Monounsaturated Fatty Acid–Enriched High-Fat Diets Impede Adipose NLRP3 Inflammasome–Mediated IL-1β Secretion and Insulin Resistance Despite Obesity

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Saturated fatty acid (SFA) high-fat diets (HFDs) enhance interleukin (IL)-1β–mediated adipose inflammation and insulin resistance. However, the mechanisms by which different fatty acids regulate IL-1β and the subsequent effects on adipose tissue biology and insulin sensitivity in vivo remain elusive. We hypothesized that the replacement of SFA for monounsaturated fatty acid (MUFA) in HFDs would reduce pro-IL-1β priming in adipose tissue and attenuate insulin resistance via MUFA-driven AMPK activation. MUFA-HFD–fed mice displayed improved insulin sensitivity coincident with reduced pro-IL-1β priming, attenuated adipose IL-1β secretion, and sustained adipose AMPK activation compared with SFA-HFD–fed mice. Furthermore, MUFA-HFD–fed mice displayed hyperplastic adipose tissue, with enhanced adipogenic potential of the stromal vascular fraction and improved insulin sensitivity. In vitro, we demonstrated that the MUFA oleic acid can impede ATP-induced IL-1β secretion from lipopolysaccharide and SFA-primed cells in an AMPK-dependent manner. Conversely, in a regression study, switching from SFA to MUFA-HFD failed to reverse insulin resistance but improved fasting plasma insulin levels. In humans, high-SFA consumers, but not high-MUFA consumers, displayed reduced insulin sensitivity with elevated pycard-1 and caspase-1 expression in adipose tissue. These novel findings suggest that dietary MUFA can attenuate IL-1β–mediated insulin resistance and adipose dysfunction despite obesity via the preservation of AMPK activity.

Intricate cellular mechanisms contribute to obesity and type 2 diabetes (T2D), wherein the development of insulin resistance (IR) is critical (1). Obesity-associated IR reflects sophisticated networks interlinking metabolic and inflammatory processes; a phenomenon aptly coined “meta-inflammation” where the nucleotide binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome is a key regulatory hub. However, the mechanisms by which different fatty acids regulate adipose tissue NLRP3 inflammasome activity and metabolic dysfunction remain ill defined.
Recent studies (2–5) have established a critical role for the NLRP3 inflammasome and interleukin (IL)-1β in peripheral IR and T2D. Furthermore, IL-1β impedes adipocyte insulin signaling (6) and adipogenesis (7). Given the highly potent nature of IL-1β, release is tightly regulated and requires two signals. First, the cell is “primed” to produce pro-IL-1β via Toll-like receptor 4 (TLR4)/nuclear factor-κB (8). The NLRP3 inflammasome, a lipid-responsive protein complex, then mediates the caspase-1-dependent processing of pro-IL-1β to mature IL-1β (9). It has been established that saturated fatty acids (SFAs), particularly palmitic acid (PA), can initiate the first “priming” signal via TLR4 (5). The second signal is likely mediated by danger-associated molecular pattern molecules such as ATP, uric acid, and reactive oxygen species (10). The role of monounsaturated fatty acids (MUFAs) in inflammation and IR remains contentious. Human studies (11,12) report conflicting evidence regarding the cardioprotective role of MUFA-rich diets. Mechanistic evidence regarding the effects of different fatty acids on IL-1mediated inflammation and insulin sensitivity in adipose tissue biology in vivo is lacking.

This present study shows that a MUFA high-fat diet (HFD) does not prime pro-IL-1β in adipose tissue concomitant with enhanced AMPK activation, a hyperplastic adipose morphology, and partial protection from IR. Furthermore, MUFAs can impede ATP-induced IL-1β secretion from lipopolysaccharide (LPS)-primed cells in an AMPK-dependent manner in vitro. Additionally, high dietary SFA intake, but not high MUFA intake, is associated with IR and inflammation in humans with T2D. This study demonstrates that dietary MUFAs can attenuate IL-1β-mediated IR and adipose tissue dysfunction despite obesity.

RESEARCH DESIGN AND METHODS

Materials

Cell culture material was purchased from Lonza (Slough, U.K.). All other reagents, unless otherwise stated, were from Sigma-Aldrich Ireland Ltd. (Wicklow, Ireland).

