Abstract  The NLRP3 inflammasome is involved in many obesity-associated diseases, such as type 2 diabetes, atherosclerosis, and gouty arthritis, through its ability to induce interleukin (IL)-1β release. The molecular link between obesity and inflammasome activation is still unclear, but free fatty acids have been proposed as one triggering event. Here we reported opposite effects of saturated fatty acids (SFAs) compared with unsaturated fatty acids (UFAs) on NLRP3 inflammasome in human monocytes/macrophages. Palmitate and stearate, both SFAs, triggered IL-1β secretion in a caspase-1/ASC/NLRP3-dependent pathway. Unlike SFAs, the UFAs oleate and linoleate did not lead to IL-1β secretion. In addition, they totally prevented the IL-1β release induced by SFAs and, with less efficiency, by a broad range of NLRP3 inducers, including nigericin, alum, and monosodium urate. SFAs did not affect the transcriptional effect of SFAs, suggesting a specific effect on the NLRP3 activation. These results provide a new anti-inflammatory mechanism of UFAs by preventing the activation of the NLRP3 inflammasome and, therefore, IL-1β processing. By this way, UFAs might play a protective role in NLRP3-associated diseases.

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Keywords: obesity • inflammation • innate immunity • interleukin-1beta • palmitate • stearate • oleate • linoleate

Interleukin-1β (IL-1β) is a proinflammatory cytokine involved in many obesity-associated diseases, such as type 2 diabetes, atherosclerosis, and gouty arthritis (1). Its release is tightly regulated at both transcriptional and posttranscriptional levels at different steps, including synthesis, processing, and secretion (2). IL-1β is produced as an inactive precursor, the pro-IL-1β, which is cleaved by caspase-1 into the biologically functional form. Caspase-1-mediated IL-1β maturation occurs mostly through the assembly of multiprotein complexes called inflammasomes (3). Several NOD-like receptor (NLR) family members were identified to form inflammasomes, including NLRP1, NLRP3, NLRP7, and NLRC4, but non-NLR inflammasomes exist, such as the AIM2 inflammasome (3).

Recent studies suggest a role of NLRP3 in obesity and its complications, especially in type 2 diabetes (4, 5). NLRP3 interacts with the adaptor protein ASC [apoptosis-associated speck-like protein containing a CARD (caspase recruitment domain)], mediating recruitment and auto-activation of caspase-1. NLRP3 is mainly expressed in immune cells from the myeloid lineage, such as monocytes, dendritic cells, and macrophages (6). However, its expression is low compared with ASC and caspase-1 (7). The main caspase-1 substrate, the pro-IL-1β, is not expressed or is very weakly expressed in these cells. As NLRP3 and pro-IL-1β are the limiting factors of the NLRP3 inflammasome, a first signal commonly called the priming is necessary to increase their transcriptions (8). Next, a second stimulus is required to induce NLRP3 inflammasome assembly, caspase-1 activation, and, therefore, IL-1β maturation.

Macrophages, whose NLRP3 and pro-IL-1β expression are easily inducible (8), infiltrate the adipose tissue in correlation with fatness (9). In obese individuals, the adipose tissue macrophages surround the dead adipocytes (10) and have a proinflammatory phenotype called M1-polarization or “classically activated macrophages” (11). IL-1β, one of the various cytokines secreted by M1-polarized macrophages,

Unsaturated fatty acids prevent activation of NLRP3 inflammasome in human monocytes/macrophages.

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Supplementary key words obesity • inflammation • innate immunity • interleukin-1beta • palmitate • stearate • oleate • linoleate

Abbreviations: ASC, apoptosis-associated speck-like protein containing a CARD; CARD, caspase recruitment domain; DSS, disuccinimidyl suberate; FACS, fluorescence-activated cell sorting; IL, interleukin; LPS, lipopolysaccharide; MDM, monocyte-derived macrophage; MDP, muramyl dipeptide; MSU, monosodium urate; NF-κB, nuclear factor-kappa B; NLR, NOD-like receptor; PBMC, peripheral blood mononuclear cell; PVD, pyrin domain; SFA, saturated fatty acid; SN, supertant; UFA, unsaturated fatty acid.

