The Inflammasome and Caspase-1 Activation: A New Mechanism Underlying Increased Inflammatory Activity in Human Visceral Adipose Tissue

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The immune competent abdominal adipose tissue, either stored viscerally [visceral adipose tissue (VAT)] or sc [sc adipose tissue (SAT)], has been identified as a source of IL-1β and IL-18. To become active, the proforms of these cytokines require processing by caspase-1, which itself is mediated by the inflammasome. In this descriptive study, we investigate the expression of inflammasome components and caspase-1 in human fat and determine whether caspase-1 activity contributes to the enhanced inflammatory status of VAT. Paired SAT and VAT biopsies from 10 overweight subjects (body mass index, 25–28 kg/m^2) were used to study the cellular composition and the intrinsic inflammatory capacity of both adipose tissue depots. The percentage of CD8$^+$ T cells within the lymphocyte fraction was significantly higher in VAT compared with SAT (41.6 *vs.* 30.4%; $P < 0.05$). Adipose tissue cultures showed a higher release of IL-1β (10-fold; $P < 0.05$), IL-18 (3-fold; $P < 0.05$), and IL-6 and IL-8 (3-fold, $P < 0.05$; and 4-fold, $P < 0.05$, respectively) from VAT compared with SAT that was significantly reduced by inhibiting caspase-1 activity. In addition, caspase-1 activity was 3-fold ($P < 0.05$) higher in VAT compared with SAT, together with an increase in the protein levels of the inflammasome members apoptosis-associated speck-like protein containing a C-terminal caspase-recruitment domain (2-fold; $P < 0.05$) and nucleotide-binding oligomerization domain-like receptor pyrin domain containing 3 (2-fold; nonsignificant). Finally, caspase-1 activity levels were positively correlated with the percentage of CD8$^+$ T cells present in adipose tissue. Our results show that caspase-1 and nucleotide-binding oligomerization domain-like receptor pyrin domain containing 3 inflammasome members are abundantly present in human VAT. The increased intrinsic caspase-1 activity in VAT represents a novel and specific inflammatory pathway that may determine the proinflammatory character of this specific depot. (*Endocrinology* 152: 3769–3778, 2011)

Chronic low-grade inflammation has now been recognized as one of the key steps in the pathogenesis of obesity-induced insulin resistance and type 2 diabetes mellitus. The metabolically active abdominal adipose tissue secretes a wide variety of cytokines, that may promote the development of peripheral insulin resistance (1). Obesity-induced enlargement of adipose tissue is accompanied by elevated plasma levels of cytokines, including IL-6, IL-8, IL-1β, IL-18, and TNFα that affect insulin sensitivity in peripheral tissues (2–6). It has been suggested that especially visceral adipose tissue (VAT), rather than sc adipose tissue (SAT), contributes to the elevated circulating levels of inflammatory cytokines in obese individuals (7, 8) that may be attributed to enhanced influx of immune cells,

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Abbreviations: ASC, Apoptosis-associated speck-like protein containing a C-terminal caspase-recruitment domain; BMI, body mass index; FACS, fluorescence-activated cell sorter; NLR, nucleotide-binding oligomerization domain-like receptor; NLRP3, NLR pyrin domain containing 3; SAT, sc adipose tissue; SVF, stromal vascular fraction; TLR, Toll-like receptor; VAT, visceral adipose tissue.

including macrophages, monocytes, and B and T cells (9, 10). However, efforts to identify possible mechanisms underlying the enhanced inflammatory capacity of VAT compared with SAT are scarce.

Recently, it was shown that the Toll-like receptor (TLR)-4 inflammatory pathway is activated in the adipose tissue during obesity and affects insulin responsiveness (11). The expression levels of multiple TLR family members were enhanced in VAT compared with SAT, suggesting that the TLR signaling pathway may contribute to an enhanced inflammatory capacity of VAT (12). IL-1β and IL-18 have been linked to the development of obesity and insulin resistance, and they partly originate from adipose tissue (4, 13). To become active, the proform of IL-1β and IL-18 is processed by a cysteine protease named caspase-1. Activation of caspase-1 itself is mediated by a multiprotein complex entitled the inflammasome (6, 14). Upon stimulation by exogenous (bacterial products) or endogenous (uric acids crystals, hyperglycemia, or cholesterol crystals) signals, formation of the inflammasome complex consisting of a nucleotide-binding oligomerization domain-like receptor (NLR) family member and the adaptor protein apoptosis-associated speck-like protein containing a C-terminal caspase-recruitment domain (ASC) occurs (15–19). To date, activation and function of the NLR pyrin domain containing 3 (NLRP3) inflammasome composed of NLRP3, the adaptor molecule ASC and caspase-1, is most fully characterized and responsible for recognition of invading pathogens and nonmicrobial molecules that eventuates into IL-1β and IL-18 production (20, 21).

Inasmuch adipose tissue has been identified as a significant source of IL-18 and IL-1β, this suggests the presence of the NLRP3 inflammasome machinery at the tissue level. Indeed, we have recently described that caspase-1 is well expressed in adipose tissue of obese animals and in human SAT (22, 23), yet nothing is known about the NLRP3 inflammasome expression in human VAT compared with SAT. Therefore, we set out to study the presence of the NLRP3 inflammasome components and caspase-1 in human fat and to determine whether caspase-1 activity contributes to the enhanced inflammatory status of VAT *vs.* SAT.

