgre1* mRNA levels also tended to be higher in the HFD-fed mice (Fig. 2I). The plasma LPS level did not show a significant correlation with the mWAT *Lbp* mRNA level and plasma LBP level (Fig. 2J and 2K, respectively), whereas the mWAT *Lbp* mRNA level showed a significant positive correlation with the mWAT FFA content and plasma LBP level (Fig. 2L and 2M, respectively).

The HFD-induced increase of Lbp gene expression in mWAT was apparent in GF mice (experiment 3)

Experiment 3 examined whether the HFD-induced increase of *Lbp* gene expression in mWAT requires gut microbiota. Three days of HFD feeding significantly increased the final body weight as compared with NFD feeding in both the CV and GF mice, whereas the presence of gut microbiota did not influence the final body weight (Table 1). The liver weight was significantly lower in the GF mice than in the CV mice. The HFD-fed mice showed a significantly higher mWAT weight than the NFD-fed mice in both the CV and GF mice, and the mWAT weight was significantly higher in the CV mice fed the HFD than in the GF mice fed the HFD. The results of two-way ANOVA for tissue mRNA, mWAT FFA, and plasma LBP and LPS levels are summarized in Supplementary Table 3. The two diets and the presence of gut microbiota did not influence the hepatic *Lbp* mRNA level (Fig. 3A). The *Lbp* mRNA level (Fig. 3B) and total FFA content (Fig. 3C) in mWAT were significantly higher in the HFD-fed mice than in the NFD-fed mice in both the CV and GF mice, and the *Lbp* mRNA level was significantly lower in the HFD-fed GF mice than in the HFD-fed CV mice. The two diets and the presence of gut microbiota did not influence the plasma LBP level (Fig. 3D). No plasma LPS was detected in the GF mice and the NFD-fed CV mice, and the plasma LPS level was significantly higher in the HFD-fed CV mice than in the other three groups (Fig. 3E). Three days of HFD feeding significantly increased the *Ccl2* and *Cxcl8* mRNA levels (Fig. 3F and 3G, respectively) in mWAT as compared with NFD feeding in both the CV and GF mice. No differences in the *Il1b*, *Il6,* and *Adgre1* mRNA levels (Fig. 3H, 3I, and 3K, respectively) in mWAT were observed between the NFD- and HFD-fed mice in either the CV or GF mice. In the CV mice, the *Tnf* mRNA levels in mWAT were significantly higher in the HFD-fed mice than in the NFD-fed mice, whereas no difference was observed between the NFD- and HFD-fed GF mice (Fig. 3J). A significant positive correlation was observed between the total FFA content and the *Lbp* mRNA level in mWAT (Fig. 3L).

Inhibition of hypoxia did not affect the HFD-induced increase of Lbp gene expression in mWAT (experiment 4)

Experiment 4 examined whether hypoxia contributes to the HFD-induced increase of *Lbp* gene expression in mWAT. In comparison to NFD feeding, 7 days of HFD feeding significantly increased the final body weight and mWAT weight in both the PBS- and CATr-treated mice; however, no significant interaction was seen between CATr administration and these parameters (Table 1). Immunohistochemistry of mWAT showed a positive signal for pimonidazole adducts in the PBS-treated and HFD-fed mice, whereas only faint signals were observed in the NFD-fed mice and the CATr-treated and HFD-fed mice (Fig. 4A). In both the PBS- and CATr-treated mice, the total FFA content (Fig. 4B) and the *Lipe*, *Lbp*, and *Ccl2* mRNA levels (Fig. 4D, 4F, and 4G, respectively) in mWAT were significantly higher in the HFD-fed mice than in the NFD-fed mice. The HFD-fed and PBS-treated mice showed a significantly higher *Hif1a* mRNA level in mWAT as compared with other mice (Fig. 4C). HFD feeding and CATr treatment had no significant influence on the *Pnpla2* mRNA level (Fig. 4E). A significant positive correlation was observed between the total FFA content and the *Lbp* mRNA level in mWAT (Fig. 4H).