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is strongly incriminated in numerous obesity-associated
diseases in a NLRP3-dependent way. Indeed, NLRP3-
deficient mice are protected against obesity-induced insulin
resistance (4, 5), atherosclerosis (12), and gouty arthri-
tsis (13).

The molecular link between obesity and NLRP3-mediated
IL-1β release is not well established. Free fatty acids (FFAs),
usually elevated in plasma of obese people, have been
proposed as one triggering event (1). Recently, the
saturated fatty acid (SFA) palmitate demonstrated its
ability to activate the NLRP3 inflammasome in murine
macrophages (14). In the present study, we tested the
four most important FFAs present in blood (15) on hu-
man macrophages: two SFAs, palmitate and stearate, and
two unsaturated fatty acids (UFAs), oleate and linoleate.
We identified stearate as a new physiological NLRP3 in-
ducer, acting with the same efficiency as palmitate on the
caspase-1/ASC/NLRP3 pathway. UFAs, which did not
activate the NLRP3 inflammasome, revealed an un-
expected anti-inflammatory property through prevent-
ing NLRP3 inflammasome activation by SFAs and by
various inducers including nigericin, alum, and mono-
sodium urate (MSU).

MATERIALS AND METHODS
Preparation of FFA solutions
The palmitic acid (#P0500), stearic acid (#S4751), oleic acid
(#O1008), and linoleic acid (#L1376) were purchased from Sigma.
A 100 mM stock solution of sodium salt was prepared by
dissolving fatty acids in 0.1 M NaOH at 30°C, 37°C, 65°C, and
75°C for linoleic acid, oleic acid, palmitic acid, and stearic acid,
respectively. Stock solutions were aliquoted and stored at
−20°C for less than one year. A 5% fatty acid free, low endotoxin BSA
(Sigma, #A8806) solution was prepared in RPMI 1640. The FFA
stock solution and the 5% BSA solution were mixed together
to obtain a 2.5 mM working solution with FFA:BSA molar ratio
at 3.4:1. After pH adjustment, the working solution was fi ltered
to obtain a 2.5 mM working solution with FFA:BSA molar ratio

Generation of stable THP-1 cell line expressing esiRNA
The THP-1 cells stably expressing shRNA were obtained by lentiviral transduction carried out by the GIGA-Viral Vectors
Platform (GIGA, Liege, Belgium). In summary, shRNA and pro-
moter sequences were amplified by PCR from commercial plasmids encoding a shRNA against human NLRP3 (Sigma,
#TRCN0000427726) or a nontarget sequence (Sigma, #SHC002)
and cloned into pHRSin-CSGW plasmid expressing GFP [a kind gift from Dr. Els Veroyen (University of Lyon)]. New lentiviral
plasmids were cotransfected with pSPAX2 (Addgene, #12260)
and a VSV-G-encoding plasmid (16) in Lenti-X 293T cells (Clon-
tech, #632180). Viral supernatants were collected, fi ltered, and
concentrated 100× by ultracentrifugation. Finally, THP-1 cells
were transduced with these lentiviral vectors and GFP-positive
cells were sorted by FACS.

esiRNA transfection
Cells were transfected by using the HiPerFect transfection re-
agent (Qiagen) according to the manufacturer’s instructions for
suspension cell lines, with minor modifications. THP-1 cells (0.2 ×
10^5) were plated in a 24-well plate in 100 µl of supplemented
medium. Transfection complexes were prepared in RPMI 1640
without serum by adding 3 µl of HiPerFect and 400 ng of esiRNA
in a fi nal volume of 100 µl and mixed with suspension cells. After
6 h, 400 µl of supplemented medium were added. The next day,
differentiation was performed with PMA as previously described.
The esiRNAs used were from Sigma: esiPYCARD (#EHU066851)
for ASC, and esiNLRP3 (#EHU07121) and esiEGFP (#EHEUGFP)
for negative control.