Animals

C57BL/6 male mice (8–9 weeks of age) were purchased from Harlan U.K. Ltd. Ethical approval was obtained from the University College Dublin Ethics Committee, and mice were maintained according to European Union and Irish Department of Health regulations. Two feeding studies were completed. 1) Mice were fed SFA-HFD (45% kcal PA), MUFA-HFD (45% kcal oleic acid [OA]) (Research Diets Inc., New Brunswick, NJ), or standard chow diet (5.2% fat: 0.9% SFA, 1.3% MUFA, 3.4% PUFA) (Teklad; Harlan U.K. Ltd.) ad libitum for 24 weeks (dietary composition is outlined in Supplementary Table 1). Fasted (6 h) mice were injected with either saline or insulin (1.5 units/kg; Actrapid; Novo Nordisk, Bagsvaerd, Denmark) 15 min mice prior to euthanasia by cervical dislocation. 2) Mice were fed SFA-HFD for 16 weeks, and were either maintained on an SFA-HFD or switched to a MUFA-HFD for a further 16 weeks.

Intraperitoneal Glucose and Insulin Tolerance Test

Fasted mice (6 h) were injected intraperitoneally with 25% (weight for volume) glucose (1.5 g/kg) (B Braun Medical Ltd., Dublin, Ireland) or insulin (0.5 units/kg). Blood glucose levels were measured before and after a glucose/insulin challenge. In a separate procedure, overnight-fasted mice were administered glucose (1.5 g/kg), and blood samples were collected by tail vein bleed sampling. Plasma insulin levels were determined using an ultra-sensitivity insulin ELISA (Crystal Chem Inc., Downers Grove, IL).

Stromal Vascular Fraction Culture

To separate the stromal vascular fraction (SVF) from the adipocyte fraction, epididymal adipose tissue was minced and collagenase (2 mg/mL) digested prior to centrifugation. The adipocyte (200 mL packed volume/mL) and SVF were seeded (1 × 106 cells/mL), cultured in complete media (DMEM, 10% FBS, and 1% penicillin/streptomycin), and treated with or without ATP (5 mmol/L) for 24 h, or with or without LPS (10 ng/mL) for 3 h. Protein lysates and culture media were harvested for further analysis. Separately, the SVF was cultured for 7 days to remove nonadherent cells, promoting a preadipocyte-enriched SVF, and then was incubated with adipogenic media (10% FBS, 0.5 mmol/L isobutylmethylxanthine, 1 μmol/L dexamethasone and 1 μg/mL insulin) for 24 h. Cells were harvested in TRI Reagent for gene expression analysis.

Bone Marrow–Derived Macrophages

Bone marrow–derived cells were isolated from tibias and fibulas of mice, and were cultured in media supplemented with 30% L929 conditioned medium for 7 days prior to cell culture experiments. Bone marrow–derived macrophages (BMMs) (1 × 106 cells/mL) were primed with LPS (10 ng/mL) for 3 h, then were treated with either PA or OA (250 μmol/L) for 24 h, followed by ATP (5 mmol/L) stimulation for 1 h. For NLRP3 inhibitor experiments, preprimed BMMs were treated with the cytokine release inhibitory drug CRID3 (50 nmol/L) for 30 min prior to ATP (5 mmol/L) stimulation for 1 h. For AMPK inhibitor experiments, preprimed BMMs were treated with compound C (10 μmol/L) for 1 h, incubated with OA (250 μmol/L) for 24 h, then stimulated with ATP (5 mmol/L) for 1 h. For AMPK agonist experiments, preprimed BMMs were treated with compound A (10 μmol/L) for 1 h, incubated with OA (250 μmol/L) for 24 h, then stimulated with ATP (5 mmol/L) for 1 h. Protein lysates and culture media were harvested for further analysis.

Lentiviral Short Hairpin RNA AMPKα1 Knockdown

AMPKα1(1–4) mission lentiviral short hairpin RNA (shRNA) vector constructs (PLCO.1 [mission shRNA]) and scrambled control lentiviral vectors were generated by cotransfecting with pMD2.G and psPAX2 viral envelope and packaging vectors (courtesy of the Trono Laboratory, Lausanne,
Switzerland) into HEK293 cells. Lentivirus-containing media were harvested, filtered, and used to infect human THP-1 monocytes. Successfully transduced cells were selected with puromycin (0.5 μg/mL). Gene knockdown of cells was confirmed by immunoblotting.

