The ATP-P2X<sub>7</sub> Signaling Axis Is Dispensable for Obesity-Associated Inflammasome Activation in Adipose Tissue

Shengyi Sun,1 Sheng Xia,2 Yewei Ji,2 Sander Kersten,2,3 and Ling Qi1,2

Inflammasome activation in adipose tissue has been implicated in obesity-associated insulin resistance and type 2 diabetes. However, when and how inflammasome is activated in adipose tissue remains speculative. Here we test the hypothesis that extracellular ATP, a potent stimulator of inflammasome in macrophages via purinergic receptor P2X<sub>7</sub>, ligand-gated ion channel, may play a role in inflammasome activation in adipose tissue in obesity. Our data show that inflammasome is activated in adipose tissue upon 8-week feeding of 60% high-fat diet (HFD), coinciding with the onset of hyperglycemia and hyperinsulinemia as well as the induction of P2X<sub>7</sub> in adipose tissue. Unexpectedly, P2X<sub>7</sub>-deficient animals on HFD exhibit no changes in metabolic phenotypes, inflammatory responses, or inflammasome activation when compared with the wild-type controls. Similar observations have been obtained in hematopoietic cell-specific P2X<sub>7</sub>-deficient animals generated by bone marrow transplantation. Thus, we conclude that inflammasome activation in adipose tissue in obesity coincides with the onset of hyperglycemia and hyperinsulinemia, respectively, is not mediated by the ATP-P2X<sub>7</sub> signaling axis. The nature of the inflammasome-activating danger signal(s) in adipose tissue in obesity remains to be characterized. Diabetes 61:1471–1478, 2012

Obesity is a major health threat in both developing and developed countries and poses a significant risk factor for type II diabetes, arthritis, cardiovascular diseases, and even cancer. The progression of obesity is strongly associated with chronic inflammation in white adipose tissue (WAT) (1–4). Genetic studies in mice and clinical studies in humans have shown that downregulation of the inflammasome in WAT is associated with improvement of systemic glucose homeostasis and insulin sensitivity (5).

Proinflammatory cytokines such as interleukin-1β and -18 (IL-1β and -18) secreted by adipose tissue have been linked to insulin resistance during obesity (6–9). The maturation of IL-1β and IL-18 requires the activation of inflammasomes, including NLRP1 (Nod-like receptor family, pyrin domain containing 1), NLRP3, NLRC4 (Nod-like receptor family, caspase activation and recruitment domain containing 4), and AIM2 (absent in melanoma 2) inflammasome (10). The most well-studied inflammasome is NLRP3 inflammasome, a multiprotein complex consisting of NLRP3, ASC (apoptosis-associated speck-like protein containing COOH-terminal caspase activation and recruitment domain), and a cysteine protease caspase-1. The activation of inflammasome induces the cleavage of procaspase-1 to active caspase-1, which in turn processes pro–IL-1β and pro–IL-18 to bioactive IL-1β and IL-18 to be released (11). Several recent studies have demonstrated that the NLRP3 inflammasome is important for obesity-associated inflammation and the development of insulin resistance (12–15). Indeed, mice deficient in caspase-1 or NLRP3 were protected against obesity-associated inflammation and insulin resistance with reduced circulating IL-18, reduced IL-1β, and effector T cells in adipose tissue, and improved systemic insulin sensitivity (12–15). However, it remains unclear when and how inflammasome is activated in high-fat diet (HFD) feeding.

Studies on the immune system have revealed that the activation of inflammasome, or synthesis, processing, and release of mature IL-1β by macrophages, ex vivo requires two stimuli: first, an inflammatory stimulus, such as lipo-polysaccharide (LPS), primes cells to transcribe and synthesize pro–IL-1β, and then a second stimulus, such as ATP, nigericin, or bacterial toxins, is required for optimal inflammasome activation and maximal IL-1β release (10). An outstanding question with regards to inflammasome activation in obesity is what the two activating stimuli are in adipose tissue. Stimuli including ATP, glucose, or lipids such as palmitate and ceramides have been suggested to play a role in inflammasome activation in obese adipose tissue (12,14–16). However, physiological relevance and importance of these stimuli have not been documented with in vivo studies.