Immunoblot analysis
Cells were washed, scraped in lysis buffer [50 mM Tris-HCl
pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Igepal, 0.25% sodium
decylsulfate, 1 mM PMSF, and complete protease inhibitor
cocktail (Roche Applied Science)], and centrifuged to remove
remnant pellets. Supernatants were stored at −20°C. For phos-
phorylation experiments, cells were lysed in total phospho lysis buf-
fier (62.5 mM Tris-HCl at pH 6.8, 10% glycerol, 2% SDS, 3% 
β-mercaptoethanol, 0.03% bromophenol blue, 1 mM DTT,
1 mM sodium orthovanadate, 25 mM β-glycerophosphate, 15 mM

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sodium fluoride, 1 mM PMSF, and complete protease inhibitor cocktail), sonicated, and stored at −20°C. Culture supernatants were also collected at −20°C after a centrifugation to remove cells. Proteins (40 µg) or supernatant (20 µl) were subjected to SDS-PAGE. After electrophoresis, proteins were transferred from gel to a PVDF membrane (GE Healthcare). The primary antibodies used were as follows: anti-ASC (Adipogen, #AG-25B-0006), anti-cleaved IL-1β (Cell Signaling, #2021), anti-IL-1β propeptide (R & D Systems, #MAB6904), anti-IL-1β (R & D Systems, #AF-201-NA), anti-NLRP3 (Sigma, #HPA012878), anti-phospho-IkBα (Cell Signaling, #9246), and anti-phospho-p65 (Cell Signaling, #3031). The secondary antibodies conjugated to HRP were anti-rabbit (Cell Signaling, #7074), anti-goat (Dako, #P0160), and antimouse (Dako, #P0447). The detection was performed with ECL or ECL plus Western blotting substrate (Pierce) by using the digital imaging system ImageQuant LAS 4000 (GE Healthcare). Quantification was achieved with the ImageQuant TL software (version 7.0, GE Healthcare).

ELISA

Mature IL-1β was quantified in supernatants by ELISA with Quantikine for human IL-1β (R & D Systems) according to the manufacturer’s recommendations.

qRT-PCR

Total RNAs were extracted with RNeasy Mini Kit (Qiagen) according to the manufacturer’s protocol, followed by DNase treatment (Roche). After quantification by spectrophotometer Nanodrop 1000 (Thermo scientific), 500 ng of RNA were reverse-transcribed by using the moloney murine leukemia virus reverse transcriptase 1000 (Thermo scientific), 500 ng of RNA were reverse-transcribed by using the moloney murine leukemia virus reverse transcriptase (Invitrogen). Obtained cDNA was submitted to qRT-PCR on a LightCycler 480 (Roche Applied Science). Relative gene expression was calculated using the \( 2^{-\Delta\Delta CT} \) method (17) with HPRT1 as housekeeping gene. Results are representative from three independent experiments quantified in triplicate. The following primers were used: IL-1β-Fw: 5′-CCATTGGCGCTCAAGGAAAA-3′; IL-1β-Rv: 5′-CTCCACGCTGTAGTGCTGCTTA-3′; ASC-Fw: 5′-CCGGGAGGTGTCGAAGAOCT-3′; ASC-Rv: 5′-TGCTCATGCGAGACCTT-3′; Caspase-1-Fw: 5′-GCGAAGGTTCAAGTTTCA-3′; Caspase-1-Rv: 5′-ACTCTTTCATGCGGCGACT-3′; NLRP3-Fw: 5′-CTGTAACATTGGGATTTGGT-3′; NLRP3-Rv: 5′-GACCGAAGAGTGCTCGAACA-3′; HPRT1-Fw: 5′-TGACACTGGCAAACATAGCA-3′; HPRT1-Rv: 5′-GCTTGGCCACCTTAACTCT-3′.

Measurement of caspase-1 activity

A 1 mM stock solution of fluorescence-quenching substrate for caspase-1 was prepared by dissolving the (7-methoxycoumarin-4-yl)acetyl-L-tyrosyl-L-valyl-L-alanyl-L-asparyl-L-alanyl-L-prolyl-Nε(2,4-dinitrophenyl)-L-lysine amide (MOCA-YVADPK(Dnp)-NH2) (PepT nova, #3183a) in DMSO, and then kept on ice. To remove nuclei, samples were filtered through 5 µm pore size membrane filter. Crosslinked complexes were precipitated by centrifugation at full speed for 3 min at 4°C, and pellets were resuspended in total phospho lysis buffer before immunoblot analysis.