**Human Study**

A subcohort of T2D subjects (n = 160–184) from the CORDIOPREV study (NCT00924937) (13) were categorized based on tertiles of baseline fasting plasma SFA and MUFA concentrations. The association of habitual dietary fat composition with insulin sensitivity was determined by the insulin sensitivity index (ISI) and HOMA-IR. Baseline fasting plasma C-reactive protein levels were measured. This work was approved by the Hospital Universitario Renò Sofia (Córdoba, Spain). Informed consent was obtained from all subjects. The effect of dietary MUFA intervention and habitual dietary MUFA intervention and dietary MUFA concentrations. The association of habitual dietary fat composition with insulin sensitivity was determined by the insulin sensitivity index (ISI) and HOMA-IR. Baseline fasting plasma C-reactive protein levels were measured. This work was approved by the Hospital Universitario Renò Sofia (Córdoba, Spain). Informed consent was obtained from all subjects. The effect of dietary MUFA intervention and habitual dietary MUFA intake on insulin sensitivity and adipose gene expression of caspase-1 was also evaluated in the LIPGENE study and is provided in the Supplementary Data.

**General Laboratory Methods**

Detailed descriptions of general methodologies, including plasma analysis, flow cytometry, immunoblotting, and real-time PCR, are included in the Supplementary Data.

**Statistical Analysis**

Data are reported as the mean ± SEM. For glucose tolerance test (GTT)/insulin tolerance test (ITT) studies with multiple time points, we performed two-way repeated-measures ANOVA to test for differences in means between groups. When significant, post hoc Bonferroni-corrected t tests were applied. For the comparison of data among three groups at a single time point, one-way ANOVA was performed with post hoc Bonferroni-corrected t testing applied. Prism version 5 (GraphPad Software Inc.) or PASW statistics version 20.0 (SPSS) was used for statistical analyses. Statistical significance is presented as *P < 0.05, **P < 0.01 and ***P < 0.001 with respect to (w.r.t.) chow; and #P < 0.05, ##P < 0.01, and ###P < 0.001 for SFA versus MUFA in all figures.

**RESULTS**

**HFDs Enriched With PA but Not OA Can Prime Pro–IL-1β Production in the SVF of Adipose Tissue In Vivo**

Ex vivo adipose profiling showed that the SFA-HFD increased IL-1β secretion (Fig. 1A) and active IL-1β protein expression (Fig. 1B) to a greater extent than the MUFA-HFD, while tumor necrosis factor-α and IL-6 secretion were equivalent (data not shown). Adipose tissue apoptosis-associated speck-like protein containing CARD (ASC) protein expression was upregulated by both HFDs while caspase-1 protein levels were unchanged (Fig. 1B). Adipose tissue NLRP3, IL-1β, and caspase-1 mRNA expression was significantly upregulated after an SFA-HFD compared with a MUFA-HFD, whereas the expressions of sirtuin-1 (SIRT-1), TLR4, and IL-18 mRNA were comparable between the HFD groups (Fig. 1C).

The separation of adipose tissue into cellular compartments demonstrated that the SVF from SFA-HFD mice secreted significantly higher amounts of ATP-induced IL-1β secretion were equivalent (data not shown). Adipose tissue C–reactive protein secretion were equivalent (Supplementary Data). Adipose tissue was reduced after a MUFA-HFD compared with an SFA-HFD compared with a MUFA-HFD and MUFA-HFD SVF compared with SFA-HFD SVF compared with chow and MUFA-HFD SVF (Fig. 1F). Proinflammatory M1 cell recruitment into adipose tissue was reduced after a MUFA-HFD compared with an SFA-HFD compared with an SFA-HFD and F. In liver tissue, mRNA expression of inflammatory markers, and levels of phosphorylated (p) Jun NH2-terminal kinase and p-extracellular signal–related kinase were equivalent across groups (Supplementary Fig. 2A–C). Further, there was no difference in IL-1β gene expression in skeletal muscle (Supplementary Fig. 2F).