Extracellular ATP is a potent inflammasome-activating signal both in vitro and in vivo in some disease settings such as cancer, graft-versus-host disease, hypersensitivity, and sterile inflammation (17–21). Among the several purinergic receptors capable of ATP sensing (P2X<sub>1</sub>, P2X<sub>3</sub>, and P2Y<sub>1</sub>–13, 11, 12), P2X<sub>7</sub> (also known as P2RX<sub>7</sub>) is unique in its ability to activate inflammasome (22). P2X<sub>7</sub> is a cell-surface ion channel receptor activated by a high level of extracellular ATP and subsequently allows rapid influx of potassium ions to activate inflammasome (17,23–25). In vivo, extracellular ATP can be released from apoptotic and necrotic cells as a “danger signal” to upregulate inflammation in a P2X<sub>7</sub>-dependent manner (18–21,26), suggesting an interesting mechanism by which dying cells regulate inflammasome activation. Intriguingly, obesity is associated with increased cell death in adipose tissue and the aggregation of macrophages around dead cells, also known as “crown-like structure” (27–30). It has been suggested that adipocyte cell death may contribute to the progression of inflammation in adipose tissue (29,31), although this remains...
controversial (30). How cell death is linked to inflammation or inflammasome activation in obesity remains a mystery.

Here we addressed whether extracellular ATP links obesity, obesity-associated cell death, and inflammasome activation in adipose tissue via its receptor P2X7. Our data show that induction of both P2X7 expression and inflammasome activation in adipose tissue started at 8-week feeding of 60% HFD, coinciding with the onset of hyperglycemia and hyperinsulinemia. However, to our surprise, the ATP-P2X7 signaling axis is dispensable for inflammasome activation in obese adipose tissue.

**RESEARCH DESIGN AND METHODS**

**Mouse models.** Wild-type (WT) C57/B6 (CD45.2+) (006644), B6.129P2-P2rx7tm1Bhk/J (P2X7−/−, 005576), B6.129P2-P2y2tm1Bhk/J (P2Y2−/−, 00132), B6.SJL-Ptprca Pepcb/J (CD45.1, 002014), and B6.V-lym-3/J (lobob, 006382) mice were purchased from The Jackson Laboratory and bred in our facility. The P2X7−/− and P2Y2−/− mice had been back-crossed to the B6 background for 7 and 12 generations, respectively. VAT tissue lysates from 16-week HFD caspase-1−/− mice were provided by Dr. Rinke Stienstra (Wageningen University). Mice were housed in our pathogen-free facility with 13% low-fat diet (LFD), with 13% low-fat, and with 25% high-fat diet (HFD). All experiments were performed in accordance with the ARRIVE guidelines. All animals were monitored and treated according to institutional guidelines and were killed by high CO2 asphyxiation (211716; BD Biosciences). Four days later, mice were killed and blood serum was collected in animals upon 4 h fasting during the day. Circulating insulin levels were measured using the ELISA kit from E Bioscience supplier’s protocol. For tissue ELISA, a piece of VAT was lysed in 1 mL Tris-based lysis buffer by homogenization and IL-1β level was normalized to total VAT weight.

**RNA extraction, Q-PCR, and RT-PCR.** RNA extraction from cells and tissues and Q-PCR were carried out as previously described (32) using Trizol plus QiAeay kit (Qiagen) for adipocytes and adipose tissues with DNase digestion (Roche). Q-PCR data collected on the Roche LightCycler 480 were normalized to ribosomal 18S gene in the corresponding sample. For RT-PCR, the equivalent amount of RNA was reverse transcribed and amplified by 30 PCR cycles (95°C for 15 s, 55–58°C for 15 s, and 72°C for 20 s). The PCR products were separated on a 1.5% agarose gel and visualized using Bio-Rad ChemiDoc XRS+ system after exposure. Supplementary Table 1 lists primer sequences used in this study.

**Western blot and quantitation.** Tissues or cells were lysed in Tris-based lysis buffer containing 1% Triton X-100. Total lysates (10–20 µg) were run on a mini–SDS-PAGE as previously described (32). Antibodies specific for p-5473 serine/threonine protein kinase Akt (92718; Cell Signaling), Akt (sc-1618; Santa Cruz), inhibitor of nuclear factor-κB (IκB) (9242; Cell Signaling), caspase-1 p35, a marker for caspase-1 activation (13,16,34,35), and beta-actin (A2228; Sigma) were used at 1:1,000 dilutions. The secondary antibody goat anti-rabbit IgG horseradish peroxidase (HRP) and goat anti-mouse IgG HRP (1:10,000) were purchased from Bio-Rad. Quantification of band density was carried out using the ImageLab software of the Bio-Rad ChemiDoc XRS+ system after exposure.