NF-κB/AP-1 activity assay

THP1-XBlue™ cells were differentiated and treated as described above. After treatment, supernatants were mixed with QUANTI-Blue™ (InvivoGen) according to the manufacturer’s instructions to detect alkaline phosphatase activity. Absorbance was measured at 630 nm on the EnSpire 2300 Multilabel Reader.

Statistical analyses

All statistical analyses were carried out using GraphPad Prism 5 for Windows (GraphPad Software, Inc.) and presented as means ± SEM. The Student t-test was performed for simple comparison (two groups) and the ANOVA test followed by Bonferroni post-test for multiple comparisons (more than two groups). Significance is indicated by a symbol.

RESULTS

SFA induce IL-1β secretion by activating the NLRP3 inflammasome in human monocytes/macrophages

FFAs are poorly soluble compounds in aqueous solution, and most of them are bound to serum albumin in human physiology (18). For in vitro experiments, human serum albumin is often substituted by BSA as we did. Among FFAs, palmitate (C16:0) demonstrated its ability to induce IL-1β secretion in murine macrophages through a caspase-1/ASC/NLRP3-dependent pathway (14). In human primary monocytes primed with LPS to increase expression of both NLRP3 and pro-IL-1β (Fig. 1A, bottom), we showed that seutarate (C18:0) was also able to induce IL-1β release with similar efficiency as palmitate when compared with vehicle alone (BSA) (Fig. 1A, top). On the other hand, oleate (C18:1) and linoleate (C18:2) had no effect on IL-1β secretion, showing opposite effects of SFAs compared with UFAs. Similar findings were observed in MDMs (Fig. 1B) and in M1-polarized macrophages (Fig. 1C), the predominant phenotype of macrophages present in the adipose tissue from obese. The release of IL-1β observed after LPS-priming (Fig. 1A) or M1-polarization (Fig. 1C) is linked to pro-IL-1β synthesis. Indeed, monocytes have a constitutive caspase-1 activity (19), resulting in a background of IL-1β release related to pro-IL-1β content.

To explore the signaling pathway involved in SFA-induced IL-1β release, we differentiated the human monocyte cell line THP-1 into macrophage-like cells by using
PMA. This classical (20, 21) and highly responsive model allowed us to easily detect large amounts of IL-1β in supernatant by Western blot and to focus on the maturation process. As in primary monocytes/macrophages, SFAs increased the IL-1β secretion in PMA-differentiated THP-1 cells in a dose-dependent manner (Fig. 2A). The 17 kDa form of IL-1β observed in Fig. 2A is known to be mainly produced after a proteolytic cleavage by caspase-1 (2, 3). To investigate caspase-1 involvement, the PMA-differentiated THP-1 cells were treated with SFAs alone or along with a pan-caspase inhibitor (Z-VAD) or with a caspase-1-specific inhibitor (Z-YVAD). Inhibition of caspasas completely abolished SFA-induced IL-1β release, while caspase-1-specific inhibition repressed it almost completely (Fig. 2B). This result suggests a key role for caspase-1 in SFA-induced IL-1β processing, even if a minor contribution of a second caspase is not excluded.

ASC is an adaptor protein commonly involved in caspase-1 processing. It is composed of two domains mediating protein-protein interactions: the pyrin domain (PYD) and the CARD domain, which is essential for the homotypic interaction with caspase-1. When ASC was knocked down by esiRNA in PMA-differentiated THP-1 cells, a decrease in IL-1β secretion was observed after SFAs treatment (Fig. 2C). ASC can participate in caspase-1 activation through formation of two types of complexes: the pyroptosome and

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**Fig. 1.** SFAs, unlike UFAs, induce IL-1β release in human monocytes/macrophages. (A–C) ELISA for IL-1β in SNs (top) and immunoblotting for pro-IL-1β and NLRP3 in cell extracts (XT) (bottom) from (A) LPS-primed monocytes, (B) MDM, or (C) M1-polarized macrophages either treated with 200 µM of FFAs or vehicle (BSA) for 8 h. Results are presented as means ± SEM of three (A) or four (B, C) independent experiments. Beta-tubulin was blotted as a loading control in cell extracts. **P < 0.01 compared with BSA by one-way ANOVA followed by Bonferroni post-test. ND, not determined.