Both LPS and palmitate promote Lbp gene expression, and recombinant LBP promotes Ccl2 and Cxcl8 gene expression in 3T3-L1 adipocytes

In differentiated 3T3-L1 adipocytes, supplementation with LPS (1.0 and 10 ng/mL) or palmitic acid (2 mM) for 24 hr significantly increased the *Lbp* mRNA level (Fig. 5A and 5C, respectively). Likewise, the LBP protein concentration in the

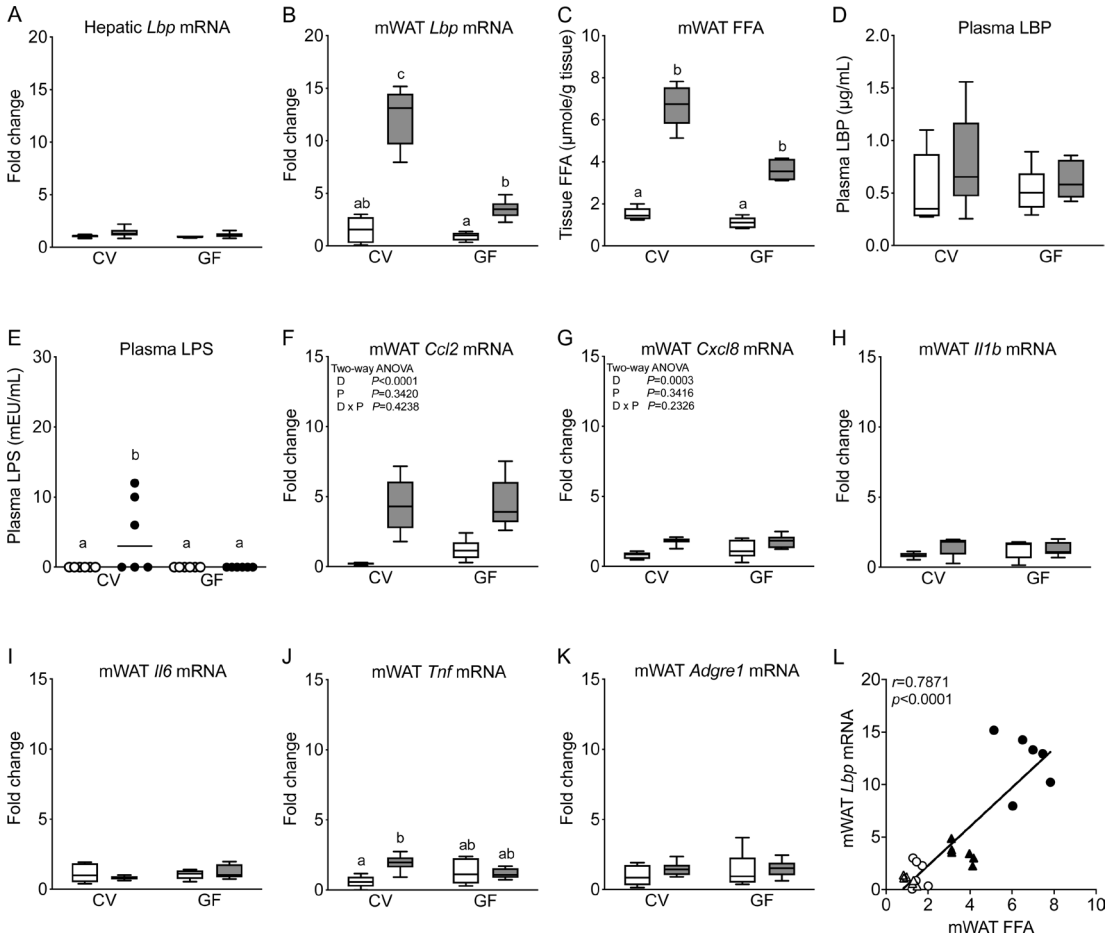

Fig. 3. The *Lbp* gene expression level in mesenteric white adipose tissue (mWAT) is associated with the local free fatty acid (FFA) content in conventional (CV) and germ-free (GF) male C57BL/6J mice fed the normal-fat diet (NFD) or high-fat diet (HFD) for 3 days. (A and B) *Lbp* mRNA levels in the liver and mWAT, respectively. (C) Total FFA content in mWAT. (D and E) Plasma LBP and LPS levels, respectively. (F–K) Messenger RNA levels of adipokine genes in mWAT. In panels A, B, and F–K, data are shown relative to the levels in the NFD-fed GF mice, which were set to 1. In panels A–D and F–K, results are presented as standard box plots showing the median and interquartile range with the minimum and maximum (n=6). White and gray boxes represent the NFD- and HFD-fed mice, respectively. In panel E, open and closed circles represent individual mice fed the NFD or HFD, respectively, and horizontal bars represent the mean values. The results of two-way ANOVA for panels A–K are summarized in Supplementary Table 3, whereas p values for two-way ANOVA are shown in panels F and G, with D, P, and D × P representing diet, period, and the interaction of diet and period, respectively. The Tukey-Kramer *post hoc* test was applied when a significant interaction was found by two-way ANOVA, and mean values with different superscript letters are significantly different ($p<0.05$). In panel L, open and closed symbols represent mice fed the NFD and HFD, respectively, and circles and triangles represent the CV and GF mice, respectively. Spearman's correlation coefficient (r) was used to evaluate the relationships.

