Ph.D.Thesis

Title

The role of CCL19-CCR7 pathway on the development of high-fat-induced obesity and insulin resistance

(CCL19－CCR7 経路が肥満およびインスリン抵抗性に及ぼす影響に関する検討)

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Abstract

Aims

Several chemokines are thought to play important roles in recruiting the monocyte/macrophage lineage into adipose tissues. We found C-C motif chemokine ligand 19 (CCL19) was highly expressed in adipocytes co-cultured with macrophages stimulated by bacterial endotoxin. This study aimed to evaluate the role of CCL19-CC chemokine receptor type 7 (CCR7) axis on obesity and insulin resistance.

Methods

Serum CCL19 concentration was first examined in obese model mice challenged by endotoxin. Next, *Ccr7*$^{-/-}$ mice and wild-type mice fed high fat diet (HFD) or normal diet were used to investigate the role of CCL19 signals on obesity-associated inflammation.

Results

Besides marked up-regulation of the gene encoding CCL19 in adipocytes co-cultured with macrophages stimulated by endotoxin, CCL19 protein was also elevated in the sera of *ob/ob* and diet-induced obese mice challenged by endotoxin. *Ccr7*$^{-/-}$ mice were protected from diet-induced obesity and subsequent insulin resistance. The expression of inflammatory genes in the adipose tissue and liver of *Ccr7*$^{-/-}$ mice was much lower than in diet-induced obese mice. Accordingly, *Ccr7*$^{-/-}$ mice were protected from fatty liver and dyslipidemia. Since CCL19 attracts activated dendritic cells (DCs), and the expression of the DC markers, CD11b and 11c, was not observed in

the adipose tissues of *Ccr7*$^{-/-}$ mice fed HFD, the absence of activated DCs appears to be a primary reason for the protection of these mice from obesity.

Conclusions

CCL19-CCR7 pathway is associated with the development of high-fat induced obesity and insulin resistance. CCL19 and its receptor, CCR7, may be potential therapeutic targets against obesity-associated inflammation.

Keywords

Adipocyte-macrophage interaction, chemokine, CCL19, CCR7, mature dendritic cells

Introduction

The hallmark of the immuno-pathophysiological point of view on obesity is a low-grade inflammation derived from the observation called the "adipocyte-macrophage interaction", in which monocytes and macrophages migrate into adipose tissue to interact with adipocytes as the adipocytes mature, thereby exhibiting exaggerated inflammatory responses, leading to a highly insulin-resistant state as well as increased cardiovascular risk [1, 2]. Several chemokines are thought to play important roles in recruiting the monocyte/macrophage lineage into adipose tissues, such as monocyte chemoattractant protein-1 (MCP-1), which is also known as C-C motif chemokine ligand 2 (CCL2) [3-7], CXCL10/Interferon gamma-induced protein 10 [8], and RANTES/CCL5 [9-11]. However, observations using mouse models deficient in CCL2 or its receptor [3-7] and CCL5 and its receptor [9-11] appear to provide conflicting results. Some of the studies suggest important roles for these chemokines in inducing insulin resistance, while others have produced negative results.

Another key finding in the adipocyte-macrophage interaction is that both cell types appear to express toll-like receptor (TLR)-2 and TLR-4 on their cell surfaces, both of which are important molecules for inducing innate immunity [12, 13]. Although the primary ligands for TLR-2 and TLR-4 are exogenous bacterial components such as lipoteichoic acid or lipopolysaccharide (LPS), some endogenous molecules such as oxidized low-density lipoprotein and/or saturated fatty acids, which are plentiful in the obese state, have also been implicated as

ligands for these receptors [14]. Therefore, determining which critical chemokines recruit the monocyte and macrophage to migrate into adipose tissues when these cells are stimulated by TLR ligands may provide new therapeutic targets against obesity-induced inflammation and subsequent insulin resistance. In this study, besides CCL2 and RANTES, we found marked up-regulation of the gene encoding CCL19 in adipocytes co-cultured with macrophages stimulated with bacterial endotoxin. Thus, we wished to evaluate the role of CCL19-CCR7 axis on obesity and insulin resistance.

Methods

Co-culture experiments

Quantification of mRNA in 3T3-L1 adipocytes co-cultured with RAW 264.7 murine macrophages with or without LPS stimulation was performed according to the same methods used in our previous study [15]. The expression of CXCL1, CXCL5, CXCL9, CXCL10, CCL2, CCL5, CCL7, CCL11, CCL19, and CCL22 mRNA were quantified at indicated time points. The primer sequences for quantitative real-time polymerase chain reaction (PCR) are listed in electronic supplementary material (ESM) Table 1. Data were normalized against internal expression level of glyceraldehydes 3-phosphate dehydrogenase (GAPDH) and expressed as fold expression of basal expression in the cells without LPS stimulation (0h).

Determination of CCL19 proteins in obese-model mice

Methods used for the measurement of serum CCL19 concentration in obese (*ob/ob* and high-fat-fed) and control (*ob/-* and normal diet-fed) mice were essentially the same as in our previous study [16]. Briefly, *ob/ob* and *ob/-* (both B6.V-Lepob/J origin) were grown to 11 weeks of age; C57BL/6J mice were raised on either a high-fat diet or normal diet for 11 weeks. At 11 weeks of age, endotoxin was injected into the tail vein as indicated in our previous study, and the serum concentration of CCL19 protein was measured at baseline and 1, 2, 3, and 4 days after endotoxin challenge. As an average weight of *ob/ob* mice at 11 weeks of age is 50 g, and that of high-fat-induced obese mice is 30 g, we used 50 ng and 30 ng single injection of endotoxin via tail vein respectively so that the endotoxin dose would be 1ng/ ml/g.

Generation of Ccr7$^{-/-}$ mice

The *Ccr7*$^{-/-}$ mice were generous gift from Dr. Martin Lipp at Department of Tumor Genetics and Immunogenetics, Max-Delbrück-Center of Molecular Medicine, Germany. The detailed generation of *Ccr7*$^{-/-}$ mice was reported in a previous study; the mice were kept under specific pathogen-free conditions until use [17].