**Fig. 2.** SFAs activate the NLRP3 inflammasome in PMA-differentiated THP-1 cells. (A–C) IL-1β release in SNs from PMA-differentiated THP-1 cells determined by immunoblotting after 8 h of treatment (A) with various concentrations of FFAs, (B) with 200 µM of SFAs along with caspase inhibitor Z-VAD or caspase-1 inhibitor Z-YVAD, or (C) with 200 µM of SFAs after ASC or NLRP3 silencing by esiRNA. (D) PMA-differentiated THP-1 cells were treated with 200 µM of SFAs for 4 h. Cell extracts were crosslinked with DSS, and immunoblotting was performed using anti-ASC antibody. (E) IL-1β release in SNs from PMA-differentiated THP-1 cells stably expressing shRNA against NLRP3 (shNLRP3) or a nontarget shRNA (shNT) determined by immunoblotting after 8 h of treatment with 200 µM of SFAs. NLRP3 and ASC were blotted as a control for RNAi. Beta-tubulin or HSP60 was blotted as a loading control in cell extracts. LPS (1 µg/ml) was used as positive control in (D), and alum (400 µg/ml) in (A) and (E). A nonspecific band (NS) at 10 kDa was used as loading control in SNs. XT, cell extract. NS, nonspecific band.
the inflammasome. The pyroptosome is produced after dimerization of ASC through its pyrin domain, allowing recruitment and activation of caspase-1 (7). To examine pyroptosome assembly, PMA-differentiated THP-1 cell extracts were crosslinked with DSS to detect ASC dimers. Compared with LPS at high concentration, a well-characterized pyroptosome inducer in PMA-differentiated THP-1 cells (7), neither palmitate nor stearate led to ASC dimerization (Fig. 2D). This indicates that ASC involvement in SFA-induced IL-1β secretion is not due to pyroptosome formation.

The second complex formed with ASC protein is the inflammasome. This complex appears by interaction between the PYD domain of ASC and the PYD domain of a second protein, such as NLRP3 or AIM2 (3). As for pyroptosome, ASC interaction leads to caspase-1 recruitment. Among the inflammasomes, the NLRP3 inflammasome is the most extensively studied, and a wide variety of compounds is known to activate it (3). The knockdown of NLRP3 in PMA-differentiated THP-1 cells by esiRNA led to a strong inhibition of SFA-induced IL-1β release (Fig. 2C). Similar results were obtained in THP-1 cells stably expressing shRNA against NLRP3 (Fig. 2E), confirming that both palmitate and stearate lead to a NLRP3 inflammasome-dependent IL-1β processing.

UFAs prevent SFA-induced IL-1β maturation

Unlike SFAs, UFAs possess double bonds that confer their particular properties. As described previously, they cannot activate the NLRP3 inflammasome compared with SFAs. To determine the impact of UFAs on SFA-induced IL-1β release, we treated LPS-primed primary monocytes with SFAs alone or in combination with UFAs. Interestingly, the presence of UFAs totally abolished the IL-1β secretion induced by SFAs, with equal efficiency for both oleate and linoleate (Fig. 3A). In M1-polarized macrophages, UFAs also decreased SFA-induced IL-1β release (Fig. 3B, C).

The affinity of long-chain fatty acids for BSA weakly differs according to each FFA (22). Therefore, if SFAs have more affinity to BSA than do UFAs, the addition of BSA to the inflammasome. The pyroptosome is produced after dimerization of ASC through its pyrin domain, allowing recruitment and activation of caspase-1 (7). To examine pyroptosome assembly, PMA-differentiated THP-1 cell extracts were crosslinked with DSS to detect ASC dimers. Compared with LPS at high concentration, a well-characterized pyroptosome inducer in PMA-differentiated THP-1 cells (7), neither palmitate nor stearate led to ASC dimerization (Fig. 2D). This indicates that ASC involvement in SFA-induced IL-1β secretion is not due to pyroptosome formation.