cell culture supernatant was significantly increased by the supplementation with LPS (10 ng/mL) or palmitic acid (2 mM; Fig. 5B and 5D, respectively). In addition, supplementation with recombinant LBP (1,000 ng/mL) for 24 hr increased the *Ccl2* and *Cxcl8* mRNA levels (Fig. 5E and 5F, respectively). In the medium supplemented with palmitic acid and recombinant LBP, the LPS level was below the detection limit.

DISCUSSION

The present study showed no correlation between the plasma LPS level and the mWAT *Lbp* mRNA level in mice fed an NFD or HFD for 14 weeks. In addition, HFD feeding increased the *Lbp* mRNA level in mWAT of the GF mice, which lack gut microbiota, the source of LPS. Furthermore, considering that intraperitoneal administration of LPS has been reported to increase the *Lbp* mRNA level in both the liver and mWAT in mice and rats [3, 14, 15], circulating LPS levels should trigger *Lbp* gene expression in both mWAT and the liver. However, we observed that the hepatic *Lbp* gene expression level was not affected by HFD feeding and obesity-related metabolic disorders. Likewise, a previous study showed that the hepatic *Lbp* mRNA level was the same between genetically obese *ob/ob* mice and lean wild-type mice [16] even though *ob/ob* mice have been reported to have higher circulating LPS levels [17]. Taken together, it seems unlikely that the HFD- and obesity-related increase of *Lbp* gene expression in mWAT is related to circulating LPS.

Increased adipocyte lipolysis has been reported to be an early characteristic of the obese state [18], which would result in increased availability of local FFAs in WAT. Indeed, Lee et al. [6] reported elevated total FFA levels in epididymal WAT at 3 days of HFD feeding. Likewise, the present study showed an