Experiments using Ccr7$^{-/-}$ mice

Male *Ccr7*$^{-/-}$ mice and wild-type C57BL/6J mice were separated into two groups. One group was

fed a high-fat diet (HFD-60, Oriental Yeast Co., Ltd., Tokyo), and the other group was fed a normal diet (MF, Oriental Yeast Co., Ltd., Tokyo) from 7-9 weeks of age. Body weights were monitored and food intake was recorded by subtracting the amounts of food left in the cage from the daily amounts given. Insulin tolerance tests and glucose tolerance tests were performed at 19-21 weeks and 21-23 weeks of age, respectively, according to the methods described previously [18]. At 22-24 weeks of age, blood samples were obtained for the measurement of biochemical markers. At 23-25 weeks of age, the mice were sacrificed to remove the liver and epididymis adipose tissues for subsequent experiments. Real-time PCR was performed according the methods described above. The expression of *Ccl2* (*Mcp-1*), *interleukin-6* (*Il-6*)*, tumor necrosis factor-α* (*Tnf-α*)*, F4/80, Ccl19, Ccr7, CD11b, CD11c, adiponectin, leptin, sterol regulatory element-binding protein* (*Srebp*)*-1c, and glucose transporter type4* (*Glut4*) mRNA in the adipose tissues were quantified, while the expression of *Ccl2*, *Il-6*, *Tnf-α*, *Ccl19*, *Ccr7*, *CD11c*, *AdipoR1*, *AdipoR2*, C*arnitine palmitoyltransferase* (*Cpt*) *1α*, *Srebp-1c, Srebp-2, stearoyl-coA desaturase* (*Scd*)*-1, Acetyl-CoA carboxylase* (*Acc*) and *glucose 6-phosphatase* (*G6Pase*) mRNA expression in the liver were quantified. The primer sequences are listed in ESM Table 1. Data were normalized against *Gapdh* and were calculated as fold expression of the genes in control wild-type normal-diet-fed mice. Liver and adipose tissue sections were stained with hematoxylin and eosin for histological examination. Serum adiponectin and leptin levels were measured by enzyme-linked immunosorbent assay, while insulin, cholesterol and triglyceride

levels were measured as described previously [18, 19].

Statistical Analysis

Data are expressed as means±SE. Statistical analyses were performed using Student's t-test. Values of $p < 0.05$ were considered significant.

Results

Ccl19 expression is markedly up-regulated in adipocytes co-cultured with macrophages stimulated by endotoxin, and its serum level increased in obese model mice when challenged by endotoxin.

We have previously performed *in vitro* studies to detect which genes are highly expressed in adipocytes when co-cultured with macrophages, with or without endotoxin (LPS) stimulation [15]. We observed the up-regulation of many chemokine genes when the cells were treated with endotoxin. A summary of the chemokines up-regulated by LPS treatment in our previous experiments are listed in Table 1. Based on these observations, we tried to confirm the gene expression of each chemokine using real-time PCR analysis (Fig. 1A-J). We verified that the gene expressions of all these chemokines are up-regulated in endotoxin-stimulated adipocytes, when co-cultured with macrophages. In addition to CCL2 (Fig. 1E) and CCL5 (Fig. 1F), which have been previously extensively studied in relation to obesity-associated inflammation, we noted a

markedly elevated expression of the gene encoding macrophage inflammatory protein-3β (*Mip-3β*), also known as *Ccl19* (Fig. 1I). Since CCL19 is known to attract activated DCs, and is considered to be involved in DC homing into lymphoid tissues, we hypothesized that CCL19 may recruit activated DCs into adipose tissues in a mechanism similar to DC homing. Twenty-four hours after challenge with intravenous LPS, we found increased levels of serum CCL19 proteins in both diet-induced obese mice (Fig. 2A) and genetically obese *ob/ob* mice (Fig. 2B), compared to their lean controls. Furthermore, in *ob/ob* mice, base line serum CCL19 concentration appeared already higher than control lean mice. Thus, both *in vitro* and *in vivo* experiments suggests that mature adipose tissue may be a major source of the elevated serum CCL19 when stimulated by TLR ligands. Based on these observations, we then decided to investigate the role of CCL19 signals on obesity-associated inflammation, using knockout mice deficient *Ccr7*, the receptor for CCL19.

***Ccr7$^{-/-}$* mice are protected from diet-induced obesity and insulin resistance.**

Ccr7$^{-/-}$ mice did not develop obesity when fed a high-fat diet, unlike the control wild-type mice, despite the fact that food intake was not impaired (Fig. 3A-C). Accordingly, adipose tissue and liver weights in the high-fat-fed *Ccr7$^{-/-}$* mice were much lower than those of the high-fat-fed wild-type controls (Fig. 3D, E). Furthermore, the size of adipocytes in the adipose tissue of high-fat-diet *Ccr7$^{-/-}$* mice was much smaller than that in the wild-type mice fed a high-fat diet

(Fig. 3G, H). When the liver and adipose tissue were examined histologically (Fig. 3F, G), severe fatty liver was observed in the wild-type mice fed a high-fat diet, but not in the wild-type mice fed a normal diet, or in the *Ccr7*$^{-/-}$ mice fed either a high-fat or normal diet (Fig. 3F). Both insulin tolerance and glucose tolerance tests demonstrated that insulin sensitivity was markedly improved in *Ccr7*$^{-/-}$ mice fed a high-fat diet, compared to wild-type mice fed the same diet (Fig. 4A-J). Therefore, we next investigated the gene expression profile in both adipose tissue and liver in each test group.

Inflammatory gene expression was markedly suppressed in adipose tissues of Ccr7*$^{-/-}$ *mice fed high fat.

Inflammatory genes such as *Mcp-1/Ccl2, Il-6*, and *Tnf-*α were more highly expressed in the adipose tissue of wild-type mice fed a high-fat diet than in wild-type mice fed a normal diet or *Ccr7*$^{-/-}$ mice (Fig. 5A-C). *Ccl19*, *F4/80, CD11b,* and *CD11c* genes were also highly expressed in the high-fat diet-fed wild-type mice (Fig. 5D, E, G, H). Especially, *CD11c* expression was markedly up-regulated in the high-fat-fed wild-type mice. *Adiponectin* gene expression was suppressed, while *Leptin* gene expression was up-regulated in high-fat-fed wild-type controls, but not in *Ccr7*$^{-/-}$ mice fed a high-fat diet (Fig. 5I, J). *Srebp-1c* gene expression was also up-regulated in high-fat-fed wild-type mice but not in *Ccr7*$^{-/-}$ mice (Fig. 5K), while *Glut4* gene expression was down-regulated in high-fat-fed wild-type mice (Fig. 5L).

Inflammatory gene expression was markedly suppressed in liver of Ccr7$^{-/-}$ mice fed high fat.

Similar to the inflammatory gene expression profile in adipose tissue, *Mcp-1/Ccl2*, *Il-6*, and *Tnf-α* gene expression was markedly up-regulated in the liver of only the wild-type mice fed a high-fat diet (Fig. 6A-C). *AdipoR1* and *AdipoR2* gene expression was down-regulated in high-fat-fed wild-type controls, but not in the other groups of mice (Fig. 6G, H). *Cpt1α* gene expression was down-regulated in high-fat-fed wild-type mice, while *Srebp-1c, Srebp-2, Scd-1, Acc,* and *G6Pase* gene expressions were all up-regulated, but only in the wild-type mice fed a high-fat diet (Fig. 6I-N).