Materials and Methods

Subjects

Paired SAT and VAT (omentum) samples were obtained according a standardized procedure from 10 patients (five females and five males) undergoing a cholecystectomy or an inguinal hernia surgery. Inclusion criteria were age between 40–60 yr and body mass index (BMI) of 25–28 kg/m^2. Subjects were normoglycemic and had a mean waist to hip ratio of 0.90 (Supplemental Table 1, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). Metabolic diseases, endocrine diseases, and chronic and/or acute inflammatory diseases (high sensitivity C-reactive protein above 1 mg/liter) were excluded. The tissue samples were collected after written informed consent, and the protocol was approved by the ethical committee of the Radboud University Nijmegen Medical Centre.

Ex vivo stimulation experiments with human adipose tissue

Intact human adipose tissue fragments from paired SAT and VAT were used to study the presence of the NLRP3 inflammasome components and the intrinsic caspase-1 activity as well as the cytokine release of IL-1β and IL-18 during a 24-h culture using standard conditions (DMEM supplemented with 10% fetal calf serum containing 5 mM glucose) with or without the addition of the caspase-1 inhibitor pralnacasan (100 μM) (24).

Part of the freshly collected SAT and VAT samples was disaggregated using collagenase digestion to isolate mature adipocytes and the stromal vascular fraction (SVF). Purity of the two different fractions was confirmed with the markers adiponectin, leptin (adipocyte specific), and CD45 (hematopoietic cell line marker) (Supplemental Fig. 1). The separate cellular fractions were subsequently used for cell culture using standard conditions for 24 h, fluorescence-activated cell sorter (FACS) analysis, and RNA isolation, followed by real-time PCR analysis.

RNA isolation and PCR analysis

RNA was extracted from total SAT and VAT or different adipose tissue cell fractions using TRIzol reagent (Invitrogen, Carlsbad, CA). RNA concentration was determined using a NanoDrop (NanoDrop Technologies, Wilmington, DE), and cDNA synthesis was performed using the iScript cDNA Synthesis kit (Bio-Rad Laboratories, Hercules, CA). Real-time PCR was done using Power-SYBR Green master mix and the 7300 Real-Time PCR system (Applied Biosystems, Warrington, UK). Expression of genes was normalized to β2M gene expression levels. Primer sequences are available upon request.

Protein analysis

Protein lysates from total adipose tissue of both depots were prepared to determine the presence of caspase-1 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA), NLRP3 (Abcam, Cambridge, MA), and ASC (Abcam) by Western blotting. Secretion of IL-1β, IL-6, IL-8, IL-18, TNFα, and adiponectin was analyzed by ELISA (R&D Systems, Minneapolis, MN). Bioactive IL-1 secretion was quantified in a bioassay using the murine thymoma cell line EL4/NOB-1 that produces IL-2 in response to bioactive IL-1 (25). IL-2 levels were measured by ELISA (R&D Systems).

Caspase-1 activity assay

Caspase-1 activity in total SAT and VAT protein lysates was determined with a caspase-1 fluorometric kit (BioVision, Mountain View, CA) by measuring the cleavage of 50 μl of the caspase-1 substrate YVAD-AFC. The fluorescence of the cleaved substrate was measured every 90 sec using a fluorometer (Polarstar BMG, fluostar galaxy; BMG Labtech, Ortenberg, Germany).

FACS analysis

SVF of both SAT and VAT were analyzed by flow cytometry (FC500; Beckman Coulter, Brea, CA). To this purpose, 100,000 cells/100 μl PBS + 1% BSA were incubated in three separate cocktails with the following conjugated monoclonal antibodies: anti-CD14 (Ph imm27074)-ECD, anti-CD45 (A 07785)-PCy5, anti-CD3 (A 07747)-PE, anti-CD8 (737659)-ECD, anti-CD4 (6604727)-ECD (Beckman Coulter), anti-F4/80 (ab60343-50)-FITC (Abcam). Blood contamination of the samples was prevented by treating the SVF with an erythrocyte lysis buffer.

Statistical analysis

Data are presented as mean ± SEM. Comparisons between SAT and VAT parameters were calculated using the nonparametric Wilcoxon rank test. Correlations were determined using a Spearman correlation test. The cut-off for statistical significance was set at a *P* value of 0.05 or below. All statistics were performed using SPSS software (version 16.0; SPSS, Inc., Chicago, IL).

Results

Cellular composition of SAT and VAT

Because adipose tissue-resident macrophages represent potent inflammatory cytokine producers during obesity (26, 27), we set out to study the macrophage content in the SVF of both SAT and VAT obtained by flow cytometry (Table 1). Our study revealed that the numbers of macrophages were equally distributed throughout SAT and VAT. Subsequent FACS analysis of the SVF of the adipose tissue to determine the cellular immune cell composition, including monocytes and granulocytes, did not show significant differences between both fat depots. However, the percentage of $CD8^+$ (cytotoxic) T lymphocytes was significantly increased in VAT compared with SAT, whereas the $CD4^+$ cell number was not different in both depots (Table 1). Supplemental Fig. 2, A and B, shows representative dot plots of flow cytometry data of the different immune cells within the SVF of SAT and VAT, respectively.