doi: 10.12938/bmfh.2021-061

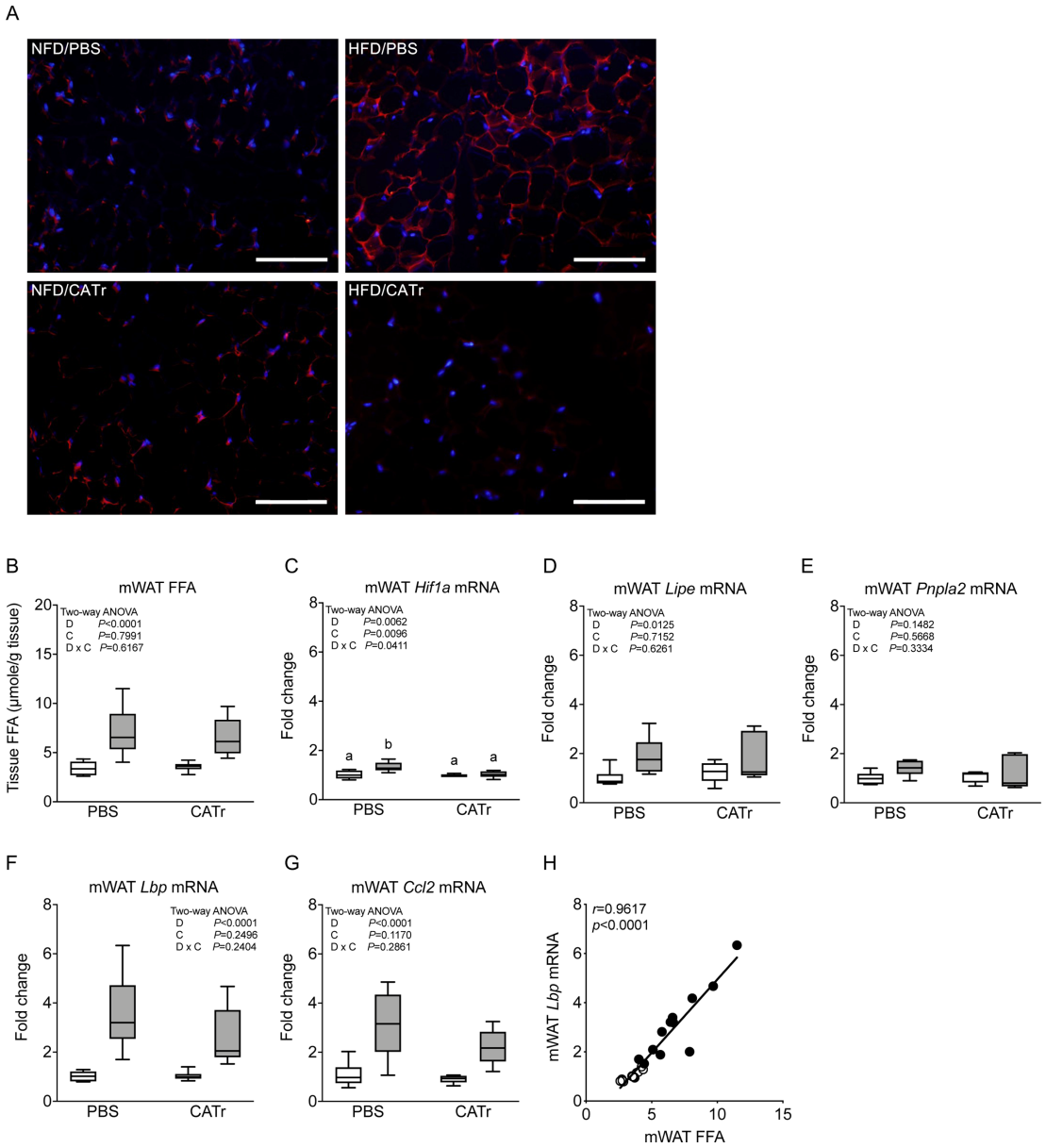

Fig. 4. The *Lbp* gene expression level in mesenteric white adipose tissue (mWAT) is associated with the local free-fatty acid (FFA) content, but not tissue hypoxia, in male C57BL/6J mice fed the normal-fat diet (NFD) or high-fat diet (HFD) for 7 days. The mice were intraperitoneally administered daily either carboxyatractyloside potassium salt (CAtr) or vehicle (PBS). On day 7, mice were intraperitoneally administered pimonidazole hydrochloride and then euthanized 60 min later. (A) Representative immunostaining of pimonidazole adducts. (B) Total FFA content in mWAT. (C–G) Messenger RNA levels of the *Hif1a*, *Lipe*, *Pnpla2*, *Lbp*, and *Ccl2* genes in mWAT, respectively. In panel A, signals for pimonidazole adducts (red) with DAPI counter staining (blue) are shown, and the bar represents 200 μm. In panels C–G, data are shown relative to the levels in the NFD-fed and PBS-administered mice, which were set to 1. In panels B–G, results are presented as standard box plots showing the median and interquartile range with the minimum and maximum (n=6). White and gray boxes represent the NFD- and HFD-fed mice, respectively. P values for two-way ANOVA are shown in panels B–G, with D, C, and D × C representing diet, CATr, and the interaction of diet and CATr, respectively. In panel H, open and closed circles represent mice fed the NFD and HFD, respectively, and Spearman's correlation coefficient (*r*) was used to evaluate the relationships.

increase in the total FFA content in mWAT at 3 days, 7 days, and 14 weeks of HFD feeding. In line with this increase, HFD feeding increased the mRNA expression of *Lipe*, which encodes a lipolytic enzyme, HSL, in mWAT, suggesting that lipolysis increased by HSL mediates the HFD-induced increase of total FFA in mWAT. Furthermore, we repeatedly observed a significant positive correlation between the total FFA content and the *Lbp* mRNA level in mWAT. These findings suggest that the elevated availability of local FFAs in WAT is responsible for the HFD- and obesity-related increase of *Lbp* gene expression. Supporting this idea, we observed that palmitic acid (2 mM) promoted *Lbp* gene expression in differentiated 3T3-L1 adipocytes. Based on the total FFA levels observed in the mWAT of the HFD-fed mice in the present study, the doses of palmitic acid supplementation used for the 3T3-L1 adipocytes could be physiologically relevant.