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UFAs inhibit NLRP3 inflammasome

To overcome this problem, a different approach was used. As explained previously, the NLRP3 inflammasome is known to be activated by various compounds (3). Therefore, we tested UFAs outcomes on the most common NLRP3 activators such as nigericin, ATP, alum crystals and MSU in LPS-primed monocytes (Fig. 5A, B) and in PMA-differentiated THP-1 cells (Fig. 5C, D). Cotreatment with UFAs significantly inhibited the IL-1β release induced by SFAs but also by nigericin, alum, and MSU (Fig. 5B, D). On the other side, ATP seemed to be insensitive to UFAs after 8 h of cotreatment in both LPS-primed monocytes and PMA-differentiated THP-1 cells.

Several less frequently used compounds were described to activate the NLRP3 inflammasome, such as doxorubicin (23), tunicamycin (20), muramyl dipeptide (MDP) (24), antimycin A (21) and monensin (25). Cotreatment with UFAs significantly inhibited the IL-1β release induced by all these compounds in PMA-differentiated THP-1 cells (supplementary Fig. I-A, B). The NLRP3 inflammasome involvement was confirmed for all these compounds by using the shNLRP3 THP-1 cells (supplementary Fig. I-C).

NLRP3 is one protein among many others leading to an inflammasome assembly. To explore other inflammasomes, we next treated LPS-primed monocytes with NLRC4, AIM2 or NLRP7 activators, respectively flagellin, poly(dA:dT) or FSL-1 (Fig. 6A). UFAs cotreatment had no significant effect on IL-1β release induced by these compounds (Fig. 6B). Taken together, these results suggest a specific action on the NLRP3 inflammasome, probably upstream of inflammasome assembly.

UFAs decrease NLRP3 inflammasome activation by various inducers

The hallmark of NLRP3 inflammasome formation is the interaction between NLRP3 and ASC. Unfortunately, the low amount of endogenous NLRP3 and the lack of good antibodies against the human form make this interaction difficult to observe without over-expressing a tagged NLRP3 protein. To overcome this problem, a different approach was used. As explained previously, the NLRP3 inflammasome is known to be activated by various compounds (3). Therefore, we tested UFAs outcomes on the most common NLRP3 activators such as nigericin, ATP, alum crystals and MSU in LPS-primed monocytes (Fig. 5A, B) and in PMA-differentiated THP-1 cells (Fig. 5C, D). Cotreatment with UFAs significantly inhibited the IL-1β release induced by SFAs but also by nigericin, alum, and MSU (Fig. 5B, D). On the other side, ATP seemed to be insensitive to UFAs after 8 h of cotreatment in both LPS-primed monocytes and PMA-differentiated THP-1 cells.

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**Fig. 5.** UFAs downregulate NLRP3 activation by classical inducers in primed-monocytes. (A) ELISA for IL-1β in SNs from LPS-primed monocytes after cotreatment with various NLRP3 inducers and 200 µM of UFAs or BSA. Cells were cotreated with nigericin (5 µM), ATP (5 mM), MSU (100 µg/ml) or alum (400 µg/ml) for 8 h. Results are presented as means ± SEM of four independent experiments. (B) Normalization of A to induction with each agent cotreated with BSA. (C) IL-1β release in SNs from PMA-differentiated THP-1 cells determined by immunoblotting after cotreatment with various NLRP3 inducers and 200 µM of UFAs or BSA. Cells were cotreated with nigericin (5 µM), ATP (10 mM), MSU (100 µg/ml), or alum (400 µg/ml) for 8 h. (D) Quantification of IL-1β in SN from C. Results are presented as means ± SEM of at least three independent experiments. A nonspecific band (NS) at 10 kDa was used as loading control in SN. HSP60 was blotted as a loading control in cell extracts. *P < 0.05; **P < 0.01; ***P < 0.001; NS, nonsignificant by t-test. XT, cell extract.
UFAs reduce IL-1β processing but not transcription