Serum adiponectin level was kept higher in Ccr7$^{-/-}$ mice, while dyslipidemia was not induced in Ccr7$^{-/-}$ mice.

Finally, we investigated the profile of serum biochemical markers. Although fasting insulin (Fig. 7A), leptin (Fig. 7B), total cholesterol (Fig. 7C), and triglyceride levels (Fig. 7D) were all elevated in the wild-type mice fed a high-fat diet, their serum concentration in *Ccr7*$^{-/-}$ mice remained low (Fig. 7A-D). These data indicate that *Ccr7*$^{-/-}$ mice, fed high-fat diet, protected not only from insulin resistance but also from dyslipidemia. In contract to insulin and leptin, the adiponectin concentration in *Ccr7*$^{-/-}$ mice remained high, which the level was much lower in high-fat-fed wild-type mice, compared to the other test groups (Fig. 7E).

Discussion

In this study, we found many chemokine genes were highly expressed in adipocytes co-cultured with macrophages stimulated by bacterial endotoxin including CCL2 (MCP-1) and CCL5 (RANTES). Actually, CCL2 and RANTES can attract monocytes via CCR2 and CCR3, respectively [20]. However, results of the several previous studies appeared to be somewhat inconsistent (Table 1). Besides these chemokines, we found high expression of *Ccl19* gene and protein in adipocytes and in sera of two kinds of obese model mice challenged by endotoxin. *Ccl19* (*MIP-3β*) is another CC family chemokine primarily attracting mature DCs via CCR7 [20]. It is generally believed that CCL19 primarily attracts activated DCs of myeloid origin, and is usually involved in the homing of activated DCs to the lymphoid organs, and, therefore, usually expressed in lymph nodes and thymus [20]. Since *Ccl19* was highly expressed in adipocytes co-cultured with macrophages in the presence of TLR ligands, we hypothesized that CCL19-CCR7 system might play some important roles in the patho-physiology of adipose tissue inflammation.

Surprisingly, *Ccr7*$^{-/-}$ mice fed high-fat diet did not develop obesity. Changes in body weight as well as general appearances indicated no developmentally observed physical retardation in *Ccr7*$^{-/-}$ mice when compared with wild type mice, as both gained weights in a similar way by normal diet. Accordingly, adipocyte size was not enlarged and insulin sensitivity was kept normal in *Ccr7*$^{-/-}$ mice fed high-fat. It should be noted that, when we compare insulin

sensitivity between wild type and *Ccr7*$^{-/-}$ mice fed normal diet, sensitivity in *Ccr7*$^{-/-}$ mice appeared significantly superior to that in wild type mice despite the fact that body weights in both test groups did not differ (Fig. 4E, I). We speculate that, even under non-obese conditions, small numbers of DCs are already infiltrated in adipose tissue, and, therefore, complete loss of function of CCR7 resulted in complete lack of DCs in adipose tissues, thereby inducing better insulin sensitivity.

Understanding the underlying mechanism by which CCR7 deficiency contributes to the protection of mice from obesity when fed a high-fat diet, and how the incorporated energy is expended is an important issue. One possible explanation is a stable adiponectin gene expression and serum level in *Ccr7*$^{-/-}$ mice, as adiponectin has been reported to accelerate energy expenditure in central [21] and peripheral (muscle and liver) organs [22, 23]. For example, adiponectin appeared to transmigrate through blood brain barrier into cerebrospinal area, resulting in increased thermogenesis, weight loss and reduction in serum glucose and lipid levels, phenotype very similar to our *Ccr7*$^{-/-}$ mice fed high-fat [21]. Adiponectin or its analog has also reported to increase fatty acid combustion, while decrease gluconeogenesis in the liver [23]. In this study, to support these observations, *Cpt1α* expression was down-regulated, while *Srebp-1c, Srebp-2, Acc and G6Pase* expression was up-regulated in wild type mice fed high-fat, but not in *Ccr7*$^{-/-}$ mice fed high-fat. Interestingly, *AdipoR1* and *AdipoR2* gene expression in the lever was down-regulated in wild type mice fed high-fat, but not in *Ccr7*$^{-/-}$ mice, suggesting that anti-obese

action of adponectin in the liver is also regulated at receptor level.

As discussed, *Ccl19* is a chemokine which primarily attracts activated DCs of myeloid origin, and is usually involved in the homing of activated DCs to the lymphoid organs [20]. The fact that inflammatory adipose tissue expresses a higher level of *Ccl19* indicates that activated DCs migrate to adipose tissue in a similar manner to lymphocyte homing, like “false homing”. In fact, *CD11c* expression was markedly up-regulated in the high-fat-fed wild-type mice, suggesting that the major cell type infiltrating the adipose tissue of obese mice is $CD11b^{weak}/CD11c^{high}$ activated DCs, based on the gene expression profile in adipose tissue. These adipose tissue DCs are probably monocyte-derived myeloid DCs, initially activated in the peripheral circulation via TLRs by endogenous ligands such as oxidized LDL and/or saturated fatty acids [14], and they play an important role in inducing adipose tissue inflammation, impaired metabolism, and subsequent insulin resistance. In fact, several recent studies have demonstrated the presence of DCs in mature adipose tissue in both rodents and humans [24, 25], and especially, the latter suggested an important role of DCs in inducing obesity. In this study, we, for the first time, demonstrated that CCL19 played a crucial role in recruiting mature DCs into adipose tissues via CCR7. Thus, our current study suggests that CCL19 or its receptor CCR7, can be a potential therapeutic target against high-fat-induced obesity and insulin resistance.

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I'm grateful to all lab members.

And I would like to thank Dr. Martin Lipp at Department of Tumor Genetics and Immunogenetics, Max-Delbrück-Center of Molecular Medicine, for kindly providing us CCR7 deficient mice.

Figure Legends

Fig. 1 Chemokine gene expression in adipocytes co-cultured with macrophages with or without LPS stimulation.

A-J: 3T3-L1 adipocyte *Cxcl1* (A), *Cxcl5* (B), *Cxcl9* (C), *Cxcl10* (D), *Ccl2* (E), *Ccl5* (F), *Ccl7* (G), *Ccl11* (H), *Ccl19* (I), and *Ccl22* (J) mRNA expression with (black bars) and without (white bars) LPS stimulation (1ng/ml) co-cultured with RAW264.7 were quantified by real time PCR. *p<0.05, **p<0.01, ***p<0.001 (n=3), Student's t-test.

Fig. 2 Serum CCL19 concentrations in obese mice after LPS injection.

Changes in serum CCL19 concentrations after LPS challenge via the tail vein in high-fat diet-induced obese mice and their controls (A), and in genetically obese *ob/ob* mice and control *ob/-* mice (B) were measured. Each serum sample was obtained by heart puncture following LPS injection at indicated time periods, and serum CCL19 concentration was measured by commercial ELISA kit. ***$p<0.001$ (n=3), Student's t-test.

