Caspase-1 Deficiency Promotes High-Fat Diet-Induced Adipose Tissue Inflammation and the Development of Obesity

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ABSTRACT

Caspase-1 is a cysteine protease responsible for the processing of the proinflammatory cytokine interleukin-1β and activated by the formation of inflammasome complexes. Although several investigations have found a link between diet-induced obesity and caspase-1, the relationship of remains controversial. Here, we found that mice deficient in caspase-1 were susceptible to high-fat diet-induced obesity with increased adiposity as well as normal lipid and glucose metabolism. Caspase-1 deficiency clearly promoted the infiltration of inflammatory macrophages and increased the production of C-C motif chemokine ligand 2 (CCL2) in the adipose tissue. The dominant cellular source of CCL2 was stromal vascular fraction rather than adipocytes in the adipose tissue. These findings demonstrate a critical role of caspase-1 in macrophage-driven inflammation in the adipose tissue and the development of obesity. These data provide novel insights into the mechanisms underlying inflammation in the pathophysiology of obesity.

Key words: adipocytes; cytokines: inflammasome; macrophages; stromal vascular fraction
INTRODUCTION

Obesity is a major risk factor for insulin resistance, hyperglycemia, dyslipidemia, and hypertension, which are collectively known as metabolic syndrome, and is a growing global public health concern. These metabolic disorders increase the risk for cardiovascular disease and type 2 diabetes and contribute to increased mortality and morbidity. Although the pathogenesis of obesity is complex and involves various factors, recent studies have revealed that sterile inflammation in the adipose tissue is involved in the development of obesity (3, 5). Indeed, the infiltration of macrophages into the adipose tissue is observed in animal models of obesity as well as in obese human subjects with metabolic syndrome. These cells are the major source of inflammatory cytokines and chemokines including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and C-C motif chemokine ligand 2 (CCL2, also known as monocyte chemoattractant protein-1 [MCP-1]). These factors in turn recruit inflammatory cells and further promote adipose tissue inflammation. However, the mechanisms of adipose tissue and sterile inflammation in obesity have not been fully understood.

Caspase-1, also known as an IL-1β-converting enzyme (ICE), is a cysteine protease responsible for the proteolytic cleavage of the proinflammatory cytokine IL-1β (4, 17). The role of caspase-1 is well characterized in the context of inflammasomes. Inflammasomes are intracellular large multiprotein complexes that regulate the release of IL-1β release and subsequent inflammation (3, 16). We have recently shown that the nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3 (NLRP3, also known as NALP3 and cryopyrin) inflammasome-mediated sterile inflammation is involved in various cardiovascular and renal diseases (9, 12, 13, 23, 26, 27, 29). Our findings are further supported by recent works showing that NLRP3 inflammasomes are key mediators of other diseases, including gout, pseudogout, asbestosis, silicosis, Alzheimer’s disease, metabolic syndrome, and type 2 diabetes (3, 16). However, inconsistent results have been reported with respect to the link between diet-induced obesity and caspase-1 (14, 22, 28). Furthermore, several studies have identified numerous proteins as potential substrates for caspase-1, suggesting that caspase-1
exerts inflammasome-independent functions. Thus, the function of caspase-1 remains under
debate. Therefore, its role in the development of obesity is currently controversial.

In the present study, we investigated the effect of caspase-1 deficiency on the
development of obesity and found that caspase-1-knockout (Casp1-KO) mice are susceptible to
the development of high-fat diet (HFD)-induced obesity with increased adiposity and
inflammation. Furthermore, we found that elevated of CCL2 mediate increased infiltration of
macrophages in the adipose tissue by caspase-1 deficiency. These findings indicate that
caspase-1 contributes to the development of obesity via a CCL2/C-C chemokine receptor 2
(CCR2) axis in the adipose tissue and provide insights into the mechanism underlying
inflammation in the pathophysiology of obesity.