Nuclear factor-kappa B (NF-κB) family members are critical regulators of gene expression in mammals (26). These transcription factors bind as dimers to κB response elements in diverse gene promoters. Without stimulation, this dimer is sequestered in the cytoplasm by the inhibitory protein IκBα. Upon activation of the canonical pathway, IκBα is phosphorylated, ubiquitinated, and finally submitted to proteasomal degradation allowing the p50/p65 heterodimer translocation to nucleus. Various posttranslational modifications are known to change the transcriptional activity of p50/p65, such as the activating phosphorylation in the transactivation domain of p65 (26). SFAs are known to activate the canonical NF-κB pathway in nondifferentiated THP-1 cells (27). In PMA-differentiated THP-1-XBlue cells, a THP-1 cell line stably expressing a reporter gene under the control of NF-κB and AP-1 transcription factors, an increase of the transcriptional activity was reported after SFA but not after UFA treatment (Fig. 7A). Since IL-1β also activates the NF-κB pathway and to exclude the artifact related to its release, subsequent experiments were performed before IL-1β secretion. IL-1β release occurred at around 4 h and became significant at 6 h post-treatment (Fig. 7B). After 3 h, SFAs increased phosphorylation of IκBα and p65 in PMA-differentiated THP-1 cells (Fig. 7C). No increase in phosphorylation was observed after UFA treatment.

Because NLRP3 and IL-1β are both NF-κB-dependent genes (8), SFAs could likely play a priming role as it was previously described in dendritic cells (28). Although IL-1β and NLRP3 genes were already induced by PMA in THP-1 cells, a further induction of NLRP3 and IL-1β mRNA was observed by qRT-PCR after treatment with SFAs but not with UFAs (Fig. 7D), suggesting a “second priming” concomitantly to NLRP3 inflammasome activation in PMA-differentiated THP-1 cells. ASC and caspase-1 mRNA did not change. This low induction in gene expression is specific of the PMA-differentiated THP-1 model, and no further induction of NF-κB activation was observed in LPS-primed monocytes (data not shown).

We next examined the impact of UFAs on this further induction of gene expression. Addition of UFAs did not change the phosphorylation of IκBα and p65 induced by SFAs in PMA-differentiated THP-1 cells (Fig. 8A). No significant effect was observed on IL-1β (Fig. 8B) or NLRP3 (Fig. 8C) mRNA levels when UFAs were present. Taken together, these results strongly suggest that i) SFAs increase little or no gene expression in primed cells, confirming that the IL-1β release observed is mainly due to an enhancement in processing; ii) the decrease in IL-1β secretion by cotreatment with UFAs is not linked to reduction of transcription but to reduction of IL-1β processing.

**DISCUSSION**

When the immune system is activated, all the resources are mobilized to eradicate the aggressor. At term, the inflammatory response ends and the immune system comes back to normal. In particular cases, the immune system is ineffective to resolve the trouble and a chronic inflammation appears. In obesity, an unexplained chronic, low-grade inflammation is present (29) in which SFAs could play a role. As reported here, both SFAs palmitate and stearate activated the NLRP3 inflammasome and led to IL-1β secretion. Unlike SFAs, the UFAs oleate and linoleate were unable to activate the NLRP3 inflammasome, showing opposite effects of SFAs versus UFAs. Our results confirm in human monocytes/macrophages previous work demonstrating the NLRP3 activation in mouse macrophages by palmitate (14) and identify stearate as a new inducer. Stearate is the second most important SFA in blood and represents with palmitate 90% of total SFAs (15), while other SFAs, such as laurate and myristate, are present in very low concentrations. This proinflammatory effect of SFAs in vitro corroborates interventional studies describing that SFA-rich diets increase IL-1β production (4, 30) as well as also other inflammatory processes that may be related to IL-1β (31–34).

In PMA-differentiated THP-1 cells, we demonstrated a weak induction of IL-1β and NLRP3 gene expression by SFAs. Even if it was low, this result means that the increase in IL-1β secretion after SFA treatment can also be, in part, of transcriptional origin. The lack of further induction reported in LPS-primed monocytes is likely inherent to the
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They also tested non-omega-3 fatty acids, such as oleate (but not linoleate), but they failed to observe an inhibition. This surprising result is likely due to their experimental conditions. Indeed, they used a very low concentration of oleate, lower than the physiological level, to work with a concentration corresponding to omega-3.