TABLE 1. Immune cell composition of SAT and VAT from seven subjects

Immune cells in SVF	SAT (%)	VAT (%)
Granulocytes (% of $CD45^+$ cells)	29.0 ± 5.6	31.0 ± 6.5
Monocytes (% of $CD45^+$ cells)	3.7 ± 0.8	2.8 ± 0.7
Macrophages (% of $CD45^+$ cells)	5.7 ± 1.5	3.6 ± 1.3
Lymphocytes (% of $CD45^+$ cells)	39.4 ± 6.0	47.3 ± .6.0[a]
T cells (% of $CD45^+$ cells)	29.2 ± 4.6	38.6 ± 5.4[a]
CD4 T cells (% of $CD45^+$ cells)	14.7 ± 3.0	16.4 ± 3.2
CD8 T cells (% of $CD45^+$ cells)	10.8 ± 2.0	16.6 ± 2.4[b]

Number of immune cells (percentage) part of the innate immune system (granulocytes, monocytes, and macrophages) or adaptive immune system [total lymphocytes and T ($CD4^+$ or $CD8^+$) lymphocytes] in the SVF of SAT and VAT. Data are presented as mean ± SEM ($n = 7$).

[a] $P < 0.05$.

[b] $P < 0.01$.

Enhanced release of bioactive IL-1β and IL-18 from VAT compared with SAT

To examine the production capacity of IL-1β, IL-18, and other cytokines by VAT and SAT, total adipose tissue was brought into culture, and cytokine production was measured after 24 h. Interestingly, secretion of both total IL-1β and bioactive IL-1, as determined by ELISA and the NOB-1 bioassay, respectively, was significantly higher ($P < 0.05$) in VAT compared with SAT. In addition, IL-18 production from VAT was also significantly enhanced (Fig. 1A). The production of other proinflammatory cytokines, including IL-6, IL-8, and IL-1Ra, was also elevated in VAT compared with SAT explants (3-fold, $P <$ 0.05; 4-fold, $P < 0.05$; and 2-fold, $P < 0.05$, respectively), and secretion of adiponectin, a protein known for its insulin-sensitizing action (28), was reduced by VAT compared with SAT ($P < 0.05$) (Fig. 1B). Noticeably, secretion levels of the proinflammatory cytokine TNFα were comparable between VAT and SAT (Fig. 1B).

To determine gene expression levels of different cytokines in VAT and SAT, quantitative PCR analysis was performed. Although IL-1β gene expression levels were similar in both depots, IL-18 mRNA levels were significantly up-regulated in VAT (Fig. 2A). Gene expression levels of IL-6, IL-8, and adiponectin did not differ between both fat depots (data not shown). Fractioning of VAT into mature adipocytes and the SVF component revealed that IL-1β and IL-18 mRNA were significantly more expressed in the SVF (Fig. 2B). In accordance with the gene expression profile, IL-1β production was elevated in the SVF compared with the mature adipocyte fraction in both fat depots. However, the production of IL-1β by mature adipocytes and SVF was higher in VAT compared with the correspondence fractions isolated from SAT (Fig. 2C).

Inflammasome expression and caspase-1 activation are increased in VAT compared with SAT

Inasmuch the adipose tissue is able to secrete IL-1β and IL-18 (Fig. 1A), it suggests the presence of active caspase-1 in human adipose tissue. Indeed, the active form of caspase-1 was detectable in both fat depots (Fig. 3, A and B). Although caspase-1 gene expression was similar in both fat depots (data not shown), a 3-fold increase in caspase-1 protein levels was observed in the VAT samples from the 10 study subjects as determined by Western blot analysis [Fig. 3, A (Western blot analysis image from one subject) and B (all subjects)]. In addition, caspase-1 activity was enhanced in VAT compared with SAT as deter-