**Microarray analysis.** Microarray analyses of WT were performed as previously described (33) with four groups (n = 3–4 mice each): age- and sex-matched WT and P2X7−/− mice under either LFD or 12 weeks of HFD. Genes were filtered according to expression value >20 in greater than two arrays with a minimum of 10 probe sets per gene. This resulted in 16,001 genes that were used to generate the scatter plot. Thereafter, genes were selected that were differentially expressed between HFD and LFD using q < 0.001 and fold change >1.5, resulting in 3,097 genes that were used to generate the heat map. The data have been deposited into the GEO database (GSE9659).

**Statistical analysis.** Results are expressed as mean ± SEM. Comparisons between groups were made using either unpaired, two-tailed Student t test of the EXCEL software for two-group comparisons or the two-way ANOVA test of the PRISM software for multigroup comparisons. P < 0.05 was considered statistically significant.

**RESULTS**

**Inflammasome activation in VAT coincides with the onset of hyperglycemia and hyperinsulinemia.** HFD feeding with 60% calories from fat progressively increased BW, gonadal WAT weight, fasting blood glucose, and insulin levels (Fig. 1A). Upon 8 weeks of feeding of 60% HFD, mice developed mild hyperglycemia and hyperinsulinemia. RT-PCR analysis revealed that components of NLRP3 inflammasome such as Nlrp3, Casp1, Asc, Il1b, and Il18 were highly expressed in mouse epididymal adipose tissues, whereas other types of inflammasome sensors such as Nlrp1, Nlrc4, and Aim2 were expressed at much lower levels (Fig. 1B). Furthermore, most NLRP3 inflammasome components including Nlrp3, Casp1, Asc, and Il18 were expressed at comparable levels in both adipocytes and SMCs in VAT, whereas Il1b was predominantly present in the SVC fraction of VAT (Fig. 1C).

Intriguingly, most NLRP3 inflammasome components, including Nlrp3, Asc, and Casp1, were highly responsive to HFD feeding and elevated starting at 6 weeks HFD (Fig. 1C), as were other types of inflammasome sensors such as Nlrp1 and Nlrc4 (Supplementary Fig. 1). Interestingly, the Il1b mRNA level was not changed whereas Il18 was decreased with HFD (Fig. 1C). In support, at the protein levels, pro-caspase-1 p45 was increased fivefold after 8 weeks HFD (Fig. 1D and E). The cleaved fragment of caspase-1 p35, a marker for caspase-1 activation (13,16,94,35),
exhibited a similar expression pattern (Fig. 1D and E). The specificity of the caspase-1 antibody was confirmed using adipose tissue from caspase-1-/- animals (Fig. 1F). Consistently, the IL-1β level in epididymal adipose tissue was significantly increased after 12 weeks, but not 6 weeks, HFD (Fig. 1G). Intriguingly, at 8 weeks, not 6 weeks, HFD, cell death in WAT was significantly increased, as measured by levels of cleaved caspase-3 (Fig. 1H and I), as well as the crown-like structures (Supplementary Fig. 2). Thus, we conclude that inflammasome activation in adipose tissue coincides with increased cell death and the onset of hyperglycemia and hyperinsulinemia.

**P2X7 expression in adipose tissue coincides with the activation of inflammasome.** Early studies have implicated a significant role of ATP-P2X7 signaling in inflammasome activation in macrophages and dendritic cells and in the clearance of dead cells (18,21,26). However, whether P2X7 plays a role in adipose tissue remains unclear. RT-PCR analyses revealed that among all P2X family members P2X1–7, P2X7 was expressed at relatively high levels in both adipocytes and SVCs of mouse epididymal adipose tissue (Fig. 2A). Both mRNA and protein levels of P2X7 were significantly increased in the WAT of 13-week-old ob/ob mice relative to age-matched WT lean mice (Fig. 2B and C). Similar observation was obtained in the liver but not muscle or pancreas (Fig. 2D).

We next asked when the induction of P2X7 occurred in adipose tissue using HFD-feeding mouse models. Intriguingly, induction of P2X7 protein also occurred at 8 weeks HFD feeding (Fig. 2D and F), a pattern strikingly similar to that of inflammasome activation. Preceding the induction of its protein, P2X7 mRNA level was elevated starting at 6 weeks HFD (Fig. 2G). Thus, P2X7 protein is induced in adipose tissue after 8 weeks feeding of 60% HFD, coinciding with the activation of inflammasome and the onset of hyperglycemia and hyperinsulinemia.