Fig. 3 Protection from diet-induced obesity in mice lacking *Ccr7*.

Wild-type (WT) or $Ccr7^{-/-}$ (KO) mice fed either a normal (ND) or high-fat diet (HFD) for 16 weeks. Food intake (n=4) (A), changes in body weight (n=5) (B) were monitored. Representative photos (C), adipose tissue weight (D), liver weight (E) of mice fed a normal or a high-fat diet for 16 weeks (n=5). F and G: HE staining of the liver (F) and epididymal adipose tissue (G) in each test group. Scale bar is 50 μm. H: The average adipocyte size of each group. **$p<0.01$, ***$p<0.001$, Student-t test.

Fig. 4 Insulin tolerance and glucose tolerance test in each test group.

A and B: Insulin tolerance test results (A) and glucose tolerance test results (B) from all test groups. C-F: Comparison of insulin tolerance test results between wild-type mice fed a normal diet (ND) or a high-fat diet (HFD) (C), $Ccr7^{-/-}$ fed either a normal diet (KO/ND) or a high-fat diet (KO/HFD) (D), wild-type mice fed a normal diet (WT/ND) and $Ccr7^{-/-}$ mice fed a normal diet (KO/HFD) (F). G-J: Comparison of insulin tolerance test results between wild-type mice fed a

normal diet (ND) or a high-fat diet (HFD) (G), *Ccr7*$^{-/-}$ fed either a normal diet (KO/ND) or a high-fat diet (KO/HFD) (H), wild-type mice fed a normal diet (WT/ND) and *Ccr7*$^{-/-}$ mice fed a normal diet (KO/HFD) (I), and wild-type mice fed a high-fat diet (WT/HFD) and *Ccr7*$^{-/-}$ mice fed a high-fat diet (KO/HFD) (J). *p<0.05, **p<0.01, ***p<0.001 (n=4), Student's t-test.

Fig 5 Gene expression in adipose tissues.

Adipose tissue *Ccl2/Mcp-1*(A), *Il-6* (B), *Tnf-α* (C), *F4/80* (D), *Ccl19* (E), *Ccr7* (F), *CD11b* (G), *CD11c* (H), *adiponectin* (I), *leptin* (J), *Srebp-1c* (K), and *GlutT4* (L) mRNA expression in wild-type mice fed either a normal (WT/ND) or a high-fat diet (WT/HFD) and *Ccr7*$^{-/-}$ mice fed either a normal (KO/ND) or a high-fat diet (KO/HFD) were quantified by real-time PCR. *p<0.05, **p<0.01, ***p<0.001 (n=3), Student's t-test.

Fig. 6 Gene expression in the liver.

Liver *Ccl2/Mcp-1* (A), *Il-6* (B), *Tnf-α* (C), *Ccl19* (D), *Ccr7* (E), *CD11c* (F), *AdipoR1* (G), *AdipoR2* (H), *Cpt1α* (I), *Srebp-1c* (J), *Srebp-2* (K), *Scd-1* (L), *Acc* (M), and *G6Pase* (N) mRNA expression in wild-type mice fed either a normal (WT/ND) or a high-fat diet (WT/HFD) and *Ccr7*$^{-/-}$ mice fed either a normal (KO/ND) or a high-fat diet (KO/HFD) were quantified by real-time PCR. *p<0.05, **p<0.01, ***p<0.001 (n=3), Student's t-test.

Fig. 7 Serum biochemical markers in each test group.

Serum fasting insulin (A), leptin (B), total cholesterol (C), triglyceride (D), and adiponectin (E) concentrations in wild-type mice fed either a normal diet (WT/ND) or a high-fat diet (WT/HFD)

and *Ccr7* knockout mice fed either a normal (KO/ND) or a high-fat diet (KO/HFD) were measured. p**<0.01, ***p<0.001 (n=4), Student's t-test.

ESM Table 1. Primer sequences for real time PCR analysis

primer	Forward	Reverse
GAPDH	AATGTGTCCGTCGTGGATCTGA	GATGCCTGCTTCACCACCTTCT
CXCL1	CACCCAAACCGAAGTCATAG	AAGCCAGCGTTCACCAGA
CXCL5	GGTCCACAGTGCCCTACG	GCGAGTGCATTCCGCTTA
CXCL9	GAACCCTAGTGATAAGGAATGCA	CTGTTTGAGGTCTTTGAGGGATT
CXCL10/IP-10	TCCCTCTCGCAAGGAC	TTGGCTAAACGCTTTCAT
CCL2/MCP-1	GAAGGAATGGGTCCAGACAT	ACGGGTCAACTTCACATTCA
CCL5/RANTES	ATGAAGATCTCTGCAGCTGCCCTC	CTAGCTCATCTCCAAATAGTTGATG
CCL7	CTCATAGCCGCTGCTTTCAGCATC	GTCTAAGTATGCTATAGCCTCCTC
CCL11	GCGCTTCTATTCCTGCTGCTCACGG	GTGGCATCCTGGACCCACTTCTTC
CCL19	GGTGCTAATGATGCGGAAGAC	ATAGCCCCTTAGTGTGGTGAACA
CCL22	CAGGTCCCTATGGTGCCAAT	AACGTGATGGCAGAGGGTG
IL-6	GGACCAAGACCATCCAATTC	ACCACAGTGAGGAATGTCCA
TNF-α	GACAGTGACCTGGACTGTGG	TGAGACAGAGGCAACCTGAC
F4/80	TGCATCTAGCAATGGACAGC	GCCTTCTGGATCCATTTGAA
CCR7	CCAGCAAGCAGCTCAACATT	GCCGATGAAGGCATACAAGA
CD11b	AAACCACAGTCCCGCAGAGA	CGTGTTCACCAGCTGGCTTA
CD11c	CCTGAGGGTGGGCTGGAT	GCCAATTTCCTCCGGACAT
adiponectin	AAGGACAAGGCCGTTCTCT	CGCACGATTTCCCTCTCAGCTG
leptin	TCTCCGAGACCTCCTCCATCT	TTCCAGGACGCCATCCAG
SREBP-1c	CGGAAGCTGTCGGGGTAG	GTTGTTGATGAGCTGGAGCA
GULT4	AGAGTCTAAAGCGCCT	CCGAGACCAACGTGAA
adipoR1	ACGTTGGAGAGTCATCCCGTAT	CTCTGTGTGGATGCGGAAGAT
adipoR2	GCCCAGCTTAGAGACACCTG	GCCTTCCCACACCTTACAAA
CPT1α	CCAGGCTACAGTGGGACATT	GAACTTGCCCATGTCCTTGT
SREBP-2	GCGTTCTGGAGACCATGGA	CACAAGTTGCTCTGAAAACAAATCA
SCD-1	GATGTTCCAGAGGAGGTACTACAAGC	ATGAGCACATCAGCAGGAGG
ACC	AACTTGCCAGAGCAGAAGGCA	GGATCTACCCAGGCCACATTG
G6Pase	TCCTGGGACAGACACACAAG	CAACTTTAATATACGCTATTGG