**MUFA-HFD Partially Protects Against Obesity-Induced IR and Hyperinsulinemia**

MUFA-HFD mice were partly protected from IR compared with SFA-HFD mice (Fig. 2A), despite equivalent glucose tolerance (Fig. 2B). MUFA-HFD mice had significantly lower fasting glucose and insulin concentrations, and attenuated insulin secretion in response to glucose challenge (Supplementary Fig. 5B and Fig. 2C). Adipocyte insulin receptor substrate (IRS)-1 and GLUT-4 mRNA expression were significantly reduced by an SFA-HFD, but not a MUFA-HFD (Fig. 2D). Adipose tissue from MUFA-HFD–fed mice displayed elevated tyrosine pIRS-1 (Fig. 2E) and pAKT (Fig. 2F) levels, compared with SFA-HFD in response to insulin. Liver GLUT-2, IRS-1, IRS-2, G-6-P-P, and Pepck mRNA expression, and protein pAKT levels were unchanged across groups (Supplementary Figs. 2D and E and 5A). Similarly in skeletal muscle, GLUT-4, IRS-1, and IRS-2 mRNA expression was equivalent between HFD groups (Supplementary Fig. 2F).

MUFA-HFD mice became significantly obese compared with chow-fed controls, but gained less weight than SFA-HFD mice (Supplementary Fig. 3A). Adipose tissue depot weights were equivalent (Fig. 3C), but liver weight was reduced in MUFA-HFD mice (Supplementary Fig. 3B). MUFA-HFD mice displayed elevated energy expenditure, with increased VO2 and heat production (Supplementary Fig. 3C and D), but no difference in locomotor activity or respiratory exchange rate (data not shown). mRNA expression of markers of mitochondrial biogenesis, including uncoupling protein-1, uncoupling protein-2, peroxisome proliferator–activated receptor-γ (PPARγ) coactivator 1–α (PGC-1α), and acetyl-CoA carboxylase were comparable between HFD groups in white and brown adipose tissue, skeletal muscle, and liver (Supplementary...
MUFA-HFD Impedes Adipose IL-1β Secretion and IR

MUFA-HFD mice exhibit a hyperplastic adipose phenotype, with enhanced AMPK activation compared with SFA-HFD mice. MUFA-HFD mice displayed adipose hyperplasia compared with SFA-HFD mice (Fig. 3A and B). The expression of adipogenic markers, levels of PPARγ and PGC-1α, were increased in MUFA adipocytes (Fig. 3D). We demonstrate that preadipocyte-enriched MUFA-SVF exhibited greater PGC-1α and Forkhead box protein class O1 (FOXO1) mRNA expression in response to adipogenic media compared with SFA-SVF (Fig. 3E). Previous studies demonstrated that AMPK activation regulates the NLRP3 inflammasome.

Fig. 4A–D). No significant difference in hepatic citrate synthase activity was observed (Supplementary Fig. 4E).

MUFA-HFD mice maintained significantly lower ITT results and insulin secretion when weight matched to SFA-HFD mice (Supplementary Fig. 3E and F), indicating that improved insulin sensitivity was independent of body weight. Fasting plasma leptin and adiponectin levels increased in SFA-HFD mice compared with MUFA-HFD mice, while plasma triacylglycerol, nonesterified fatty acid, cholesterol, IL-1β, and IL-6 were equivalent (Supplementary Table 2).

Figure 1—HFDs enriched with PA can prime pro-IL-1β production in the SVF of adipose tissue in vivo. A: Adipose tissue from mice fed a chow diet, a MUFA-HFD, or an SFA-HFD for 16 weeks was cultured in complete media (100 μg/mL) for 24 h, and levels of IL-1β secreted into culture media were measured by ELISA (n = 5–6). B: Protein levels of mature ASC, procaspase-1, active IL-1β, and control GAPDH in adipose tissue were determined by immunoblot analysis. Protein bands were quantified by densitometry and normalized to GAPDH levels (n = 7–8). C: Gene expression analysis of SIRT-1, TLR4, NLRP3, IL-1β, and caspase-1 in adipose tissue (n = 6–8, normalized to chow control) was quantified by real-time PCR. D: Adipocytes and SVFs were isolated from epididymal fat pads by collagenase digestion. IL-1β secretion from the SVF (1 × 10⁶ cells/mL) and adipocytes (200 μL packed volume/mL) cultured in complete media for 24 h with or without ATP (5 mmol/L) was determined by ELISA (n = 7–8). E: The SVF was seeded at a density of 1 × 10⁶ cells/mL and cultured for 24 h with or without ATP (5 mmol/L). Caspase-1 activity was determined. F: Protein expression of pro-IL-1β, mature IL-1β, and control GAPDH in the SVF treated with LPS (10 ng/mL) for 3 h was determined by immunoblot analysis. A representative immunoblot is shown. Protein bands were quantified by densitometry and normalized to GAPDH levels (n = 7–8). *P < 0.05, **P < 0.01, ***P < 0.001, chow-fed vs. HFD-fed mice; #P < 0.05, ##P < 0.01, ###P < 0.001 MUFA vs. SFA for all graphs. White bars, chow-fed mice; striped bar, MUFA-HFD–fed mice; black bars, SFA-HFD–fed mice in all graphs.
complex (14) and that MUFA can enhance AMPK activity (15). We thus speculated that differential AMPK activity after HFDs may account for alterations in adipose IL-1β levels. Equivalent levels of pAMPK were observed in adipose tissue from chow-fed and MUFA-HFD–fed mice, but levels were significantly reduced after the SFA-HFD (Fig. 3F).