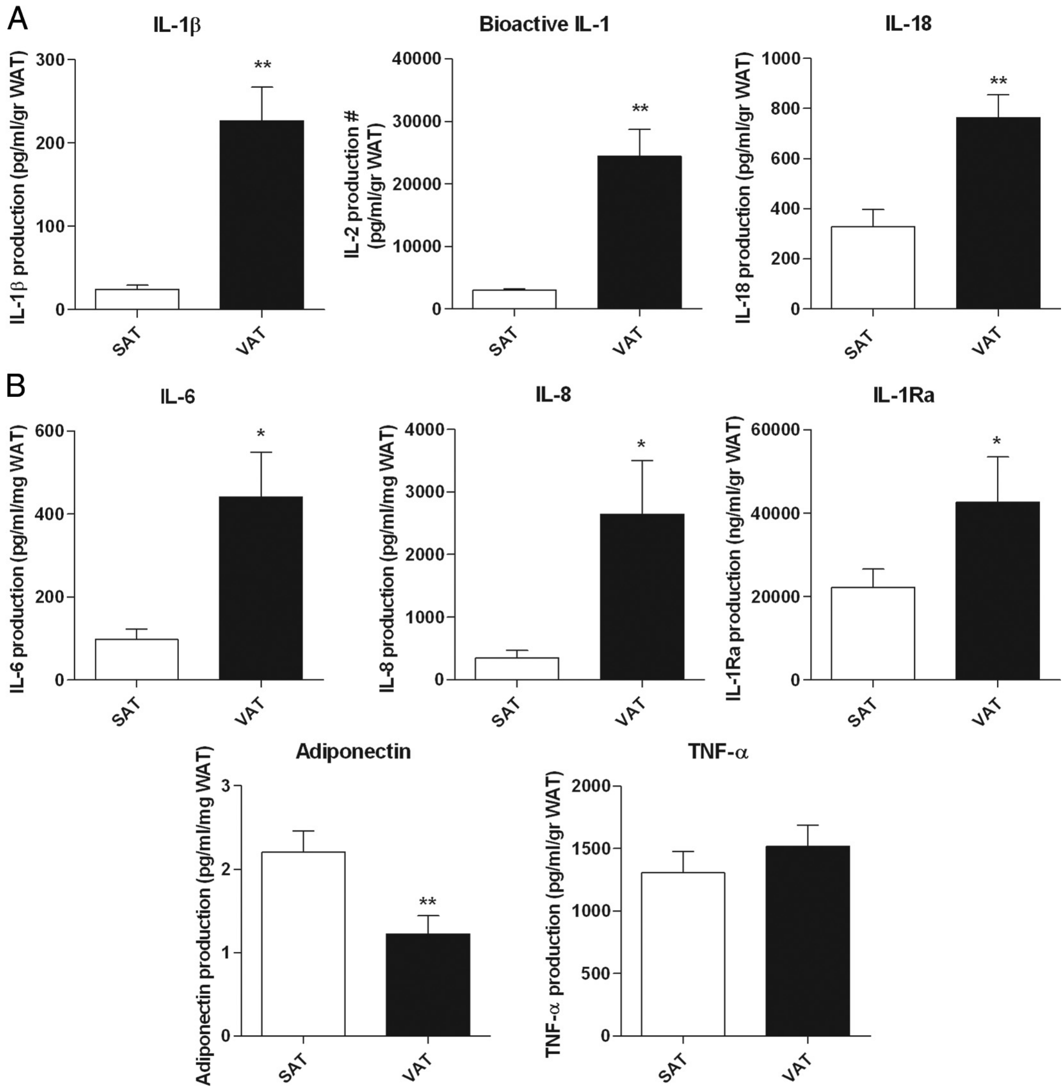

FIG. 1. Enhanced production of IL-1β, IL-18, IL-6, IL-8 yet not TNFα from VAT. A, Secretion of IL-1β, bioactive IL-1 (#, measured as IL-2 production from NOB-1 cells in response to bioactive IL-1), and IL-18 in intact SAT and VAT fragments cultured for 24 h (n = 5; BMI range, 25–28). B, Secretion levels of IL-6, IL-8, IL-1Ra, adiponectin, and TNFα by VAT and SAT after 24 h of culturing (n = 5). *, $P < 0.05$; **, $P < 0.01$ using a Wilcoxon rank test.

mined by a functional caspase-1 activity assay in freshly isolated adipose tissue from two patients (Fig. 3C).

The abundant activity of caspase-1 in VAT (Fig. 3, A–C) implies the presence of the inflammasome machinery in human adipose tissue. Therefore, we tested whether the inflammasome components NLRP3 and ASC were present in the 10 human VAT and SAT samples. Similar to caspase-1, ASC protein was detected in both depots, yet significantly up-regulated in VAT [Fig. 3, A (Western blot analysis image from one subject) and B (all subjects)]. Due to a large interindividual variation in NLRP3 protein levels between SAT and VAT, the expression levels of this protein only tended to be higher in VAT without reaching statistical significance. (Fig. 3, A and B). To investigate the cellular origin of the inflammasome components in human adipose tissue, quantitative PCR analysis of fractionated VAT revealed that caspase-1 gene expression mainly originated from mature adipocytes, whereas ASC mRNA expression levels were higher in the SVF (Fig. 3D). NLRP3 transcription levels were equally distributed between the two fractions of VAT (Fig. 3D).

Blocking of caspase-1 inhibits cytokine release of VAT

To determine the potential of caspase-1 blockage to reduce the inflammatory trait of VAT, caspase-1 activity was blocked by the specific inhibitor pralnacasan. In Fig. 4A, the enhanced release of both IL-1β and IL-18 in VAT was significantly reduced when caspase-1 was blocked. The diminished IL-1β production in VAT was observed in both adipocytes and SVF cells, illustrating that caspase-1 is functionally active in both cellular fractions (Fig. 4B). Interestingly, inhibition of caspase-1 activity also limited the boosted production of IL-6 ($P = 0.06$) and IL-8 ($P <$