MATERIALS AND METHODS

Animals

All animal experiments were approved by the Use and Care of Experimental
Animals Committee of the Jichi Medical University Guide for Laboratory Animals, and were
carried out in accordance with the Jichi Medical University guidelines. C57BL/6J wild-type
(WT) mice were purchased from Japan SLC, Inc. (Hamamatsu, Japan). Casp1-KO mice were
generated as described previously (15, 25). IL-1β-KO mice were kindly provided by Dr.
Yoichiro Iwakura (Tokyo University of Science, Chiba, Japan) (6). The male mice (9–10
week-old) were fed with either 60 kcal% HFD (D12492: Research Diets, Japan LSG, Tokyo) or
a normal chow diet (CE-2; CLEA Japan Inc., Osaka). Each mouse was weighed every 2 weeks
and at terminal points, the mice were fasted for 6 h, weighed again, and then blood was
collected to obtain serum. After perfusion, the tissues were carefully excised and weighed.

Pair-feeding experiments

The results of the food intake were obtained from the mice individually housed in
cages; the weight of the freely available HFD in a jar was measured every 3 or 4 days, and the
daily food intake was calculated. Based on these results, we set up a pair-feeding experiment to
compare the body weight changes of mice fed with the same amount (2.6 g) of HFD every day
for 56 days.

Micro-CT analysis

A micro-CT analysis was performed using LaTheta LCT-200 (Hitachi Aloka Medical, Tokyo, Japan). The mass of muscle, visceral, and subcutaneous fat was analyzed using the LaTheta software (Hitachi Aloka Medical).

Biochemical test

Blood was collected from the tail vein of 6 h-fasted mice. The blood glucose levels were measured using a Terumo MEDISAFE™ Blood Glucose Meter (Terumo Co., Tokyo, Japan). Serum levels of the total cholesterol (TCHO) and triglycerides (TG) were measured using a FUJI DRI-CHEM system (Fujifilm, Tokyo, Japan).

Glucose tolerance test (GTT)

A GTT was performed in mice fed a normal chow or HFD for 8 weeks. Mice were fasted for 6 h before the test with free access to water. Blood glucose was then measured just before the intraperitoneal glucose injection (1 g/kg body weight in saline) and subsequently at 15, 30, 60, 90, and 120 min post-administration.

Flow cytometric analysis

Infiltrating leukocytes in the epididymal white adipose tissue (WAT) were analyzed by flow cytometry as described previously (11). The data on flow cytometry was obtained using FACSVerse (BD Biosciences, San Jose, CA) and analyzed using FlowJo software (Treestar, Inc., San Carlos, CA).
Histology

Hematoxylin and eosin (HE) staining was performed as described previously (18). Digital images of the HE-stained histopathology of WAT were captured using a light microscope FSX-100 (Olympus, Tokyo, Japan). The size (relative area) of adipocytes was measured by NIH imaging software (Image J 1.44p, National Institute of Health).

Respiratory gas and locomotor analyses

Mice were housed individually in a metabolic chamber for 48 h to allow them to adapt to the environment and to attain a constant respiratory exchange ratio (RER). Respiratory gas (O2 and CO2) analysis was performed using an open-circuit metabolic gas analysis system connected directly to a mass spectrometer (Arco2000; ArcoSystem, Chiba, Japan). Oxygen consumption (VO2), and carbon dioxide production (VCO2) of each mouse were measured every 5 min in each cage. RER was calculated as the ratio VCO2/VO2. Locomotor activity of each mouse was determined using an activity monitoring system (ACTIMO-100; Shinfactory, Fukuoka, Japan) combined with individual metabolic chambers.

Measurement of inflammatory cytokines

Levels of CCL2, TNF-α, IL-6, IL-12 (p70), interferon (IFN)-γ, IL-1β, IL-18, leptin, adiponectin, and insulin were assessed using a mouse enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, BioVendor, and Shibayagi Co., Ltd.) or cytometric bead array (CBA) kit (BD Biosciences). For the CBA analysis, flow cytometry (FACSVerse, BD Biosciences) was used. WAT protein was extracted in RIPA buffer with protease inhibitor cocktail and CHCl3. Total protein concentration was measured using Pierce BCA protein assay kit (Thermo Scientific).