Fig.1

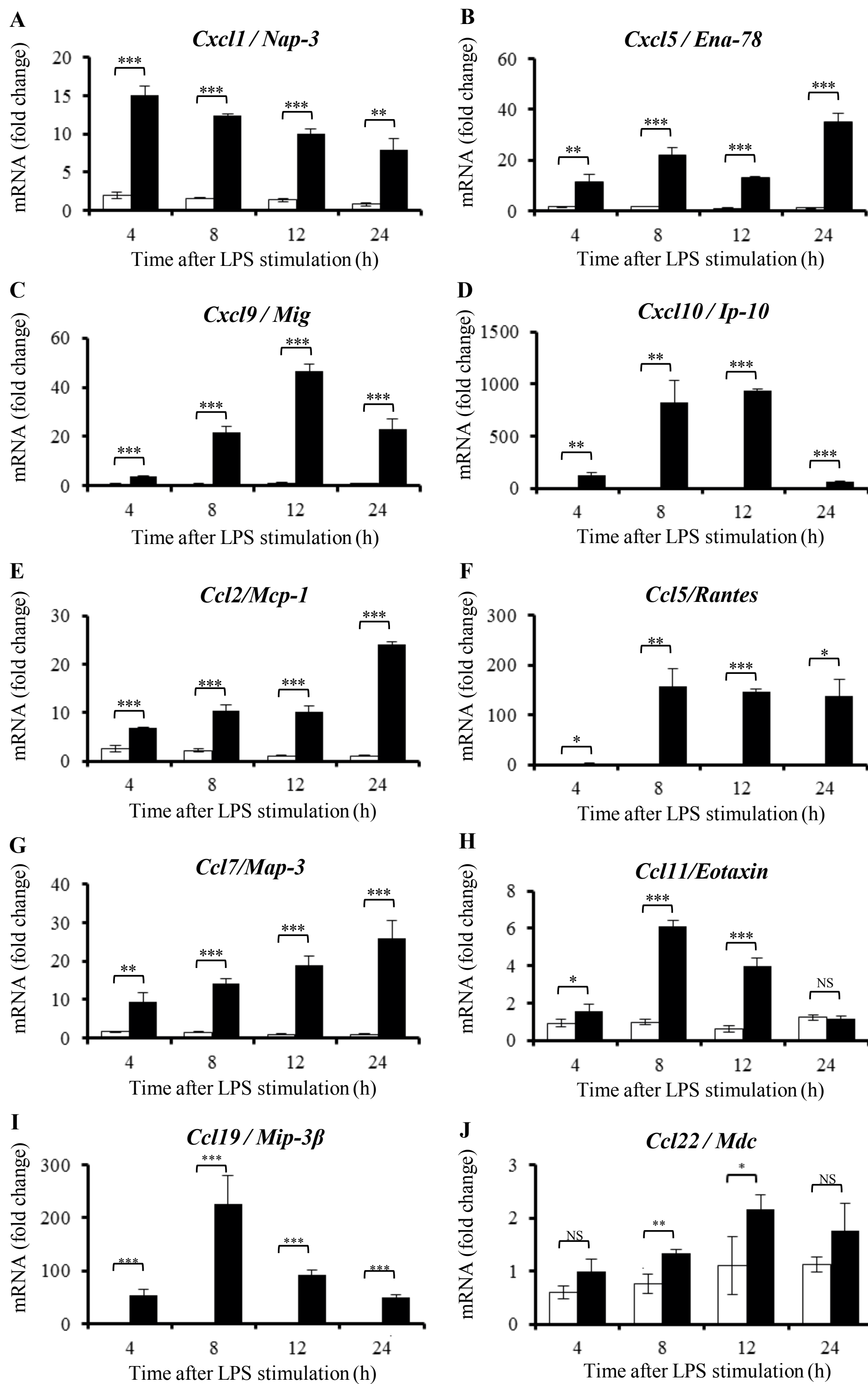

Fig.2

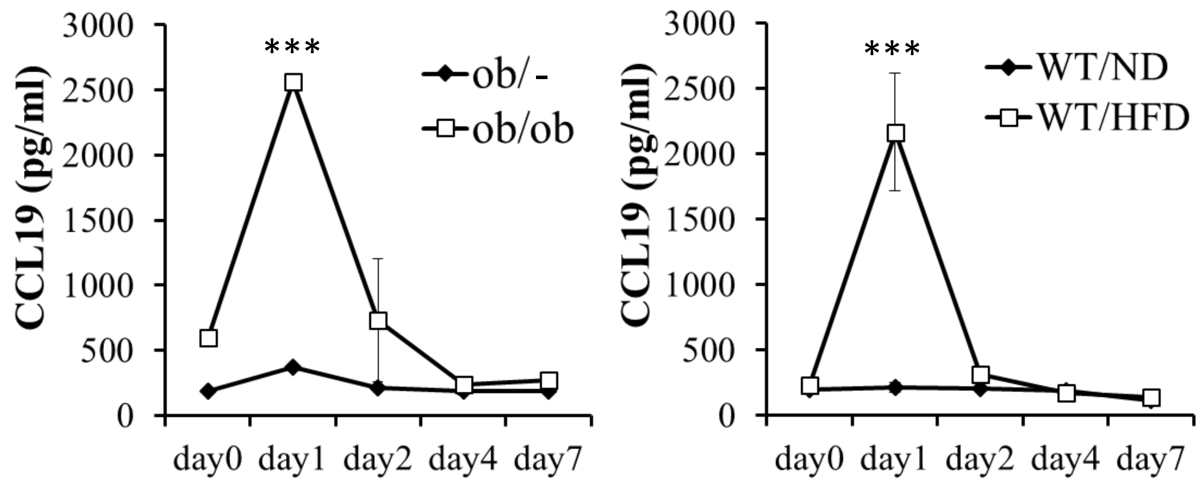

Fig.3

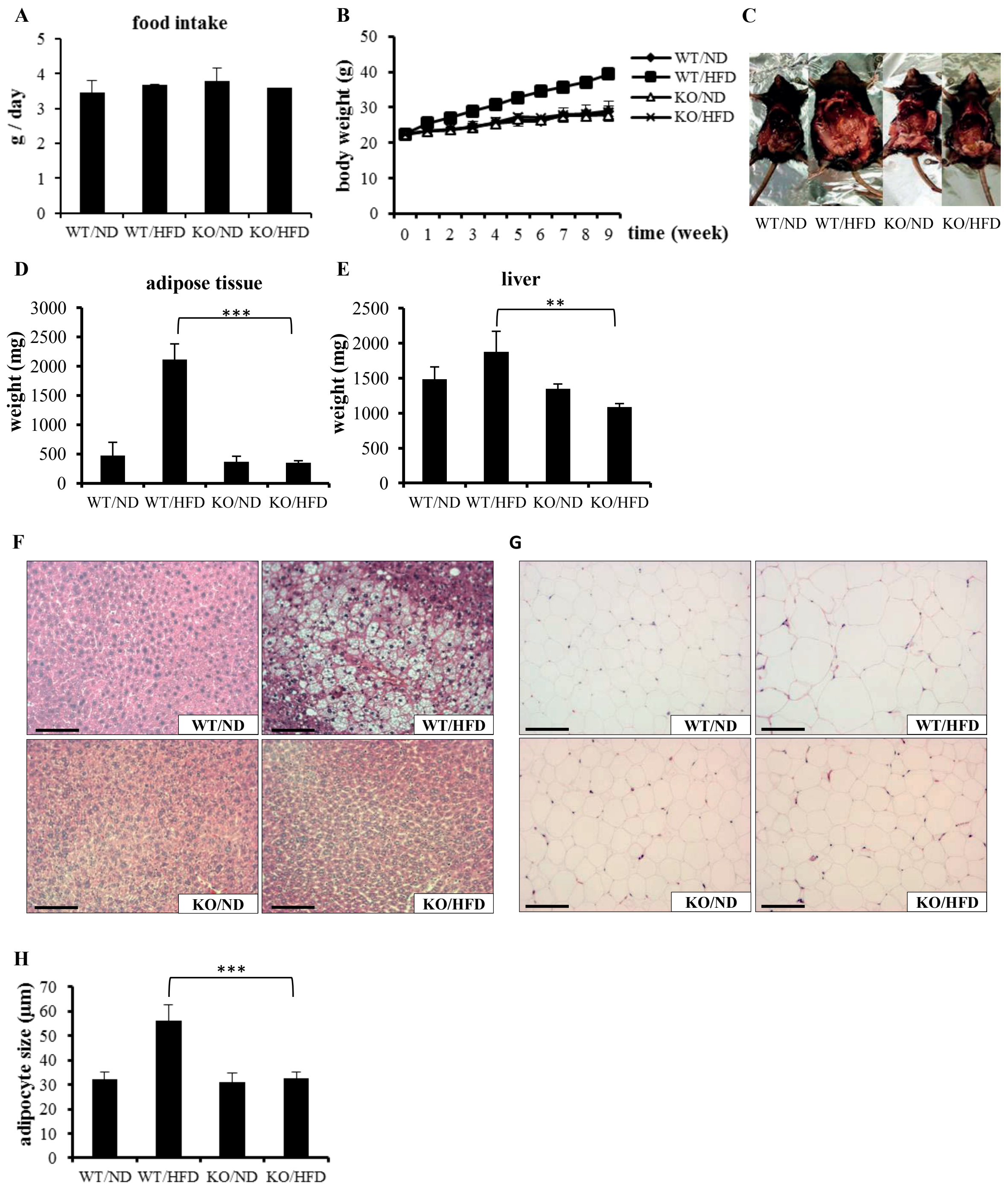

Fig.4

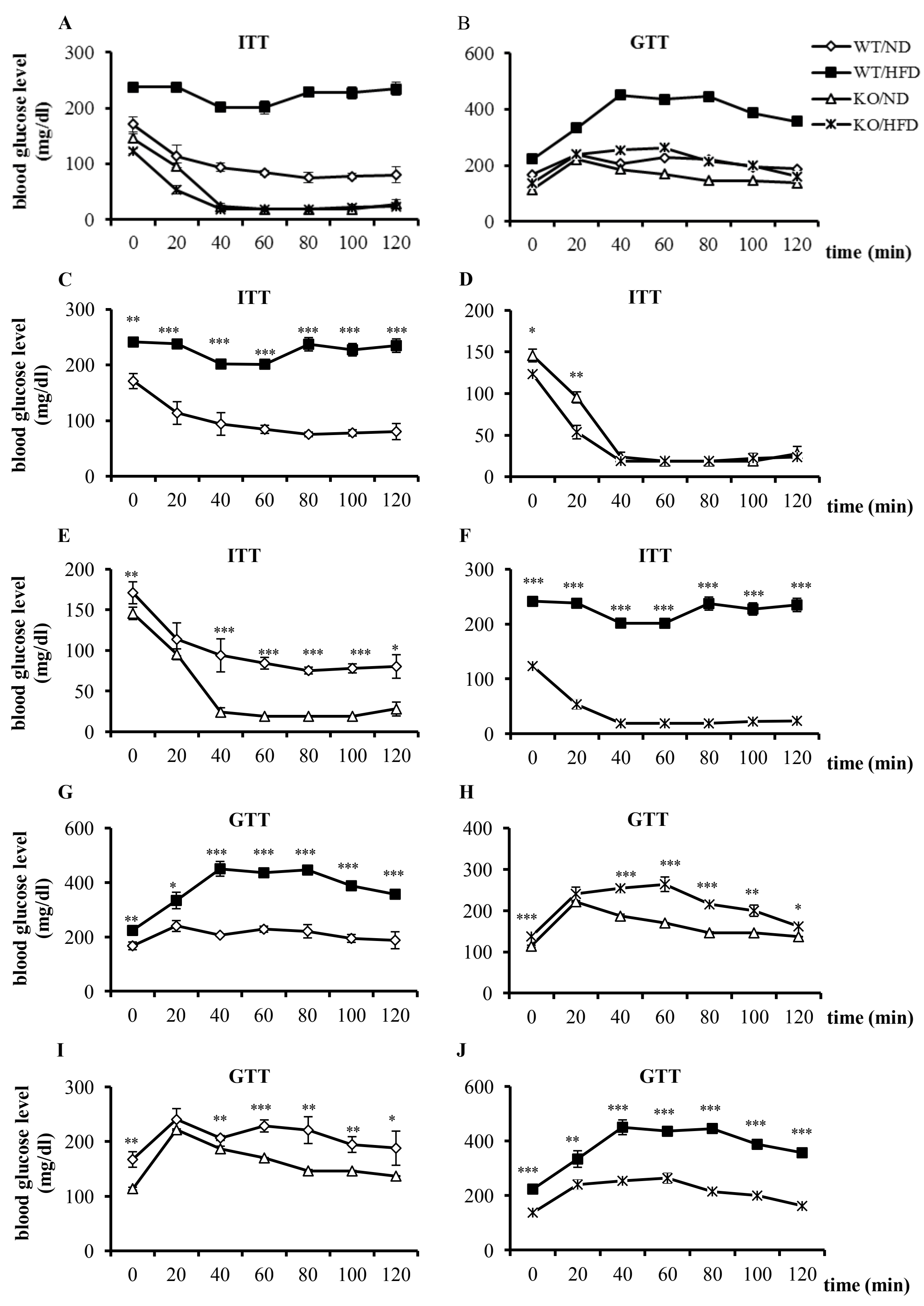

Fig.5

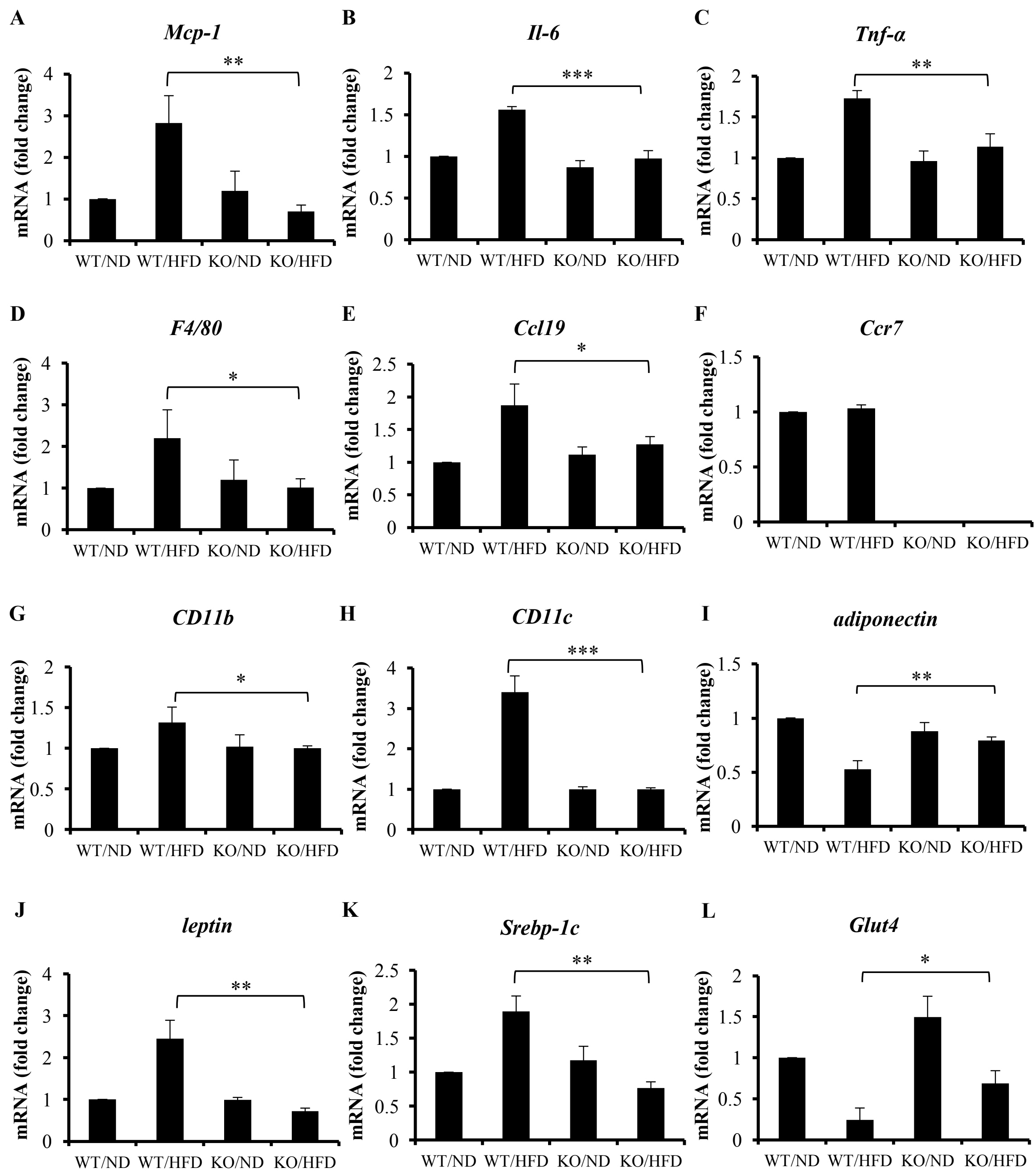

Fig.6

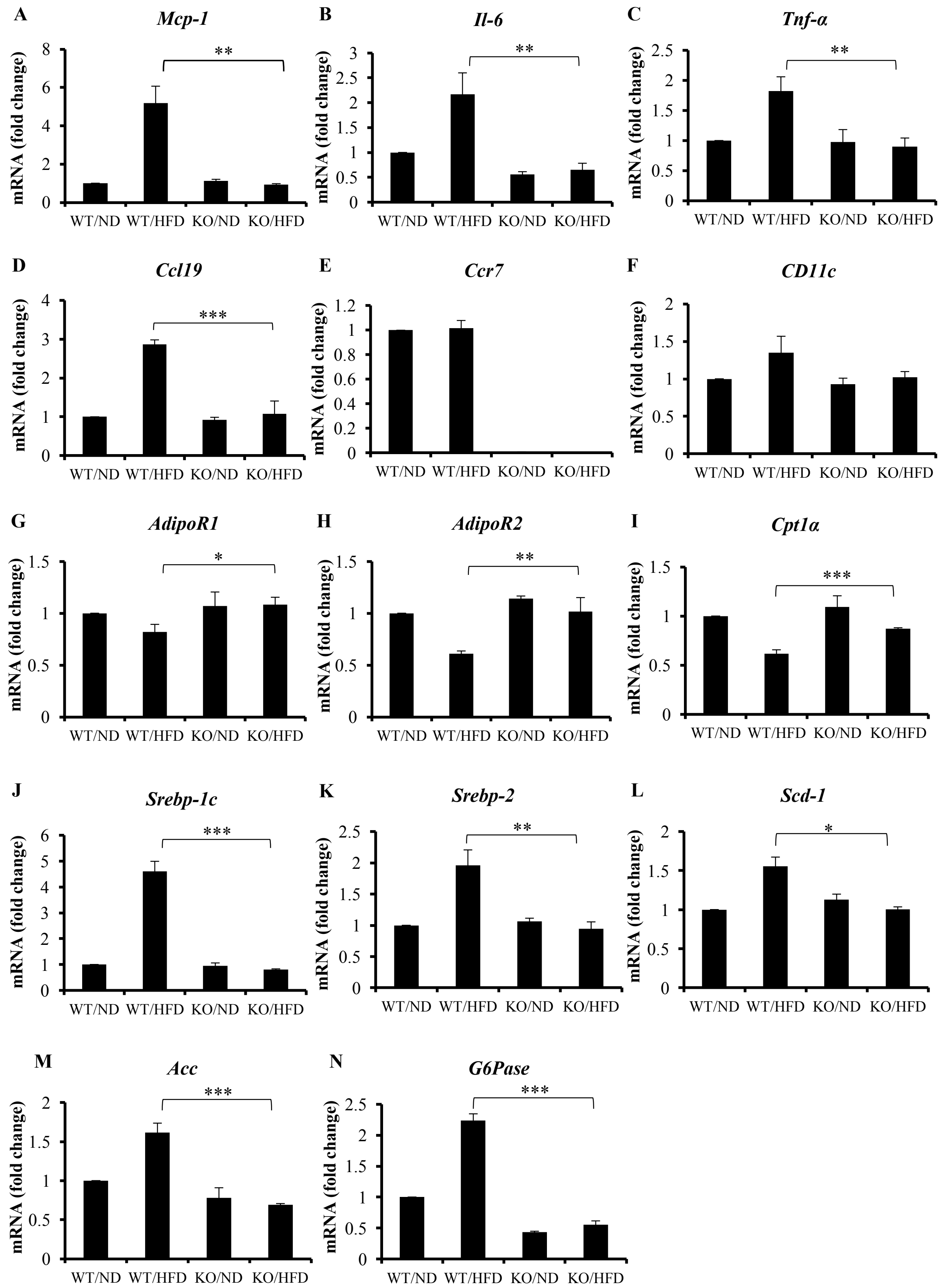

Fig.7

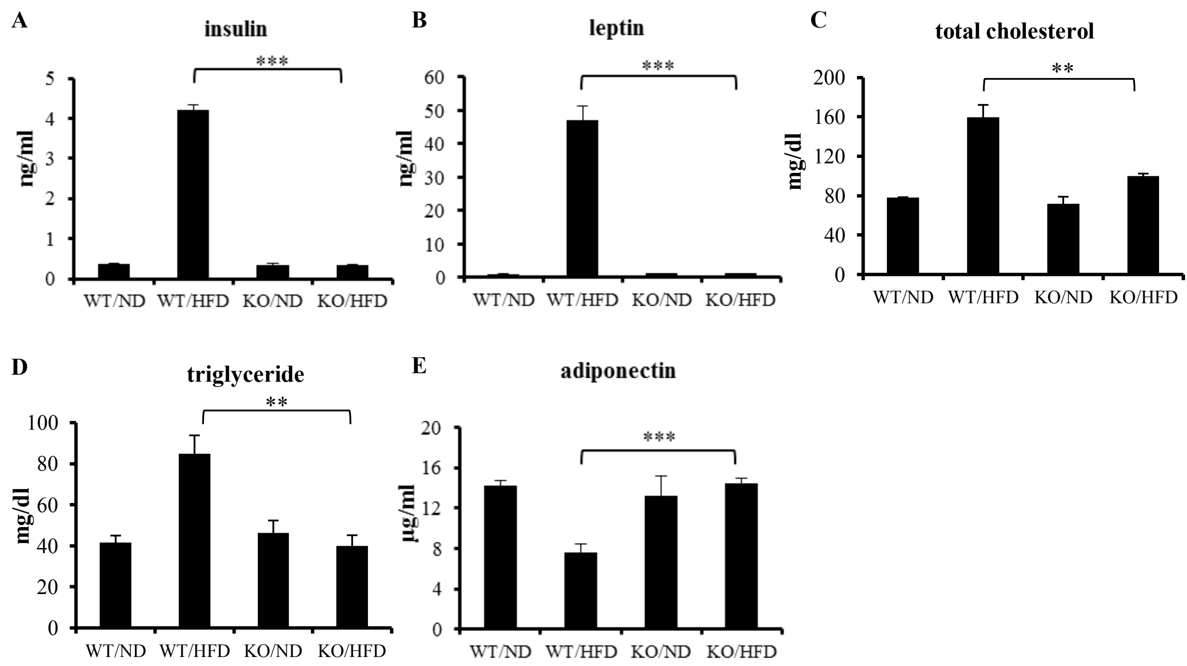

Table 1

Chemokines highly expressed in adipocytes co-cultured with macrophages in the presence of endotoxin (data picked up from reference 15).

chemokines		receptor	relative expression (LPS(+) / control)				reported observations								ref.