In spite of increased total FFA in mWAT at 14 weeks of

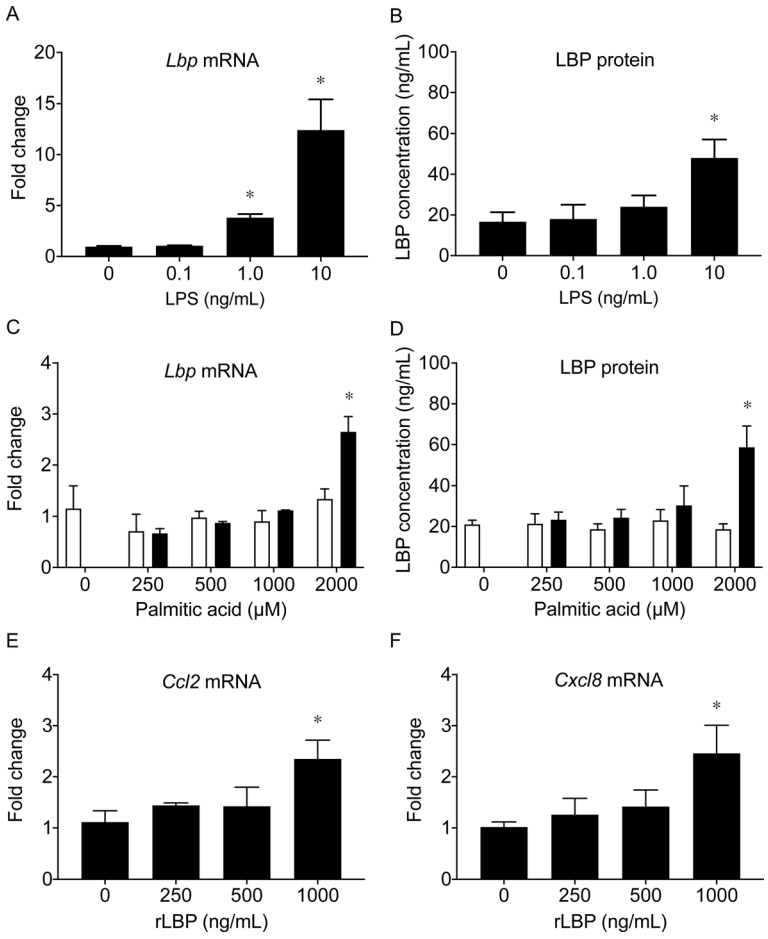

Fig. 5. *Lbp* gene expression (panels A and C), LBP protein concentration in the culture supernatant (panels B and D), and *Ccl2* and *Cxcl8* gene expression (panels E and F, respectively) in differentiated 3T3-L1 adipocytes supplemented with LPS (panels A and B), palmitic acid (panels C and D), or recombinant LBP (panels E and F) for 24 hr. In panels C and D, white and black bars represent the cells supplemented with bovine serum albumin (BSA) and palmitic acid-conjugated BSA, respectively. Results are presented as the mean ± SEM of three independent experiments. *p<0.05 vs. without supplementation, as estimated by one-way ANOVA followed by Dunnett's multiple comparison test.

HFD feeding, the plasma total FFA concentration did not differ between the NFD- and HFD-fed mice under non-fasting conditions. Raubenheimer *et al.* showed that HFD feeding for 8 weeks increased the plasma total FFA concentration under fasting conditions but not non-fasting conditions in mice [19]. Although it has been well known that the plasma FFA concentration is elevated by HFD feeding and obesity, these effects may be blunted under non-fasting conditions.

Lee *et al.* demonstrated that local FFAs in mWAT of mice fed an HFD for 3 days induced ANT-mediated uncoupled respiration and increased adipocyte oxygen consumption, thereby leading to WAT hypoxia [6]. In addition, previous studies have demonstrated that HFD-induced WAT inflammation is mediated by WAT hypoxia [6, 20–22]. We therefore examined whether hypoxia contributes to the increased WAT expression of the *Lbp* gene in HFD-fed mice. In the present study, a positive signal for hypoxia adducts was observed in mWAT of the HFD-fed mice, and CATr treatment reduced the signal. In line with these observations, the mRNA expression of *Hif1a* in mWAT was increased by HFD feeding, and CATr treatment blunted the HFD-induced increase of *Hif1a* mRNA levels. These results were consistent with those of Lee *et al.* [6] and suggest that ANT inhibition suppresses HFD-induced WAT hypoxia. Under the hypoxia-suppressed condition caused by CATr treatment, we observed that *Lbp* gene expression in mWAT was still higher in the HFD-fed mice than in the NFD-fed mice. In addition, the total FFA content in mWAT was higher in the HFD-fed mice than in the NFD-fed mice regardless of CATr administration. These results suggest that hypoxia is not responsible for the increased expression of the *Lbp* gene in the mWAT of the HFD-fed mice.