Migration assay

Resident peritoneal macrophages from the mice were harvested, and macrophage
migration in response to CCL2 was assessed using 5-μm pore size transwell plates.

Real-time RT-PCR analysis

Total RNA was prepared from the adipose tissues using ISOGEN (Nippon Gene Co., Ltd., Toyama, Japan) following the manufacturer’s instructions. Real-time RT-PCR analysis was performed using the Takara TP960 PCR Thermal Cycler Dice Detection System (Takara Bio Inc, Shiga, Japan) to detect mRNA expression. Gene expression was normalized using Actb (β-actin) expression using the software provided with the system. Sequences of primers used for real-time RT-PCR analysis have been described elsewhere (11).

Cell and adipose tissue cultures

Adipose tissues (epididymal and subcutaneous WAT tissues) were carefully excised from WT or Casp1-KO mice on normal chow diet and divided into pieces of equivalent size. The pieces were weighed and cultured overnight in 1 mL of Dulbecco’s modified Eagle’s medium (DMEM; Sigma) supplemented with 10% fetal bovine serum (FBS), and 1% antibiotic-antimycotic solution (Sigma). The next day, culture media were replaced with new media in the presence or absence of 10 ng/mL LPS, and the tissues were cultured for 24 h.

To obtain the adipocytes and stromal vascular fraction (SVF) cells, freshly isolated mouse adipose tissue from WT or Casp1-KO mice on normal chow diet was cut into small pieces and digested in 0.5 mg/mL collagenase type II (Calbiochem, San Diego, CA) in Krebs-Ringer bicarbonate-HEPES (KRBH) buffer at 37°C for 40 min. After washing twice in DMEM, tissue cells were filtrated through series of nylon mesh, and the single cell suspension was centrifuged at 750 x g at 4°C for 10 min. The floating cells were used as adipocytes, and the precipitated cells were used as SVF cells. The cells (adipocytes: 2 x10^5 cells/mL; SVF cells: 5 x10^5 cells/mL) were seeded and cultured overnight, the culture media were replaced with new media in the presence or absence of 10 ng/mL LPS, and the cells were cultured for 24 h.
Statistical analysis

Outcomes with a normal distribution (e.g., flow cytometry results) were analyzed by parametric unpaired t-tests. Outcomes without a normal distribution were analyzed by the following: comparisons of unpaired outcomes were assessed using the Mann–Whitney test; differences among more than three groups were assessed using a nonparametric Kruskal–Wallis test; and longitudinal analyses were performed for body weight and GTT. All analyses were performed using Stata software, release 13 (Stata Corp., College Station, TX).

RESULTS

Caspase-1 deficiency promoted HFD-induced WAT accumulation and obesity

We first examined whether a caspase-1 deficiency could influence the development of obesity in mice fed with a normal chow diet or HFD for 8 weeks. In WT mice, the HFD increased the body weight gain from that in mice fed a normal chow diet (Fig. 1A). Interestingly, Casp1-KO mice gained significantly more body weight than the WT mice did on the HFD. On a normal chow diet, the Casp1-KO mice tended to have greater body weight gain than the WT mice did, but this difference did not reach a statistical significance. Therefore, the following experiments were primarily conducted on HFD feeding. An abdominal CT analysis revealed that visceral and subcutaneous fat contents were also significantly increased in Casp1-KO mice, whereas the muscle content was decreased (Fig. 1B and C). Furthermore, the relative weight (tissue weight/body weight) of the epididymal and subcutaneous WATs in the Casp1-KO mice was significantly higher than that of the WT mice (Fig. 1D–F). Consistent with this finding, the increased size of the adipocytes was ascertained histologically in both types of WAT in the Casp1-KO mice (Fig. 1G, Supporting Figure 1 for reviewers only). These findings indicate that Casp1-KO mice are susceptible to HFD-induced obesity with increased adiposity.