OA Impedes ATP-Induced Secretion of IL-1β in LPS-Primed BMMs in an AMPK-Dependent Manner

BMMs mimic the IL-1–related immuno-phenotype of adipose tissue macrophages, and we thus used them in mechanistic studies (16). MUFA-HFD–derived BMMs secreted significantly less IL-1β after LPS and ATP stimulation compared with SFA-HFD–derived BMMs ex vivo (Fig. 4A). Caspase-1 activity was significantly higher in BMMs from SFA-HFD–fed mice compared with BMMs from chow-fed and MUFA-HFD–fed mice (Fig. 4B). Pretreatment of LPS-primed BMMs with an NLPR3 inflammasome inhibitor, CRID3, attenuated IL-1β secretion from BMMs from SFA-HFD–fed mice (Fig. 4C). Furthermore, in LPS-primed BMMs, PA, but not OA, increased pro-IL-1β secretion, and induced IL-1β (Fig. 4D–F) and IL-18 secretion (Supplementary Fig. 5D).

Figure 2—A MUFA-enriched HFD partially improves insulin sensitivity and hyperinsulinemia. ITT (0.5 units/kg insulin) (A) and GTT (1.5 g/kg glucose) (B) results are shown in 6-h–fasted chow-fed, MUFA-HFD–fed, and SFA-HFD–fed animals (n = 12–14). C: Insulin secretion response in overnight-fasted mice after intraperitoneal injection with 1.5 g/kg glucose. Tail-vein blood samples were taken at indicated times, and insulin levels were determined by ELISA (n = 7–12). In A–C: Black circles, chow-fed mice; white squares, MUFA-HFD–fed mice; black squares, SFA-HFD–fed mice. D: Gene expression analysis of GLUT-4 and IRS-1 in primary adipocytes from mice fed chow, a MUFA-HFD, and an SFA-HFD, as measured by real-time PCR (n = 5). E: Levels of tyrosine pIRS-1 in adipose tissue protein lysates were measured using a PathScan ELISA kit. The fold increase in response to insulin over basal levels (non-insulin stimulated) in adipose tissue for each individual mouse was calculated and is presented (n = 4–8). F: Adipose tissue explants (50 mg) were stimulated with insulin (100 nmol/L) ex vivo for 15 min, and protein lysates were prepared. Phosphorylated AKT, whole-cell AKT, and control GAPDH levels were determined by immunoblot analysis. A representative immunoblot is shown. Protein bands were quantified by densitometry and normalized to GAPDH levels (n = 6). In D–F: White bar, chow-fed mice; striped bar, MUFA-HFD–fed mice; black bar, SFA-HFD–fed mice. *P < 0.05, **P < 0.01, ***P < 0.001, chow-fed vs. HFD-fed mice ; #P < 0.05, ###P < 0.001, MUFA vs. SFA for all graphs.
We speculated that MUFA-induced AMPK may impede NLRP3 inflammasome activity. Interestingly, OA prevented ATP-induced IL-1β secretion in LPS-primed BMMs (Fig. 4D). Furthermore, PA-treated BMMs exhibited lower pAMPK levels compared with OA-treated cells, consistent with findings in adipose tissue (Fig. 4E and F). Pretreatment of LPS-primed BMMs with an AMPK inhibitor, compound C, prior to OA treatment reduced pAMPK (Supplementary Fig. 5E), and increased IL-1β and IL-18 secretion (Fig. 5A and Supplementary Fig. 5F). Conversely, pretreatment with the AMPK agonist AICAR completely reverses PA-induced IL-1β secretion (Fig. 5B). We similarly demonstrated that OA can prevent ATP-stimulated IL-1β secretion in LPS- and PA-primed macrophages (Fig. 5C). These findings demonstrate that MUFAs not only fail to prime IL-1β, but can also block ATP-induced IL-1β secretion in preprimed cells in an AMPK-dependent manner. To confirm, stable THP-1 cell lines lacking the AMPKα1 subunit were created. Phorbol myristic acid (PMA)–differentiated THP-1 macrophages secrete high levels of IL-1β in response to PA, but not to OA, similar to BMMs (Fig. 5D). Supporting our chemical inhibitor studies, knockdown of AMPKα1 increased IL-1β secretion from OA-treated cells in the presence and absence of ATP (Fig. 5E and F).