Since FFAs, particularly saturated fatty acids, induce adipocyte inflammation through the TLR4-NF-κB pathway [7], it is possible that the increased expression of the *Lbp* gene in the mWAT of the HFD-fed mice was promoted by inflammatory cytokines. In fact, TNF-α supplementation has been reported to promote *Lbp* gene expression in 3T3-L1 adipocytes [3]. However, the present study showed that the increase in *Lbp* gene expression preceded the increase in inflammatory markers, i.e., *Il1b*, *Il6, Tnf,* and *Adgre1* mRNA, in the mWAT of the HFD-fed mice. Although the mRNA level of *Tnf* in mWAT was increased by 3 days of feeding of HFD in CV mice, the degree of increase was much lower as compared with early increasing genes, i.e., *Lbp* and *Ccl2*. In addition, the present study tested whether supplementation with recombinant LBP promotes the expression of the *Ccl2* and *Cxcl8* genes in

doi: 10.12938/bmfh.2021-061

differentiated 3T3-L1 adipocytes, because the expression of these genes was increased in adipocytes, and to a lesser extent nonadipocytes, in WAT of obese mice [23, 24]. We observed that the mRNA expression of *Ccl2* and *Cxcl8* genes was increased by recombinant LBP in differentiated 3T3-L1 adipocytes. The *Ccl2* and *Cxcl8* genes encode the chemokines MCP-1 and CXCL8 (also known as IL-8), which recruit monocytes and neutrophils, respectively [25, 26]. In particular, previous findings [27] and our present data indicate that the increased production of MCP-1 in WAT is an initial event that occurs at the early stage of obesity. Thus, it seems unlikely that inflammatory cytokines mediate the increase of *Lbp* gene expression in mWAT, especially early in the course of HFD feeding.

Previous studies have reported that circulating LBP is a marker of obesity-related metabolic disorders in humans and mice [2, 28, 29]. In accordance with this idea, we observed an elevated plasma LBP level in mice with obesity-related metabolic disorders induced by 14 weeks of feeding of an HFD, and a significant positive correlation was seen between the plasma LBP level and the mWAT *Lbp* mRNA level. In contrast, no significant relationship was observed between the LBP and LPS levels in plasma. Thus, the circulating LBP level appears to reflect the *Lbp* gene expression in mWAT of mice with obesity-related metabolic disorders. In other words, it is possible that circulating LBP is a marker of WAT inflammation rather than metabolic endotoxemia. However, HFD feeding for 7 or 28 days failed to increase the plasma LBP level, despite a significant increase of *Lbp* mRNA expression in mWAT. It is possible that chronic HFD feeding and/or obesity-related metabolic disorders promote LBP production in parts of WAT other than mWAT, resulting in the elevated plasma LBP levels. Another possibility is that the efficiency of LBP clearance from the circulation may be lowered by chronic HFD feeding and/or obesity-related metabolic disorders. Further studies are needed to test these possibilities.

Because basal adipocyte lipolysis is increased in obesity and is closely associated with insulin resistance, it has been thought that the inhibition of adipocyte lipolysis may be a promising therapeutic strategy for treating insulin resistance [30]. Given that increased adipocyte lipolysis and the resultant higher local FFA content is responsible for increased LBP production, the inhibition of adipocyte lipolysis would lead to decreased LBP production and thereby reduce WAT inflammation. In other words, the improvement of insulin sensitivity via the inhibition of adipocyte lipolysis may be mediated, at least in part, by decreased LBP production.

In conclusion, we propose that local FFAs, but not circulating LPS, are the trigger for increased *Lbp* expression in mWAT of mice fed the HFD. Further studies are needed to elucidate the molecular mechanisms by which FFAs promote *Lbp* expression in adipocytes.

CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ACKNOWLEDGMENT

This study was supported by a grant from the Center of Innovation program of the Japan Science and Technology Agency (Grant Number JPMJCE1301).