			4h	8h	12h	24h	mouse model	weight	insulin	GTT	ITT	fatty acid synthesis / metabolism	macrophage infiltration	cytokine/cell marker expression	
CXC	CXCL1, NAP-3, Gro-α, GRO1	CXCR1 CXCR2	**	**	*	*									
	CXCL5, ENA-78	CXCR2	**	**	**	**									
	CXCL9, MIG, CRG-10	CXCR3		**	**	**									
	CXCL10, IP-10, CRG-2	CXCR3	****	*****	*****	***	ApoE KO, CXCL10 KO	→						F4/80↓, CXCR3↓, CD4↓, CCL22↑	8
CC	CCL2, MCP-1	CCR2 CCR4 CCR11	*	**	**	**	CCR2 KO	→	↓	↓	↓	FABP4↑, Gpam↑, Lipe↑, PPARγ↑	↓	TNF-α↓, CD68↓, EMR1↓	3
							CCL2 KO	→	↓	↓	↓	adiponectin↑, G6Pase↓, SREBP1c↓	↓	TNF-α↓, CD68↓, F4/80↓	4
							CCL2 KO	↑	↑	↑	→	adiponectin↓	↑	F4/80↑, CD11b↑	5
							CCL2 KO	↑	→	→	→	adiponectin→	↑	CD68↑, CCL7↓	6
							CCR2 KO	→	→	↓	→		↓	MPO↑, IL8RB↑, CXCL1/CXCL2↑	7
	CCL5, RANTES	CCR1 CCR3 CCR4		****	****	****	CCR5 KO	→					↓	MOMA-2↓	9
							CCR5 KO	→	↓	↓	↓	SREBP1↓, FASN↓, SCD1↓	↓	F4/80↓, CD68↓, CD11c↓, TNF-α↓	10

		CCR5					CCR5 KO	→	→	↑			↓	CD11c↓	11
	CCL7, MCP-3, MARC	CCR1 CCR2 CCR3	*	**	**	**									
	CCL11, Eotaxin	CCR2 CCR3 CCR5		*											
	CCL19, MIP-3β, ELC, Exodus-3, CKβ11	CCR7 CCR11	***	****	***	**									
	CCL22, MDC, DC/β-CK	CCR4													

*:5-time **:10-time ***:50-time ****:100-time *****:500-time

FABP4 fatty acid binding protein 4

Gram Gestion Prévoyance Assurance Maladie

Lipe lipase, hormone sensitive

PPARɤ peroxisome proliferator-activated receptor gamma

FASN fatty acid synthase

SCD1 Stearoyl-CoA desaturase-1

EMR1 EGF module-containing mucin-like hormone receptor, EGF-like module containing mucin-like hormone receptor-like-1

MPO myeloperoxidase

IL8RB interleukin 8 receptor B

MOMA-2 Monocytes/Macrophages antibody

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