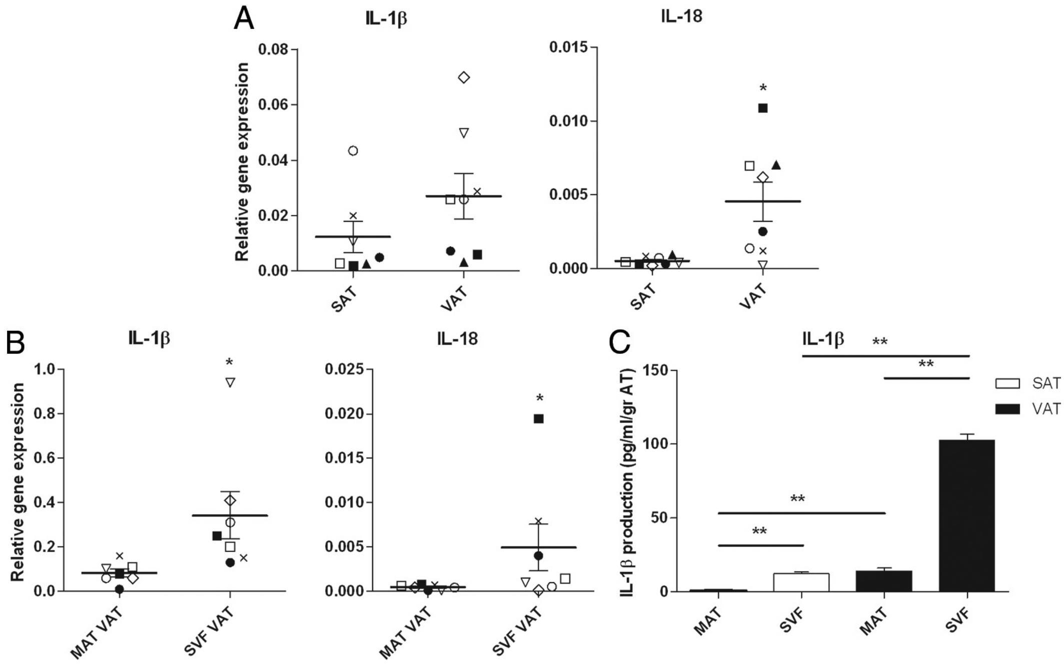

FIG. 2. Expression profile of IL-1β and IL-18 in VAT and SAT. A, Relative IL-1β and IL-18 gene expression levels in total SAT and VAT (n = 8, each specific symbol corresponds with the same individual). B, Relative IL-1β and IL-18 gene expression in mature adipocytes (MAT) and the SVF isolated from VAT (n = 7, each specific symbol corresponds with the same individual; BMI range, 25–28). C, IL-1β production by MAT and SVF isolated from 1 g of SAT and VAT cultured for 24 h (n = 4). *, $P < 0.05$; **, $P < 0.01$ using a Wilcoxon rank test.

0.05) by VAT, yet no effect was observed on adiponectin and TNFα secretion levels (Fig. 4C). These results demonstrate that caspase-1 activity is mainly responsible for the production of IL-1β and IL-18 by VAT.

The percentage of CD8$^+$ T cells in adipose tissue positively correlates with caspase-1 activity levels

Adipose tissue inflammation is partly caused by the influx of immune cells, including macrophages, monocytes, and T cells (10, 29–31). To examine whether the intrinsic activity of caspase-1 in adipose tissue is associated with the immune cell composition, we studied correlations between caspase-1 activity levels and the number of immune cells present in both adipose tissue depots as determined by FACS analysis (Table 1). As shown in Fig. 5, a significant positive correlation was observed between caspase-1 activity levels and the percentage of CD8$^+$ T cells present in adipose tissue. Noticeably, none of the other cells measured by FACS analysis (Table 1) correlated significantly with caspase-1 activity levels (data not shown). Interestingly, CD8$^+$ T cells have been shown to serve as a major contributor to adipose tissue inflammation (32). In line with the differences in caspase-1 activity levels between VAT and SAT, the number of CD8$^+$ T cells was significantly lower in SAT (Table 1). Moreover, both in SAT and VAT separately, a positive correlation was observed between caspase-1 activity levels and the CD8$^+$ T cells present in adipose tissue (data not shown). These results suggest that caspase-1 activity in adipose tissue is associated with the influx of CD8$^+$ T cells.

Discussion

In this descriptive study, we demonstrate that in paired human adipose tissue biopsies, the innate immune system represented by the NLRP3 inflammasome is abundantly present in VAT compared with SAT. In addition, intrinsic caspase-1 activity is elevated in VAT, contributing to the production of IL-1β and IL-18 as well as IL-6 and IL-8. Moreover, caspase-1 activity levels positively correlated with CD8$^+$ T cells present in the adipose tissue.

In addition to the storage of excessive amounts of energy, adipose tissue has been identified as a source of many inflammatory mediators (1). Interestingly, obesity-induced low-grade inflammation originating from expanding adipose tissue exploits similar pathways initiated by host defense mechanisms, suggestive of an important function of the innate immune system in fat (5, 33). Circulating levels of IL-1β and IL-18, both part of the innate immune response, are increased in obese and insulin resistant individuals and have robust effects on atherosclerosis and insulin resistance (34, 35). Several reports have identified adipose tissue as a potent source of IL-18 and IL-1β (4, 22, 36). Human adipose tissue depots have unique inflammatory characteristics exemplified by en-