REFERENCES

1. Zweigner J, Schumann RR, Weber JR. 2006. The role of lipopolysaccharide-binding protein in modulating the innate immune response. Microbes Infect 8: 946–952. [Medline] [CrossRef]
2. Moreno-Navarrete JM, Ortega F, Serino M, Luche E, Waget A, Pardo G, Salvador J, Ricart W, Frühbeck G, Burcelin R, Fernández-Real JM. 2012. Circulating lipopolysaccharide-binding protein (LBP) as a marker of obesity-related insulin resistance. Int J Obes 36: 1442–1449. [Medline] [CrossRef]
3. Moreno-Navarrete JM, Escoté X, Ortega F, Serino M, Campbell M, Michalski MC, Laville M, Xifra G, Luche E, Domingo P, Sabater M, Pardo G, Waget A, Salvador J, Giralt M, Rodriguez-Hermosa JI, Camps M, Kolditz CI, Viguerie N, Galitzky J, Decaunes P, Ricart W, Frühbeck G, Villarroya F, Mingrone G, Langin D, Zorzano A, Vidal H, Vendrell J, Burcelin R, Vidal-Puig A, Fernández-Real JM. 2013. A role for adipocyte-derived lipopolysaccharide-binding protein in inflammation- and obesity-associated adipose tissue dysfunction. Diabetologia 56: 2524–2537. [Medline] [CrossRef]
4. Moreno-Navarrete JM, Escoté X, Ortega F, Camps M, Ricart W, Zorzano A, Vendrell J, Vidal-Puig A, Fernández-Real JM. 2015. Lipopolysaccharide binding protein is an adipokine involved in the resilience of the mouse adipocyte to inflammation. Diabetologia 58: 2424–2434. [Medline] [CrossRef]
5. Rastelli M, Knauf C, Cani PD. 2018. Gut microbes and health: a focus on the mechanisms linking microbes, obesity, and related disorders. Obesity (Silver Spring) 26: 792–800. [Medline] [CrossRef]
6. Lee YS, Kim JW, Osborne O, Oh DY, Sasik R, Schenk S, Chen A, Chung H, Murphy A, Watkins SM, Quehenberger O, Johnson RS, Olefsky JM. 2014. Increased adipocyte O_2 consumption triggers HIF-1α, causing inflammation and insulin resistance in obesity. Cell 157: 1339–1352. [Medline] [CrossRef]
7. Suganami T, Tanimoto-Koyama K, Nishida J, Itoh M, Yuan X, Mizuarai S, Kotani H, Yamaoka S, Miyake K, Aoe S, Kamei Y, Ogawa Y. 2007. Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. Arterioscler Thromb Vasc Biol 27: 84–91. [Medline] [CrossRef]
8. Ito H, Tanabe H, Kawagishi H, Tadashi W, Yasuhiko T, Sugiyama K, Kiriyama S, Morita T. 2009. Short-chain inulin-like fructans reduce endotoxin and bacterial translocations and attenuate development of TNBS-induced colitis in rats. Dig Dis Sci 54: 2100–2108. [Medline] [CrossRef]
9. Udomsopagit T, Miwa A, Seki M, Shimbori E, Kadota Y, Tochio T, Sonoyama K. 2020. Intestinal microbiota transplantation reveals the role of microbiota in dietary regulation of RegIIIβ and RegIIIγ expression in mouse intestine. Biochem Biophys Res Commun 529: 64–69. [Medline] [CrossRef]
10. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT. 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem 55: 611–622. [Medline] [CrossRef]
11. Okumura M, Hamada A, Ohsaka F, Tsuruta T, Hira T, Sonoyama K. 2020. Expression of serotonin receptor HTR4 in glucagon-like peptide-1-positive enteroendocrine cells of the murine intestine. Pflugers Arch 472: 1521–1532. [Medline] [CrossRef]
12. Frost SC, Lane MD. 1985. Evidence for the involvement of vicinal sulfhydryl groups in insulin-activated hexose transport by 3T3-L1 adipocytes. J Biol Chem 260: 2646–2652. [Medline] [CrossRef]
13. Faul F, Erdfelder E, Lang AG, Buchner A. 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 39: 175–191. [Medline] [CrossRef]
14. Fang H, Liu A, Dirsch O, Dahmen U. 2013. Liver transplantation and inflammation: is lipopolysaccharide binding protein the link? Cytokine 64: 71–78. [Medline] [CrossRef]
15. Nakanishi K, Kaji K, Kitade M, Kubo T, Furukawa M, Saikawa S, Shimozato N, Sato S, Seki K, Kawaratani H, Moriya K, Namisaki T, Yoshiji H. 2019. Exogenous administration of low-dose lipopolysaccharide potentiates liver fibrosis in a choline-deficient L-amino-acid-defined diet-induced murine steatohepatitis model. Int J Mol Sci 20: 2724. [Medline] [CrossRef]
16. Stenman LK, Holma R, Gylling H, Korpela R. 2013. Genetically obese mice do not show increased gut permeability or faecal bile acid hydrophobicity. Br J Nutr 110: 1157–1164. [Medline] [CrossRef]
17. Brun P, Castagliuolo I, Di Leo V, Buda A, Pinzani M, Palù G, Martines D. 2007. Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. Am J Physiol Gastrointest Liver Physiol 292: G518–