Because caspase-1 is an ICE and induces IL-1β maturation, we next assessed the effect of HFD feeding on the development of obesity using IL-1β-KO mice. IL-1β-KO mice
significantly gained less body weight than WT mice on HFD feeding (Fig. 1H). In addition, the
relative weight (tissue weight/body weight) of the epididymal and subcutaneous WATs in
IL-1β-KO mice tended to be less than that of WT mice (Fig. 1I and J). These results indicate
that Casp1-KO mice are susceptible to HFD-induced obesity, independently of IL-1β.

*Caspase-1 deficiency had no effects on lipid profiles, glucose intolerance, energy expenditure
and locomotor activity*

Since obesity leads to metabolic disorders (e.g., dyslipidemia and hyperglycemia),
we next determined the levels of serum TCHO and TG, blood glucose, and plasma insulin. The
levels of these parameters were significantly increased in both WT and Casp1-KO mice by HFD
feeding (Fig. 2A–D). The levels of blood glucose and plasma insulin tended to be higher in
Casp1-KO mice than those in WT mice, but they did not reach the statistical significance.
Furthermore, the GTT on a normal and HFD feeding exhibited no differences in glucose
intolerance between the WT and Casp1-KO mice (Fig. 2E and F). We also assessed energy
expenditure and locomotor activity in both strains on HFD feeding and found no significant
differences in VO_2, RER, and locomotor activity between WT and Casp-1 KO mice (Fig. 2G-I).
These findings suggest that caspase-1 deficiency had no effects on lipid, glucose metabolism,
energy expenditure, or locomotor activity.

*Caspase-1 deficiency enhanced WAT accumulation and obesity in pair-fed mice*

We observed that the food intake tended to be slightly higher in Casp1-KO mice than
in WT mice fed a chow and HFD feeding (Fig. 3A). To ascertain whether the HFD-induced
obesity in Casp1-KO mice is attributed to increased food intake, we performed pair-feeding
experiments. Similar to the free-feeding conditions, the body weight gain was significantly
increased in the Casp1-KO mice under pair-feeding conditions from that in the WT mice (Fig.
3B). An abdominal CT analysis revealed that the subcutaneous fat contents were significantly
increased in the Casp1-KO mice (Fig. 3C and D). Epididymal fat weight was also increased in
the Casp1-KO mice from that in the WT mice, but this difference did not reach statistical
significance ($p = 0.1161$, Fig. 3E). However, there was no difference in the liver weight
between these mice. These findings indicate that caspase-1 deficiency enhanced WAT
accumulation and obesity independent of food intake.

Caspase-1 deficiency influenced serum levels of IL-18, leptin, and adiponectin

Because caspase-1 also cleaves pro-IL-18 into its mature form (4), and IL-18-KO
mice spontaneously develop obesity (19), we assessed serum IL-18 levels, and found that IL-18
was detectable in HFD-fed WT mice, whereas IL-18 was not detected in HFD-fed Casp1-KO
mice (Fig. 3F). We also assessed serum levels of leptin and adiponectin, and found that leptin
and adiponectin were significantly increased and decreased, respectively, in HFD-fed
Casp1-KO mice, compared to those in HFD-fed WT mice (Fig. 3G and H). These findings
suggest that IL-18, leptin, and adiponectin are involved in the development of HFD-induced
obesity in Casp1-KO mice.