**FIG. 1.** Inflammasome activation in WAT requires 8 weeks HFD feeding. A: Body weight, epididymal WAT weight, insulin levels (following a 4-h fast), and blood glucose (following a 16-h fast) in WT mice on 60% HFD for the indicated time periods from 1 to 24 weeks, compared with age-matched mice on LFD (13% fat); n = 6–12 mice each group. B: RT-PCR analysis of various inflammasome components in purified primary adipocytes, SVCs, epididymal WAT, liver, and peritoneal macrophages (Mac) of WT mice. The exclusive presence of Foxp3, a regulatory T cell–specific marker, in the SVC fraction indicates a clean separation of adipocytes and immune cells in SVCs. L32, a loading control; no RT, negative controls with no reverse transcription. C: Q-PCR analysis of various inflammasome components in epididymal WAT from HFD-fed WT mice for the indicated time periods, compared with 18-week-old WT mice on LFD (0 weeks on HFD); n = 5 mice per group. D: Western blot analysis of procaspase-1 p45 (procasp-1) and cleaved caspase-1 p35 in WAT from HFD-fed WT mice with quantification shown in E. F: Western blot analysis of caspase-1 in WAT from 12- and 29-week HFD-fed WT mice and 16-week HFD-fed caspase-1-/- mice, showing the specificity of anti-caspase-1 antibody. Pos. CON, LPS-ATP–treated macrophages. G: IL-1β concentration measured in WAT of WT mice on 0 or 12 weeks at 60% HFD, compared with age-matched mice on LFD; n = 4–5 mice per group. H: Western blot analysis of procaspase-3 and cleaved caspase-3 p17 and p19 in WAT from HFD-fed WT mice with quantification shown in I. For Western blots, each lane represents an individual animal or sample with HSP90 as a loading control. All experiments were repeated at least twice. Values represent mean ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.001, compared to LFD or 0 weeks HFD controls.
Whole-body P2X7 ablation does not affect metabolic status in both lean and obese mice. We next addressed whether the ATP-P2X7 signaling axis plays a role in metabolic regulation in HFD-induced obesity by placing P2X7−/− mice on 60% HFD feeding for 12 weeks. Obese P2X7−/− mice exhibited similar body and gonadal fat weights relative to the WT cohort (Fig. 3A and B). There were no gross morphological differences in WT, liver, and pancreas tissues between knockout (KO) and WT cohorts (Fig. 3C and Supplementary Fig. 3). Systemic glucose homeostasis and insulin sensitivity were not affected by P2X7 deficiency as shown by GTT, fasting insulin levels, and AKT phosphorylation, respectively (Fig. 3D–F).

Whole-body P2X7 ablation does not affect inflammatory status in obese WAT. Given the known role of P2X7 in ATP-induced inflammasome activation, we next addressed whether the ATP-P2X7 signaling axis is required for inflammasome activation in adipose tissue upon 12 weeks HFD. Unexpectedly, levels of caspase-1 and its cleavage product p35 were not affected in P2X7−/− WAT (Fig. 4A and B), nor was the IL-1β level in WAT (Fig. 4C) or mRNA levels of key inflammasome components (Fig. 4D), suggesting that P2X7 may be not involved in inflammasome activation in adipose tissue during diet-induced obesity. In direct contrast, P2X7-deficient peritoneal macrophages and mice were not able to respond to LPS plus ATP both in vitro (Fig. 4E) and in vivo (Fig. 4F), further supporting the notion that P2X7 is the only purinergic receptor that activates inflammasome in response to ATP stimulation. Consistently, protein levels of inflammatory markers such as the inhibitor of nuclear factor-κB (IkB) and Arg1 (36,37) in WAT were not affected by P2X7 deficiency (Fig. 4A). In support, the expression of several key pro- and anti-inflammatory genes was not affected by P2X7 deficiency (Fig. 4G). This was further confirmed using an unbiased cDNA microarray analysis of WAT from WT or P2X7−/− mice under LFD and 12 weeks HFD where the HFD effect was almost identical between two groups. As shown in both scatter plots and heat map, the loss of P2X7 had no significant effect on the expression of genes that were regulated by HFD (Fig. 4H and Supplementary Fig. 4A), including many inflammatory genes (not shown). Taken together, these data suggest that the ATP-P2X7 signaling axis is dispensable for obesity-associated inflammasome activation in WAT.
status in WAT of P2Y2−/− mice after 15 weeks HFD (Supplementary Fig. 5). We failed to observe any alterations in metabolic phenotypes (Supplementary Fig. 5A–E), inflammatory responses (Supplementary Fig. 5F and G), or inflammasome activation (Supplementary Fig. 5H) in adipose tissue compared with age-matched WT controls. These results excluded the involvement of another ATP receptor, P2Y2, in inflammasome activation in obese WAT.