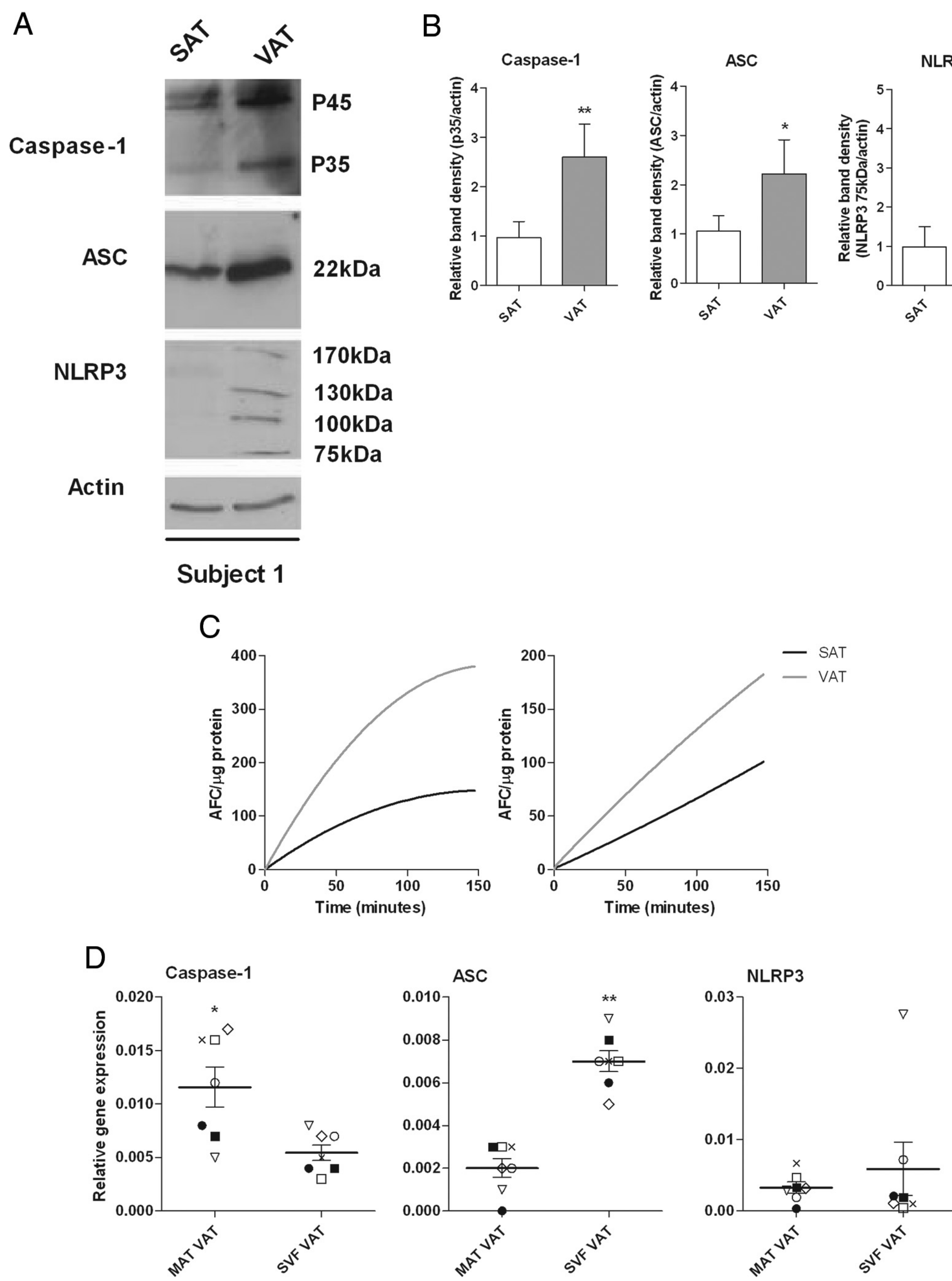

FIG. 3. Protein levels of ASC, NLRP3, and caspase-1 in VAT and SAT. A. Caspase-1, ASC, and NLRP3 protein levels in paired total SAT and VAT samples. Western blot analysis images are shown for one subject. Several bands observed for NLRP3 represents the difference in length of the leucine-rich repeats, which forms a part of NLRP3 (51). B, Mean caspase-1, ASC, and NLRP3 protein expression in paired SAT and VAT samples from 10 subjects quantified by densitometry relative to actin protein levels. C, Functional caspase-1 activity assay in paired SAT and VAT of two different subjects. D, Relative caspase-1, ASC, and NLRP3 gene expression in MAT and the SVF isolated from VAT ($n = 7$, each specific symbol corresponds with the same individual); *, $P < 0.05$; **, $P < 0.01$ using a Wilcoxon rank test.

hanced production of IL-6, IL-8, TNFα, and C-reactive protein by VAT compared with SAT, which contribute to key features of the metabolic syndrome (7, 8, 37–39). In line with these studies, we showed an increased production of IL-6, IL-8, and IL-1Ra, together with lower secretion levels of adiponectin in VAT explants.

In this study, we extended the proinflammatory properties of VAT by demonstrating that the protein levels of the inflammasome members NLRP3 and ASC were more expressed in this specific depot and that caspase-1 activation is severely increased in VAT compared with SAT resulting in a higher production of IL-1β and IL-18. In addition, secretion levels of the antiinflammatory cytokine IL-1Ra by VAT were also enhanced and may be the result of a compensatory protective response aimed at counteracting the excessive IL-1β secretion by VAT.