Caspase-1 deficiency promoted macrophage infiltration in WAT

Since infiltration of macrophages in WAT is the hallmark of obesity, we assessed
this infiltration in WT and Casp1-KO mice fed with HFD using flow cytometry analysis. The
number of infiltrated macrophages (CD11b$^{+}$F4/80$^{+}$) was significantly increased in the
epididymal and subcutaneous WAT of the Casp1-KO mice from that in the respective tissue of
the WT mice (Fig. 4A–B). The number of inflammatory macrophages (CCR2$^{+}$F4/80$^{+}$) was also
increased in the WAT of Casp1-KO mice (epididymal WAT, $p = 0.0532$; subcutaneous WAT, $p
< 0.05$). Furthermore, we performed real-time RT-PCR analysis to assess gene expression of
M1 and M2 macrophage markers (M1: Cd86 and Nos2; M2: Cd206, Cd163, and Chil3) in the
subcutaneous WAT of HFD-fed WT and Casp1-KO mice. Cd206 expression was significantly
decreased in Casp1-KO mice. Expression of other M2 markers, Cd163, and Chil3, also tended
to be decreased in Casp1-KO mice (Fig. 4C). On the other hand, no difference of M1 marker
expression was observed (Fig. 4D). These findings suggest that caspase-1 deficiency promoted the inflammatory macrophage infiltration and influenced M1 and M2 macrophage polarization in the adipose tissue.

Caspase-1 deficiency enhanced CCL2 production in WAT

There are two possibilities to explain the findings that macrophage infiltration was promoted in the WAT of Casp1-KO mice: 1) the increased migration activity of Casp1-KO macrophages; and 2) the increased production of macrophage-tropic chemokines such as CCL2.

To test the first possibility, we assessed macrophage migration using a transwell migration assay and found that there were no significant differences in the migration activity in response to CCL2 between macrophages from the WT and Casp1-KO mice (Fig. 4E). To test the second possibility, we assessed the inflammatory cytokine levels in the subcutaneous WAT because increased adiposity and macrophage infiltration were found to be more prominent in the subcutaneous WAT of the Casp1-KO mice (Fig. 3C and 4). In the subcutaneous WAT, the CCL2 levels were significantly increased the Casp1-KO mice from those in the WT mice (Fig. 5). The levels of TNF-α, IL-6, IL-12p70, IFN-γ, and IL-1β were elevated in the Casp1-KO mice, but it did not reach statistical significance. This result is in accordance with the findings that the infiltration of inflammatory macrophages was increased in the WAT of Casp1-KO mice.

To investigate the tissue and cellular sources of CCL2, the epididymal and subcutaneous WATs were isolated and cultured in the presence or absence of the prototypical inflammatory stimulus, lipopolysaccharide (LPS). LPS clearly induced CCL2 production in both types of WAT from the WT and Casp1-KO mice, but CCL2 production in response to LPS was significantly higher in the WATs of the Casp1-KO mice than in those of the WT mice (Fig. 6A and B). The WAT is composed of different cell populations including adipocytes and non-adipocytes. The non-adipocyte cells were fractionated from the WAT after collagenase digestion, known as SVF. Therefore, we cultured SVF and adipocytes, and assessed the production of CCL2 in response to LPS. A marked CCL2 production was observed in the
subcutaneous WAT-derived adipocytes and SVF (Fig. 7A and B). Furthermore, the SVF culture from the epididymal and subcutaneous WATs produced more CCL2 than the adipocyte culture did. Additionally, CCL2 production in response to LPS was more apparent in the cell fractions isolated from Casp1-KO mice. These findings suggest that SVF is the dominant cellular source of CCL2 in the WAT.

**DISCUSSION**

Inflammation plays a pivotal role in the pathophysiology of obesity. Since caspase-1 participates in the inflammasome assembly and is an inflammatory caspase, the link between diet-induced obesity and caspase-1 was demonstrated (14, 22, 28). However, the role of caspase-1 in obesity is still unclear. In this study, we found that mice deficient in caspase-1 were susceptible to HFD-induced obesity with increased adiposity, normal lipid profiles and glucose metabolism. The caspase-1 deficiency clearly promoted the infiltration of inflammatory macrophages and increased the production of CCL2 in the adipose tissue. The dominant cellular source of CCL2 was stromal vascular fraction rather than adipocytes in the adipose tissue. These findings demonstrate a critical role of caspase-1 in macrophage-driven inflammation in the adipose tissues and also provide insights into the mechanisms underlying inflammation in the pathophysiology of obesity.