P2X7 ablation in hematopoietic cells does not affect inflammatory status in obese WAT. To exclude a possible role of P2X7 in somatic cells, such as pancreatic β-cells (39) and adipocytes (40), that may affect the outcome of inflammasome activation in adipose tissue, we next specifically ablated P2X7 in hematopoietic cells using BMT. Preliminary BMT experiments using congenic CD45.1+ and CD45.2+ mice showed efficient immune cell reconstitution in SCVs of WAT (Supplementary Fig. 6). Next, lethally irradiated WT mice were reconstituted with bone marrow from P2X7−/− or WT mice. Metabolic phenotypes and inflammasome status were examined after 16 weeks HFD after 4 weeks recovery (Fig. 5A). The loss of P2X7 in hematopoietic cells did not affect BW gain, fat weight, and adipose tissue morphology (Fig. 5B–D). Liver and pancreas tissue morphologies were also comparable between experimental and control cohorts (Supplementary Fig. 7). Systemic glucose tolerance and fasting insulin levels were not affected by hematopoietic-specific P2X7 ablation (Fig. 5E and F). Moreover, mice with P2X7 deficiency in hematopoietic cells exhibited no alterations in inflammasome activity as shown by the protein levels of caspase-1 p45 and p35 (Fig. 6A and B) as well as mRNA levels of three key inflammasome components (Fig. 6C). Additionally, the overall inflammation level in WAT was not altered, as shown by protein levels of iκB (Fig. 6A) and mRNA levels of various inflammatory genes (Fig. 6D). Thus, these data suggest that hematopoietic P2X7 is not required for inflammasome activation in obese adipose tissue.

DISCUSSION

Previous studies have shown that inflammasome is activated in obese adipose tissue either after 36 weeks 60% HFD or 12 weeks 45% HFD feeding (13,15), and it remains unclear how long it takes for HFD feeding to activate inflammasome in adipose tissue. Here, we report a dramatic activation of inflammasome in adipose tissue starting at 8 weeks feeding.
of 60% HFD, coinciding with the onset of hyperglycemia and hyperinsulinemia and with cell death. This is consistent with the general timeline of inflammation during diet-induced obesity, which was reported to be up-regulated at 9 weeks in adipose tissue based on the infiltration of macrophages with 60% HFD and the global induction of inflammatory genes in microarray analysis with 45% HFD (28,41). In parallel with the onset of inflammation, cell death in adipose tissue was elevated at about 4–8 weeks 60% HFD and peaked at about 12 weeks HFD (Fig. 1H and Supplementary Fig. 2) (28). These tight correlations among HFD feeding, cell death, and inflammatory response strongly suggest that dying cells in obese adipose tissue may release cell death–mediated “danger signals” to activate inflammasome in situ.

However, our data demonstrated that extracellular ATP-P2X7 signaling is not involved in inflammasome activation in obese adipose tissue in vivo. In line with our observation, a recent study showed that P2X7 expression is increased in adipose tissue of human patients with metabolic syndrome (42). However, our data demonstrated that, although required for LPS-ATP–induced inflammasome activation both in vitro and in vivo (Fig. 4E and F), P2X7 is dispensable for inflammasome activation in obese adipose tissue in vivo. One possible explanation is that the extent of cell death in adipose tissue during obesity may be much lower than the systems used in previous studies, such as drug-induced tumor cell death, host-versus-graft disease, and sterile inflammasome (12–15). Additionally, ATP may be quickly hydrolyzed in extracellular space (22,43). Therefore, as P2X7 receptor requires a high ATP level to be activated (~5 mM) (Fig. 4E), extracellular ATP in obese adipose tissue may not reach the activation threshold for P2X7 and thus fail to activate inflammasome in vivo. This possibility is supported by a study showing that CD39/− mice, which are incapable of hydrolyzing extracellular ATP, exhibit exacerbated insulin resistance (44). Although the inflammatory status was not examined in the CD39/− mice, this report suggests that extracellular ATP may have a metabolic effect when it reaches a certain level.