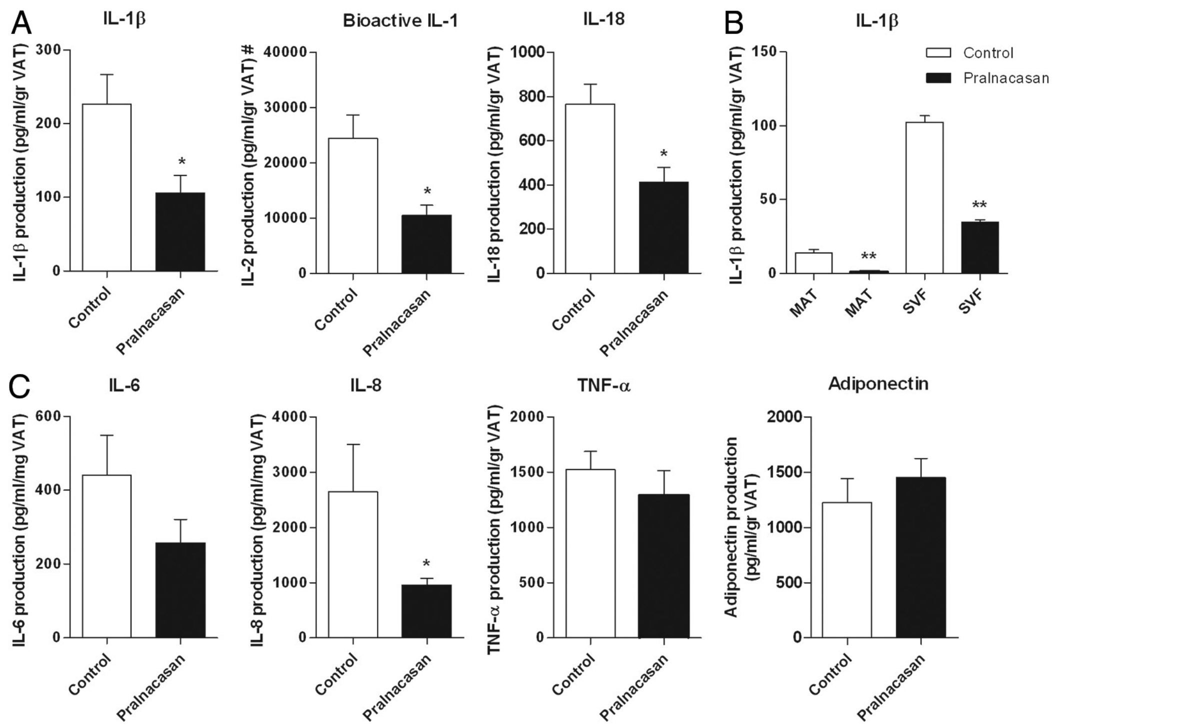

FIG. 4. Caspase-1 inhibition reduces production of IL-1β, IL-18, IL-6, IL-8 yet not TNFα from VAT. A, Secretion of IL-1β, bioactive IL-1 (#, measured as IL-2 production from NOB-1 cells in response to bioactive IL-1), and IL-18 by intact VAT (n = 5) cultured for 24 h in the presence or absence of pralnacasan (100 μM). B, IL-1β production by MAT and SVF isolated from 1 g of SAT and VAT (n = 4) cultured for 24 h in the presence or absence of pralnacasan (100 μM). C, Secretion of IL-6, IL-8, TNFα, and adiponectin by intact VAT (n = 5) cultured for 24 h in the presence or absence of pralnacasan (100 μM). *, $P < 0.05$; **, $P < 0.01$ using a Wilcoxon rank test.

Caspase-1-dependent production of IL-1β and IL-18 is supported by the observation that blocking caspase-1 activity in VAT by pralnacasan reduces the secretion of both cytokines. Furthermore, IL-1β release was reduced in both adipocytes and SVF after inhibiting caspase-1, indicating that this enzyme is involved in the IL-1β production in both fractions of VAT. The caspase-1-dependent release of IL-6 and IL-8 by VAT fits with the well-known capacity of IL-1β to enhance the production of IL-6 and IL-8 by adipose tissue (40, 41). Our results show that activation of caspase-1 controls the production of these proinflammatory proteins by VAT. However, secretion levels of TNFα from both fat depots were comparable, suggesting that the enhanced release of IL-1β and IL-18 is conveyed by a specific mechanism and does not involve a general increase in inflammatory status of VAT.

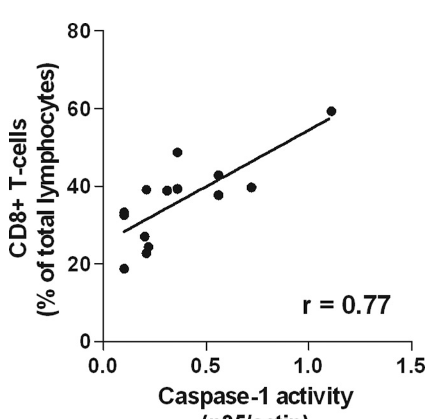

FIG. 5. Caspase-1 activity levels correlates positively with CD8$^+$ T-cell number present in both SAT and VAT. Caspase-1 activity is represented by the density of the active caspase-1 band (p35). Percentage of CD8$^+$ T lymphocytes in the SVF from both SAT and VAT was obtained by FACS analysis (n = 7, dots represent caspase-1 activity levels and CD8$^+$ T-cell number in both SAT and VAT of seven individuals); $P < 0.01$ using Spearman's rank correlation.