Regression Study: Replacing SFA-HFD With MUFA-HFD Cannot Reverse Adipose Dysfunction or IR

Mice were fed an SFA-HFD for 16 weeks to induce an obese and insulin-resistant state, and were subsequently maintained on an SFA-HFD or switched to a MUFA-HFD to establish whether we could regress established IR. Switching from a MUFA-HFD only moderately improved HOMA-IR, but it did not reach statistical significance (Fig. 6A), whereas ITT (Fig. 6B) and GTT curves were unchanged (data not shown). Nonetheless, switching from...
an SFA-HFD to a MUFA-HFD prevented further increases in fasting plasma insulin concentrations (Fig. 6C) and insulin secretion in response to glucose (Fig. 6D). Adipose morphology was equally hypertrophic between groups (Fig. 6E). Adipose M1 macrophage infiltration was also equivalent (data not shown). Pancreatic cell size was reduced after a MUFA-HFD compared with an SFA-HFD (Fig. 6F).

**Habitual Dietary MUFA Is Associated With Enhanced Insulin Sensitivity in Human Cohort**

To translate our findings from animal to humans, we reanalyzed data from two human studies with detailed...
dietary fat data wherein we could discriminate between fat quantity and composition. In T2D subjects (CORDIOPREV cohort), high habitual SFA intake, but not MUFA intake, was associated with significantly lower insulin sensitivity, as measured by ISI (Fig. 7A) and HOMA-IR (Fig. 7B). Further, hs-CRP levels increased with higher habitual SFA intake (Fig. 7C). Age, BMI, waist circumference, and systolic blood pressure were not different between dietary SFA tertiles (Supplementary Table 3).

Second, a subcohort of subjects with metabolic syndrome from the LIPGENE study was categorized according to their habitual dietary fat intake. At baseline, individuals with habitual high SFA intake (Supplementary Fig. 6A) exhibited elevated caspase-1 (P = 0.005) and Pycard-1 (P = 0.009) mRNA expression in adipose tissue (Supplementary Fig. 6B and C). Dietary intervention with MUFA for 16 weeks resulted in improvements in the acute insulin response to glucose and the first-phase insulin...
response (Supplementary Fig. 6D and E) in habitual high MUFA consumers, but not in high SFA consumers. Age, preintervention HOMA-IR, and habitual MUFA intake were the most important determinants of improved acute insulin response to glucose in response to a MUFA-HFD ($r^2 = 0.620, P = 0.015$) (Supplementary Table 4).

**DISCUSSION**

This study has demonstrated that enrichment of obesigenic HFDs with MUFA can improve insulin sensitivity, reduce adipose IL-1β-mediated inflammation, and promote adipose hyperplasia compared with diets enriched with SFA. We further demonstrate that MUFA-HFDs fail to prime IL-1β in whole adipose tissue and the SVP of adipose tissue, with reductions in both active IL-1β protein levels and ATP-induced IL-1β secretion. Lack of IL-1β coincided with the preservation of adipose AMPK activity in the MUFA group, which has previously been implicated in impeding NLRP3 inflammasome activity (5). We further demonstrate that the MUFA OA can prevent ATP-induced IL-1β secretion in both LPS- and PA-primed macrophages in vitro, indicative that MUFA can impede NLRP3 inflammasome activation. Furthermore, pretreatment of cells with an AMPK inhibitor rescued ATP-induced
IL-1β secretion in the presence of OA. These findings indicate that MUFA, via activation of AMPK, can impede ATP-induced processing of pro-IL-1β to active IL-1β. Indeed, preservation of adipose AMPK activity in MUFA-HFD–fed mice may account for reduced IL-1β in adipose tissue and improved insulin sensitivity in vivo.