Though our data suggest that extracellular ATP may not be the “danger signal” for inflammasome activation in WAT...
during obesity, it does not exclude the potential importance of cell stress and death in obese adipose tissue during the pathogenesis of obesity. Lipid spillover during adipocyte expansion and death has been linked to macrophage infiltration and obesity-associated inflammation (45). Indeed, several studies using lipodystrophy mouse models suggest that adipocyte cell death is important for macrophage infiltration into adipose tissue (46,47). Furthermore, both palmitate and ceramides, but not unsaturated oleate, have been reported to activate NLRP3 inflammasome in bone marrow–derived macrophages in vitro (14,15). As saturated fatty acid has been demonstrated to induce ceramide biosynthesis in vivo (48), it remains to be tested whether palmitate and ceramides activate inflammasome in adipose tissue via the same mechanism. Thus, the function of lipids as inflammasome-activating signals in obese adipose tissue and the underlying molecular mechanism are worth further studies.

Alternatively, inflammasome in obese adipose tissue may be activated by mechanisms unrelated to in situ cell death. A high glucose level has been suggested to induce inflammasome activation. In isolated pancreatic islets, a high glucose level was shown to induce thioredoxin interacting protein (TXNIP) expression and then activate inflammasome, which was not mediated by ATP-P2X7 signaling (12). In another independent study, similar induction of TXNIP and IL-1β by glucose was observed in adipose tissue explants and primary adipocytes (16). These reports indicate that hyperglycemia during obesity may lead to inflammasome activation. Interestingly, we observed that the onset of both hyperglycemia and inflammasome activation in adipose tissue occurred at 8 weeks HFD, whereas 6 weeks HFD feeding was not sufficient. Further studies are needed to show the potential role of high circulating glucose levels in inflammasome activation in adipose tissue in vivo.

To date, four classes of inflammasomes have been shown to activate caspase-1 in vivo, including NLRP3, NLRP1, NLRC4, and AIM2 inflammasome (10). Different classes of inflammasomes may respond to specific environmental cues. For example, the NLRC4 inflammasome is activated by gram-negative bacteria, the AIM2 inflammasome senses cytosolic double-stranded DNA, and the NLRP3 inflammasome responds to a number of pathogen-associated or endogenous danger–associated activators (10). Previous studies on inflammasomes in adipose tissue focused on NLRP3 inflammasome (13,15). However, the nature of inflammasomes in adipose tissue remains unclear and their cell-type specificity has not been examined. Here, we determined the expression of the four classes of inflammasomes in epididymal adipose tissue and liver by assessing the relative transcript levels of their sensors. Besides NLRP3, our data show that both NLRP1 and NLRC4 were also expressed in adipose tissue and were highly induced after 8 weeks HFD. In contrast, AIM2 expression was very low in adipose tissue. Thus, the role of non-NLRP3 inflammasomes in adipose inflammation in obesity requires further investigation.

Inflammasome activation generates two active caspase-1 subunits, p20 and p10, with enzymatic activities (10). In addition, as first reported by Tschopp and colleagues (94) in a cell-free system, the caspase-1 intermediate product p35 may also serve as an indicator for the activation of caspase-1. Recently, several studies by different groups have used p35 as a marker of inflammasome activation in tissues (13,16,35). Indeed, our data show that the p35 band is caspase-1 specific as it disappears in the caspase-1–deficient adipose tissue (Fig. 1F), and more importantly, that the appearance of caspase-1 p35 subunit in WAT under various physiological settings is in line with that of caspase-1 p20 (Fig. 1D and F, Fig. 4A, and Fig. 6A vs. Supplementary Fig. 8). Thus, the caspase-1 p35 fragment, albeit possibly not the active form, may be a bona fide indicator of inflammasome status in WAT. P2X7 was previously reported to regulate pancreatic β-cell functions by modulating insulin secretion and β-cell mass (39). Upon feeding with high-fat/high-sucrose Surwit diet (58% fat with majority of saturated fat, 26% carbohydrate, and 16% protein, no fiber) for 12 weeks, P2X7−/- mice developed hyperglycemia, severe glucose intolerance, decreased β-cell mass, and impaired β-cell function (39). Such β-cell–associated diabetic phenotype, however, was not observed in our study (Fig. 3 and Supplementary Figs. 3 and 7). The discrepancies may be due to specific diets used in the studies. In addition, the ATP-P2X7 signaling axis has been reported to induce lipolysis and inhibit insulin signaling in adipocytes in vitro by upregulating cyclic AMP levels or inhibiting phosphorylation on insulin receptor substrate and AKT in rat primary adipocytes (40,49,50). In contrast, others have reported that insulin signaling was down-regulated by ATP-P2X7 in astrocytes (51,52). Here in our study, animals with both global and hematopoietic-specific deletion of P2X7 did not exhibit altered systemic insulin sensitivity and adiposity, suggesting a dispensable role of ATP-P2X7 signaling in regulating adipocyte function and insulin sensitivity in vivo.