G525. [Medline] [CrossRef]
18. Nielsen S, Guo Z, Johnson CM, Hensrud DD, Jensen MD. 2004. Splanchnic lipolysis in human obesity. J Clin Invest 113: 1582–1588. [Medline] [CrossRef]
19. Raubenheimer PJ, Nyirenda MJ, Walker BR. 2006. A choline-deficient diet exacerbates fatty liver but attenuates insulin resistance and glucose intolerance in mice fed a high-fat diet. Diabetes 55: 2015–2020. [Medline] [CrossRef]
20. Halberg N, Khan T, Trujillo ME, Wernstedt-Asterholm I, Attie AD, Sherwani S, Wang ZV, Landskroner-Eiger S, Dineen S, Magalang UJ, Brekken RA, Scherer PE. 2009. Hypoxia-inducible factor 1alpha induces fibrosis and insulin resistance in white adipose tissue. Mol Cell Biol 29: 4467–4483. [Medline] [CrossRef]
21. Jiang C, Qu A, Matsubara T, Chanturiya T, Jou W, Gavrilova O, Shah YM, Gonzalez FJ. 2011. Disruption of hypoxia-inducible factor 1 in adipocytes improves insulin sensitivity and decreases adiposity in high-fat diet-fed mice. Diabetes 60: 2484–2495. [Medline] [CrossRef]
22. Krishnan J, Danzer C, Simka T, Ukropec J, Walter KM, Kumpf S, Mirtschink P, Ukropcova B, Gasperikova D, Pedrazzini T, Krek W. 2012. Dietary obesity-associated Hif1α activation in adipocytes restricts fatty acid oxidation and energy expenditure via suppression of the Sirt2-NAD+ system. Genes Dev 26: 259–270. [Medline] [CrossRef]
23. Takahashi K, Mizuarai S, Araki H, Mashiko S, Ishihara A, Kanatani A, Itadani H, Kotani H. 2003. Adiposity elevates plasma MCP-1 levels leading to the increased CD11b-positive monocytes in mice. J Biol Chem 278: 46654–46660. [Medline] [CrossRef]
24. Cimmino I, Oriente F, D'Esposito V, Liguoro D, Liguoro P, Ambrosio MR, Cabaro S, D'Andrea F, Beguinot F, Formisano P, Valentino R. 2019. Low-dose Bisphenol-A regulates inflammatory cytokines through GPR30 in mammary adipose cells. J Mol Endocrinol 63: 273–283. [Medline] [CrossRef]
25. Luo Y, Laning J, Hayashi M, Hancock PR, Rollins B, Dorf ME. 1994. Serologic analysis of the mouse β chemokine JE/monocyte chemoattractant protein-1. J Immunol 153: 3708–3716. [Medline]
26. Murphy PM. 1997. Neutrophil receptors for interleukin-8 and related CXC chemokines. Semin Hematol 34: 311–318. [Medline]
27. Ito A, Suganami T, Miyamoto Y, Yoshimasa Y, Takeya M, Kamei Y, Ogawa Y. 2007. Role of MAPK phosphatase-1 in the induction of monocyte chemoattractant protein-1 during the course of adipocyte hypertrophy. J Biol Chem 282: 25445–25452. [Medline] [CrossRef]
28. Gubern C, López-Bermejo A, Biarnés J, Vendrell J, Ricart W, Fernández-Real JM. 2006. Natural antibiotics and insulin sensitivity: the role of bactericidal/permeability-increasing protein. Diabetes 55: 216–224. [Medline] [CrossRef]
29. Sun L, Yu Z, Ye X, Zou S, Li H, Yu D, Wu H, Chen Y, Dore J, Clément K, Hu FB, Lin X. 2010. A marker of endotoxemia is associated with obesity and related metabolic disorders in apparently healthy Chinese. Diabetes Care 33: 1925–1932. [Medline] [CrossRef]
30. Morigny P, Houssier M, Mouisel E, Langin D. 2016. Adipocyte lipolysis and insulin resistance. Biochimie 125: 259–266. [Medline] [CrossRef]