Increasing evidence indicates that NLRP3 inflammasomes are implicated in sterile inflammatory diseases. Indeed, the link between NLRP3 inflammasomes and obesity has been reported, yet the role of caspase-1 in obesity remains controversial. Stienstra et al. (22) demonstrated that Casp1-KO mice were resistant to HFD-induced obesity and insulin resistance through enhanced energy expenditure. Conversely, two studies by Kotas et al. (14) and Wang et al. (28) reported that Casp1-KO mice were susceptible to HFD-induced obesity. Although the latter studies are consistent with our results, they did not evaluate any macrophage-driven inflammation. Thus, our results provide additional information regarding increased obesity in Casp1-KO mice.
Another important point to be noted is that different HFD was used in each study. In the studies by Stienstra et al. (22) and Wang et al. (28), HFD containing 45 kcal% fat was used, whereas HFD containing 60 kcal% fat was used in the study by Kotas et al. (14). Therefore, we postulate that the diet used in each study may have influenced the effect of caspase-1 deficiency on the development of obesity. Supporting this, we used the same HFD (60 kcal% fat) as in the Kotas’s study and found consistent results with their findings: increased body weight and fat mass, and no difference of glucose metabolism and energy expenditure. The differences between our study and Kotas’s study are serum levels of TG and TCHO on normal chow diet (Kotas et al. did not provide the data on HFD feeding). In this regard, recent studies demonstrated that gut flora can significantly influence lipid metabolism (1). Therefore, we speculate that the discrepancy may be partially explained by the changes of gut flora.

In this study, we showed that a caspase-1 deficiency promoted the development of obesity in an inflammasome- and IL-1β-independent manner; this finding is based on the following observations: First, the IL-1β levels in the adipose tissue were not decreased in the Casp1-KO mice. Second, IL-1β-KO mice significantly gained less body weight than WT mice on HFD feeding. In this regard, inflammasome-independent functions of NLRP3 or ASC have recently received increasing attention. For instance, Ippagunta et al. (8) reported that an inflammasome-independent role of ASC, which regulates the migration of lymphocytes, and contributes to the generation of adaptive immune responses. Shigeoka et al. (21) demonstrated that renal I/R injury was attenuated in Nlrp3-KO mice, but not in Asc-KO and Casp1-KO mice, and concluded that NLPR3 contributes to renal I/R injury through an inflammasome-independent pathway. Similarly, we have recently demonstrated that NLRP3 regulates neutrophil migration and contributes to the pathophysiology of hepatic I/R injury and hyperoxia-induced lung injury independent of the inflammasomes or IL-1β (7, 18). More recently, Bruchard et al. (2) reported that NLRP3 in CD4+ T cells was required for the differentiation of T helper type 2 cells in an inflammasome-independent manner. These findings imply that caspase-1 can function independently of the inflammasomes. Indeed previous studies
using a proteomic-based approach indicate that in addition to IL-1β, numerous proteins act as candidates for caspase-1 substrates (4, 17). Of these, IL-18 has shown to be implicated in the progression of obesity (19). In addition, IL-18 is processed in an inflammasome-dependent and independent manner (20). Therefore, we assessed serum IL-18 levels and showed that no IL-18 was detected in HFD-fed Casp1-KO mice, suggesting the contribution of IL-18 to this process. Further studies are required to elucidate the precise role of caspase-1 in adipose tissue inflammation and the development of obesity.

We found that in Casp1-KO mice, subcutaneous WAT was prone to inflammatory responses (e.g., inflammatory macrophage infiltration and CCL2 production), rather than the epididymal WAT. Additionally, we showed that the primary cellular source of CCL2 was SVF, rather than the adipocytes in the adipose tissue. The dominant cells in the SVF are leukocytes (e.g., macrophages and lymphocytes) and stromal cells (e.g., pre-adipocytes and fibroblasts), and macrophages produce a large amount of CCL2 (24). Thus, we assumed that CCL2 was produced by the infiltrating macrophages in the adipose tissue. Taken together, under HFD conditions, inflammatory macrophage infiltration is amplified via the CCL2/CCR2 axis in the adipose tissue of Casp1-KO mice. This in turn enhances adipose tissue inflammation and promotes the development of obesity.