In conclusion, our study showed that inflammasome is activated concurrently with the development of hyperglycemia and hyperinsulinemia. Unexpectedly, the ATP-P2X7 signaling axis seems not to play a critical role in this process. Further studies are required to identify and characterize the endogenous signal(s) that is responsible for inflammasome activation in adipose tissue during obesity.

ACKNOWLEDGMENTS

S.S. is supported by the International Student Research Fellowship from the Howard Hughes Medical Institute. This study was also funded by the Netherlands Nutrigenomics Centre (to S.K.), Cornell startup package, American Diabetes Association (7-08-JF-47 and 1-12-CD-04), and National Institute of Diabetes and Digestive and Kidney Diseases (R01-DK-082582 and R01-DK-082582-S1 to L.Q.).

No potential conflicts of interest relevant to this article were reported.

S.S. conceived the framework of the study, designed the experiments, performed most in vivo and in vitro studies, analyzed data, and wrote, commented on, and approved the manuscript. S.X. performed the flow cytometric analysis of BMT, analyzed data, and commented on and approved the manuscript. Y.J. helped with animal dissections and Q-PCR analysis, analyzed data, and commented on and approved the manuscript. L.Q. performed the microarray analysis, analyzed data, and commented on and approved the manuscript. L.Q. conceived the framework of the study, designed the experiments, analyzed data, and edited, commented on, and approved the manuscript. L.Q. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Parts of this study have been submitted in abstract form for presentation at the 72nd Scientific Sessions of the American Diabetes Association, Philadelphia, Pennsylvania, 8–12 June 2012.
The authors thank Dr. Rinke Stienstra for helpful suggestions and sharing Cosp1−/− WAT, Dr. Joseph Waksilag (Cornell University) and Dr. Vishva Dixit for caspase-3 and caspase-1 p20 antibodies, Dr. Qiaoming Long (Cornell University) for use of the microscope, Cindy Wang (Cornell University) for technical assistance, and other members of the Qi Laboratory at Cornell University for critical discussions/suggestions.