UFAs are known to exert a broad range of effects, such as changes in membrane composition (41), generation of anti-inflammatory compounds, such as resolvins (42), or activation of various receptors (43). G protein-coupled receptor (GPR)40 and GPR120 are both activated by long-chain fatty acids. Inhibition of the NLRP3 inflammasome by omega-3 was described to be dependent on these two receptors through initiation of \( \beta\)-arrestin-2 binding to NLRP3 (30). Therefore, one explanation could be that oleate and linoleate prevent NLRP3 inflammasome activation by binding these receptors but with less affinity than omega-3, explaining the requirement of a higher concentration. A second mechanism could involve the endoplasmic reticulum (ER). UFAs could prevent ER stress induction (44, 45), recently described to be involved in inflammasome activation (20, 46). In addition, SFAs or tunicamycin, both sensitive to UFA inhibition, are well-characterized ER stress inducers (20, 44–46).

An outstanding issue is the lack of response to UFAs when NLRP3 was activated by ATP. Numerous studies have described contradictory results depending on the activators tested (47–49). This wide heterogeneity in response to NLRP3 inducers clearly suggests that several pathways are able to trigger the NLRP3 inflammasome assembly.
Il-1
ventions could be helpful to obese people in decreasing
tagonists requiring regular injections, simple dietary inter-
arthritis. Unlike treatments with anakinra or other IL-1
involved, such as type 2 diabetes, atherosclerosis, or gouty
could play a protective role in diseases in which NLRP3 is
effect on exogenous ATP.

Extended to all the NLRP3 inducers, one likely explana-
tion could be that UFAs prevent ATP release but have no
impression of NLRP3 activators. These results could be correlated
with in vivo studies reporting anti-inflammatory properties
of a UFA-rich diet on IL-1β production. By preventing
NLRP3 inflammasome activation, UFAs might play a pro-
tective role in NLRP3-associated diseases, such as type 2
diabetes, atherosclerosis, and gouty arthritis.

Currently, the ATP-activating pathway is commonly de-
scribed as very simple with few places for UFA interfer-
tance: ATP binds to P2X7 receptor leading to K+ efflux
and cells were treated for 1 h (A) or 2 h (B, C). NS, nonsignificant
by t-test.

Albeit the mechanism is not yet fully elucidated, UFAs
could play a protective role in diseases in which NLRP3 is
involved, such as type 2 diabetes, atherosclerosis, or gouty
arthritis. Unlike treatments with anakinra or other IL-1β
antagonists requiring regular injections, simple dietary inter-
ventions could be helpful to obese people in decreasing
IL-1β secretion and inflammation. Further investigation is
required before concluding a protective effect of a high-UFA
diet on inflammation, but several lines of evidence point in
this direction. Numerous studies described a powerful anti-
flammatory effect for oleate and linoleate. Ex vivo macro-
phages from rats treated by gavage with oleate or linoleate
showed a reduction of cytokine secretion, including IL-1β,
compared with untreated rats (52), and intracerebroven-
tricular injection of oleate reduced hypothalamic inflam-
mation in rats (53). In most cases, intervention studies are
performed in animals by changing the diet. Various groups
reported an improvement in obesity-associated inflamma-
tion (32–34) and insulin sensitivity (34, 53) after a diet rich
in UFAs, partially attributing these results to oleate- and li-
oleate-rich oils. In humans, there is an association between
high-SFA and/or low-UFA concentrations and inflamma-
tion (54, 55) or metabolic syndrome (54). In some studies,
these parameters can predict metabolic syndrome develop-
ment (56, 57).

In conclusion, we demonstrated that SFAs palmitate
and stearate trigger IL-1β secretion in various models of
human monocytes and macrophages in a caspase-1/ASC/
NLRP3-dependent pathway. The UFAs oleate and li-
oleate, which did not activate the NLRP3 inflammasome,
totally prevented the NLRP3 inflammasome activation in-
duced by SFAs and, with less efficiency, by a broad range
of NLRP3 activators. These results could be correlated
in vivo studies reporting anti-inflammatory properties of
a UFA-rich diet on IL-1β production. By preventing
NLRP3 inflammasome activation, UFAs might play a pro-
tective role in NLRP3-associated diseases, such as type 2
diabetes, atherosclerosis, and gouty arthritis.

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