There are several limitations of this study that should be noted: 1) we found that a caspase-1 deficiency increased the production of CCL2. However, the molecular mechanism underlying caspase-1 regulation of CCL2 production in the adipose tissue remains to be addressed; 2) since we observed that the IL-1β levels tended to be elevated in Casp1-KO mice, we postulated that there was little contribution of the inflammasomes and IL-1β to the development of obesity in these mice. Furthermore, the Casp1-KO mice used in this study and in other studies (14, 22, 28) lack both caspase-1 and caspase-11 (10, 15). Therefore, the precise contributions of the inflammasomes and caspase-1 remain to be determined in mice deficient in the inflammasome-related molecules, such as NLRP3, ASC, IL-18, or caspase-1 alone. Thus, further investigations are necessary to elucidate the precise role of caspase-1 in the development
of obesity.

In conclusion, we clearly demonstrate that caspase-1 deficiency promotes the development of obesity with increased adiposity, as well as normal lipid profiles and glucose metabolism. Caspase-1 deficiency also promotes the infiltration of inflammatory macrophages via the CCL2/CCR2 axis in the WAT, which accelerates the development of obesity. These findings demonstrate a critical role for caspase-1 in macrophage-driven inflammation in the adipose tissue and provide novel insights into the mechanism underlying inflammation in the pathophysiology of obesity.

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DISCLOSURES

The authors have no conflict of interest to declare relating this work.

AUTHOR CONTRIBUTIONS

H.K. and M.T. designed the study and wrote the manuscript; H.K., T.K., F.U., A.K., Y.E. M.K.,
REFERENCES


FIGURE LEGENDS

**Figure 1. Caspase-1 deficiency promotes HFD-induced WAT accumulation and obesity**

(A) Body weight curves of WT and Casp1-KO mice on a normal chow (n = 7 for each) or HFD (n = 8–9 for each). (B) Representative abdominal images of WT and Casp1-KO mice fed with HFD for 4 weeks by CT analysis (blue: muscle; pink: visceral fat; yellow: subcutaneous fat). (C) Quantitative analysis of muscle, visceral fat, and subcutaneous fat contents (n = 8–9 for each). (D) Body weight, epididymal WAT, (E) subcutaneous WAT, and (F) relative WAT weights (WAT weight/body weight) of WT and Casp1-KO mice fed with HFD for 8 weeks (n = 10–12). (G) Representative images of HE staining in epididymal and subcutaneous WATs in WT and Casp1-KO mice fed with HFD for 8 weeks. (H–J) Body weight (H), epididymal and subcutaneous WAT weight (I), and relative weight of epididymal and subcutaneous WAT (WAT weight/body weight) of WT and IL-1β-KO mice fed with HFD for 8 weeks (n = 3–5). Data are expressed as mean ± SEM. *p < 0.05; **p < 0.01.

**Figure 2. Effect of Caspase-1 deficiency on lipid and glucose metabolism, energy expenditure, and locomotor activity**

(A–C) Levels of serum TCHO (A), TG (B), blood glucose (C), and plasma insulin (D) in WT and Casp1-KO mice before and 8 weeks after HFD feeding (n = 7–10 for each). (E, F) Results of GTT in WT and Casp1-KO mice on a normal chow (E) and HFD (F) (n = 6 for each). (G–I) Data on energy expenditure. VO₂ (G) and RER (H) in WT and Casp1-KO mice fed with HFD (n = 5 for each). (I) Locomotor activity in WT and Casp1-KO mice fed with HFD (n = 5 for each). Data are expressed as mean ± SEM. *p < 0.05; **p < 0.01.