REFERENCES

1. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87–91
2. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. J Clin Invest 1995;95:2409–2415
3. Xu H, Barnes GT, Yang Q, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003;112:1821–1830
4. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003;112:1706–1708
5. Olefsky JM, Glass CK. Macrophages, in obesity of multiple signalling pathways on ROS production? Nat Rev Immunol 2010;3:re1
6. Jager J, Grémeaux T, Cormont M, Le Marchand-Brustel Y, Tanti JF. Interleukin-1β-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. Endocrinology 2007;148:241–251
7. Lagathu C, Yuan-Charvet L, Bastard J-P, et al. Long-term treatment with Caspase-1 p20 antibodies, Dr. Qiaoming Long (Cornell University) and Dr. Vishva Dixit for caspase-3 and caspase-1 p20 antibodies.
8. Larsen CM, Faulenbach M, Vaag A, et al. Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N Engl J Med 2007;356:1517–1526
9. Netea MG, Joosten LAB, Lewis E, et al. Depletion of interleukin-18 in mice leads to hyperphagia, obesity and insulin resistance. Nat Med 2006;12:650–656
10. Schroeder K, Tschopp J. The inflammasomes. Cell 2010;140:821–832
11. Petrilli V, Dostert C, Maruve DA, Tschopp J. The inflammasome: a danger sensing complex triggering innate immunity. Curr Opin Immunol 2007;19:615–622
12. Zhou R, Tardivel A, Thorens B, Choi I, Tschopp J. Thioredoxin-interacting protein links oxidative stress to inflammasome activation. Nat Immunol 2010;11:136–140
13. Stienstra R, Koenen LB, Koenen T, et al. The inflammasome-mediated caspase-1 activation controls adipocyte differentiation and insulin sensitivity. Cell Metab 2010;12:503–605
14. Wen H, Gris D, Lei Y, et al. Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. Nat Immunol 2011;12:408–415
15. Vandanmagsar B, Youm Y-H, Ravussin E, et al. The NLRP3 inflammasome impairs obesity-induced insulin and insulin resistance. Nat Med 2011;17:170–188
16. Koenen TB, Stienstra R, van Tins LF, et al. Hyperglycemia activates caspase-1 and TNXP-mediated IL-1βeta transcription in human adipose tissue. Diabetes 2011;60:517–524
17. Di Virgilio F. Liaisons dangereuses: P2X(7) and the inflammasome. Trends Pharmacol Sci 2007;28:465–472
18. McDonald B, Pittman K, Menezes GB, et al. Intravascular danger signals guide neutrophils to sites of sterile inflammation. Science 2010;330:362–366
19. Weber FC, Esser PR, Müller T, et al. Lack of the purinergic receptor P2X(7) occurs through a cyclophilin D intrinsic signaling pathway independent of adipose tissue inflammation. Diabetes 2011;60:2134–2143
20. Fujiki H, Yoneda H, Ohono Y, et al. Tryptophan as a danger signal mediating caspase-1 activation. J Immunol 2005;175:851–858
21. Koenen TB, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL-1β. Mol Cell 2002;10:417–425
22. Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J. Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 2006;440:237–241
23. Bollinger J, Bollinger S, Bailleau A, et al. High pancreatic n-3 fatty acids prevent STZ-induced diabetes in fat-1 mice: inflammatory pathway inhibition. Diabetes 2011;60:1090–1099
24. Ishii M, Ven H, Corsa CAS, et al. Epigenetic regulation of the alternatively activated macrophage phenotype. Blood 2009;114:3244–3254
25. Skaper SD, Debetto P, Giusti P. The P2X7 purinergic receptor: from physiology to neurological disorders. FASEB J 2010;24:337–345
26. Glaser S, Sauter NS, Schulthess PT, Shu L, Oberholzer J, Maeder K. Purinergic P2X7 receptors regulate secretion of interleukin-1 receptor antagonist and beta cell function and survival. Diabetologia 2009;52:1579–1588
27. Ledig C, Giusti DM, Sub BC, et al. Dual roles of P2 purinergic receptors in insulin-stimulated leptin production and lipolysis in differentiated rat white adipocytes. J Biol Chem 2005;280:28556–28563
28. Kleemann R, van Erk M, Verschuren L, et al. Time-resolved and tissue-specific systems analysis of the pathogenesis of insulin resistance. PLoS ONE 2010;5:e8817
29. Madec S, Rossi C, Chiarugi M, et al. Adipocyte P2X7 receptors expression: a role in modulating inflammatory response in subjects with metabolic syndrome? Atherosclerosis 2011;219:552–558
30. North RA. Molecular physiology of P2X receptors. Physiol Rev 2002;82:1013–1067
31. Enjoji K, Kotani K, Thukral C, et al. Deletion of cd39/entpd1 results in defective adipocyte function and survival. Nat Immunol 2009;10:1526–1532
32. Kostel A, Sugaru A, Haenmueller G, et al. Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. J Clin Invest 2010;120:3466–3470
33. Fischer-Posovszky P, Wang QA, Asterholm IW, Rutkowski JM, Scherer PE. Targeted deletion of adipocytes by apoptosis leads to adipose tissue recruitment of alternatively activated M2 macrophages. Endocrinology 2011;152:3074–3081
34. Herrera L, Shapiro H, Nayer A, Lee J, Shoelson SE. Inflammation and adipose tissue macrophages in lipodystrophic mice. Proc Natl Acad Sci USA 2010;107:240–245
35. Naber VL, Bikman BT, Wang L-P, et al. Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. J Clin Invest 2011;121:1858–1870
36. Hashimoto N, Robinson FW, Shibata Y, Flanagan JE, Kono T. Diversity in caspase recruitment domain (CARD) signaling complexes in the innate immune response in subjects with metabolic syndrome? Atherosclerosis 2011;219:552–558
37. Elliott MR, Chekeni FB, Trampont PC, et al. Nucleotides released by apoptotic cells act as a find-me signal to promote phagocytic clearance. Nature 2009;461:282–286
38. Cinti S, Mitchell G, Barbadiello G, et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res 2005;46:2347–2355
39. Strissel KJ, Stanevza C, Miyoshi H, et al. Adipocyte death, adipose tissue remodeling, and obesity complications. Diabetes 2007;56:2910–2918
40. Akhouri N, Gornicka A, Berk MP, et al. Adipocyte apoptosis, a link between obesity, insulin resistance, and hepatic steatosis. J Biol Chem 2010;285:3452–3458
41. Feng D, Tang Y, Kwon H, et al. High-fat-diet-induced adipocyte cell death occurs through a cyclophilin D intrinsic signaling pathway independent of adipose tissue inflammation. Diabetes 2011;60:2134–2143
42. Pajvani UB, Trujillo ME, Combs TP, et al. Fat apoptosis through targeted activation of caspase 8: a new mouse model of inducible and reversible lipostrophy. Nat Med 2005;11:797–803