Although our study clearly demonstrated that IL-1β production was mainly derived from the SVF within VAT, mature adipocytes were also capable to release IL-1β. We hypothesize that *in vivo*, an interaction between adipocytes and various cells from the SVF determine the caspase-1-dependent cytokine-secreting capacity of the adipose tissue, hence explaining the high levels of caspase-1 gene expression in adipocytes. Additionally, these high mRNA expression levels in adipocytes may also indicate IL-1β-independent effects. Caspase-1 has been shown to regulate insulin sensitivity and to suppress peroxisome proliferator-activated receptor-γ activity in adipocytes (22, 42). Despite the enhanced IL-1β production in VAT compared with SAT and elevated protein levels of caspase-1 and ASC analyzed by Western blotting, we would like to emphasize that these outcomes can only be used as indirect measurements of inflammasome-dependent caspase-1 activation, because the inflammasome depends on protein-protein interactions to activate caspase-1.

Even though it has been clearly established that VAT displays enhanced inflammatory properties compared with SAT, much less is known about the underlying molecular mechanisms. Macrophage infiltration is known to contribute to the inflammatory status of a tissue (27). In the present study, FACS analysis revealed no differences in macrophage content in both fat depots, although we did not differentiate between the resident macrophage populations and the infiltrating macrophages that may represent the primary proinflammatory cells (43). The percentage of adipose tissue macrophages in this study was relatively low compared subjects suffering from severe obesity (44). This could be explained by inclusion of solely (healthy) overweight subjects (average BMI, 26.1 $kg/m^2 \pm$ SD 2.7). In addition to macrophages, the percentage of other immune cells that are part of the innate immune system (monocytes and granulocytes) present in the SVF of VAT and SAT did not differ. These results rule out differences in caspase-1 activity in VAT compared with SAT due to the influx of innate immune cells. However, in this study, caspase-1 activity was associated with the infiltration of cytotoxic T lymphocytes into adipose tissue. A robust positive correlation was observed between caspase-1 activity levels and the number of $CD8^+$ T cells present in adipose tissue. Moreover, differences in the percentage of $CD8^+$ T cells in VAT compared with SAT were mirrored by similar changes in caspase-1 activity levels. Although we did not study the direct effect of caspase-1 on $CD8^+$ T-cell influx in the adipose tissue, caspase-1 itself or by its activation of IL-1β and IL-18 might control the activation and number of $CD8^+$ T cells present in human adipose tissue. Inasmuch a recent study has demonstrated an important role for $CD8^+$ T cells in determining adipose tissue inflammation, the influx of these T cells may represent an important mechanism by which caspase-1 controls adipose tissue inflammation (32). However, further studies will be needed to reveal the possible role of caspase-1 in controlling the influx of $CD8^+$ T cells into adipose tissue.

Future research should be aimed at identifying possible signals that trigger caspase-1 activation specifically in VAT. The enhanced rate of lipolysis and resistance to insulin action in VAT (45, 46) may contribute to elevated activity levels of caspase-1. Hyperglycemia may also be one of the stimulators of caspase-1 in VAT (18), although it remains to be determined why high glucose levels would specifically activate caspase-1 in VAT and not in SAT. In addition to NLRP3, other members of the NLR family that can activate caspase-1, including NLRP1 and NLRC4 (interleukin-converting enzyme protease-activating factor), should be studied in human adipose tissue.

Irrespective of the cellular origin or activators of caspase-1, we demonstrate that the differences in IL-1β and IL-18 release in VAT are mediated by caspase-1. Previously, it has been suggested that expression and regulation of IL-1β are not solely dependent on inflammasome-mediated caspase-1 processing (47, 48). Several studies have identified other enzymes that can process pro-IL-1β and pro-IL-18 into their active forms, including the neutrophil-derived proteinase-3 under circumstances when neutrophils infiltrate sites of infection (49). Interestingly, it has been reported that neutrophils are activated to a greater extend in obese subjects (50), suggesting that these cells may also contribute to processing of bioactive IL-1β and IL-18. However, FACS analysis revealed no difference in granulocyte infiltration in both fat depots, making it less likely that IL-1β and IL-18 production by VAT occurred independently of caspase-1.

Although our study population was composed of a relatively small number of overweight subjects, future studies should be aimed at comparing caspase-1 levels in severely obese and nonobese individuals with or without type 2 diabetes mellitus in larger study populations. Hypothetically, enhanced caspase-1 activation in VAT of severely obese individuals may explain why an increase in this fat depot is associated with an enhanced proinflammatory status that may contribute to the progression of cardiovascular disease and type 2 diabetes mellitus.

In conclusion, we demonstrate that the inflammasome components NLRP3, ASC, and caspase-1 are present in human abdominal adipose tissue and are highly activated in VAT compared with SAT. Caspase-1 activation leads to an increased release of IL-1β and IL-18, regulates the production of other proinflammatory cytokines, including IL-6 and IL-8, and appears to be associated with the number of $CD8^+$ T cells present in adipose tissue. These findings give new insight into the important function of caspase-1 activation in determining the inflammatory characteristics of human adipose tissue.

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