**Figure 3. Caspase-1 deficiency enhances WAT accumulation and obesity in pair-fed mice**

(A) Food intake in WT and Casp1-KO mice on a normal chow and HFD (chow, n = 3; HFD, n = 8–9). Average values of each type of food intake were shown above the bars. (B) Body weight curves of pair-fed WT and Casp1-KO mice on HFD (n = 6–7 for each). (C)
Representative abdominal images of pair-fed WT and Casp1-KO mice fed HFD for 8 weeks by CT analysis (blue: muscle; pink: visceral fat; yellow: subcutaneous fat). (D) Quantitative analysis of muscle, visceral fat, and subcutaneous fat contents of pair-fed WT and Casp1-KO mice on HFD for 8 weeks (n = 3–4 for each). (E) Relative tissue weight (liver or epididymal WAT weight/body weight) of pair-fed WT and Casp1-KO mice on HFD for 8 weeks (n = 6–7). (F–H) Levels of serum IL-18 (F), leptin (G), and adiponectin (H) in WT and Casp1-KO mice on HFD for 8 weeks (n = 5–6 for each). Data are expressed as mean ± SEM. *p < 0.05; **p < 0.01.

**Figure 4. Caspase-1 deficiency promoted macrophage infiltration in WAT**

(A and B) Representative images of infiltrated macrophages (CD11b^+ F4/80^+ and F4/80^+CCR2^+) in epididymal (A) and subcutaneous (B) WATs of WT and Casp1-KO mice fed with HFD for 8 weeks, by flow cytometry analysis. Quantitative analysis of infiltrated macrophages (CD11b^+ F4/80^+ and F4/80^+CCR2^+) in epididymal WAT (A) (n = 5–6) and subcutaneous WAT (B) (n = 6–7). Data are expressed as mean ± SEM. *p < 0.05; **p < 0.01. (C and D) Expression of macrophage M2 markers (C: Cd206, Cd163, and Chil3) and M1 markers (D: Cd86, and Nos2) in subcutaneous WAT of HFD-fed WT and Casp1-KO mice (n = 4 for each). (E) Migration activity in response to CCL2 (100 ng/mL) of primary WT and Casp1-KO macrophages (n = 4 for each). Data are expressed as mean ± SEM. *p < 0.05.

**Figure 5. Caspase-1 deficiency enhances inflammatory cytokine production**

Tissue levels of CCL2, IL-1β, TNF-α, IL-6, IL-12p70, and IFN-γ, in the subcutaneous WAT of WT and Casp1-KO mice fed HFD for 8 weeks (n = 6 for each). Data are expressed as mean ± SEM. *p < 0.05.

**Figure 6. Caspase-1 deficiency increases CCL2 production in WAT**

Tissue levels of CCL2 in the culture of epididymal (A) and subcutaneous (B) WAT of WT and
Casp1-KO mice in the presence or absence of LPS (100 ng/mL) for 24 h (n = 4 for each). Data are expressed as mean ± SEM. *p < 0.05.

**Figure 7. Caspase-1 deficiency increases CCL2 production in SVF and adipocytes**

CCL2 levels in the culture supernatants of SVF (A: $5 \times 10^5$ cells/mL) and adipocytes (B: $2 \times 10^5$ cells/mL) isolated from epididymal and subcutaneous WATs of WT and Casp1-KO mice in the presence or absence of LPS (100 ng/mL) for 24 h (n = 4 for each). Data are expressed as mean ± SEM. *p < 0.05.
Figure 1. Kimura H, et al.
Figure 3. Kimura H, et al.
Figure 6. Kimura H, et al.

A  epididymal WAT

B  subcutaneous WAT

(ng/mg)

LPS  WT  Casp1-KO

-  +  -  +

Mean ± SEM
Figure 7. Kimura H, et al.

A

CCL2 in primary SVF-cell culture

B

CCL2 in primary adipocyte culture