**Figure 7**—Habitual dietary SFA, but not MUFA, promotes IR. A–C: T2D subjects from the CORDIOPREV study (NCT00924937) were categorized based on tertiles of baseline fasting plasma SFA and MUFA concentrations. A: ISI is represented according to SFA and MUFA tertiles. B: HOMA-IR is represented according to SFA and MUFA tertiles. C: C-reactive protein is represented according to SFA tertiles. Black bars, SFA. In A–C: **P < 0.01, w.r.t. tertile 1; #P < 0.05, w.r.t tertile 2; n = 160–184. D: Schematic representation illustrating the differential effects of an SFA-HFD vs. a MUFA-HFD on pro-IL-1β priming and NLRP3 inflammasome activation: 1, MUFA-HFD lacks the ability to prime pro-IL-1β in SVF; 2, MUFA-HFD maintains adipose protein pAMPK levels at those of chow-fed mice, while SFA-HFD–fed mice display reduced pAMPK levels; 3, caspase-1 activity is significantly increased in BMMs from SFA-HFD–fed mice; 4, greater levels of IL-1β are secreted from SFA-SVF compared with MUFA-SVF; 5, protein pAKT levels are reduced in SFA adipose tissue compared with MUFA adipose tissue; and 6, a MUFA-HFD induced a hyperplasic adipose morphology, while an SFA-HFD induced adipocyte hypertrophy. NF-κB, nuclear factor-κB; ROS, reactive oxygen species.
In regression studies, the replacement of SFA for MUFA did not significantly rescue IR, which is associated with IL-1β activation and hypertrophic adipose morphology, but did lower fasting plasma insulin levels at baseline and after a glucose challenge. Human studies highlight the importance of habitual dietary fat composition, suggesting that high habitual MUFA intake is associated with increased insulin sensitivity and greater flexibility in responding to different dietary interventions.

It is well acknowledged that IL-1β, which is primed by SFA-HFD and cleaved via the NLRP3 inflammasome complex, has a detrimental role in obesity-induced IR (3,5,14). Our work demonstrates that MUFA-HFD–induced obesity did not induce adipose IL-1β production, coincident with lower caspase-1 activity, and reduced NLRP3, caspase-1, IL-1β, and IL-1R1 mRNA expression in the SVF. These findings extend those of Wen et al. (5), who demonstrated that treatment of BMMs with OA did not enhance LPS-induced IL-1β ex vivo. Similarly, we show that OA does not prime IL-1β in vitro. Furthermore, we establish that the treatment of LPS-primed macrophages with OA, prior to an ATP stimulus, markedly attenuates IL-1β secretion from macrophages. These findings reveal that not only do MUFAs fail to prime IL-1β, but they also impede NLRP3–mediated processing of IL-1β in preprimed cells.

Lack of IL-1β signaling is accompanied by improved insulin sensitivity in HFD-fed mice (6). In our study, MUFA-HFD mice have improved insulin sensitivity. Markers of inflammation and insulin sensitivity are comparable in periphery tissues between HFD groups, suggesting the modulation of adipose IL-1β by MUFA is critical to the insulin-sensitive phenotype. Interestingly, MUFA-HFD fails to improve glucose tolerance. We speculated that altered hepatic glucose production may account for this phenotype; however, hepatic G-6-P and PepCK2 expression were equivalent, while basal glucose levels were reduced with MUFA-HFD. MUFA-HFD–fed mice secrete significantly lower levels of insulin in response to a glucose load and have reduced pancreatic cell death compared with SFA-HFD–fed mice. Additionally, by using a regression dietary model we display evidence that switching from an SFA-HFD to a MUFA-HFD halted the progression of HFD-induced pancreatic hypertrophy and hyperinsulinemia. Maedler et al. (17) demonstrated that MUFA, both C16:1 and C18:1, prevented the deleterious effects of palmitate on pancreatic β-cell function in vitro. We postulate that the pancreatic cells may be partially protected by mice being fed a MUFA-HFD in vivo, and as a result insulin secretion levels are not elevated to the same extent as those associated with an SFA-HFD, which may account for the lack of difference in glucose tolerance.

Hypertrophied adipose tissue is associated with adipose dysfunction, lipid spillover, and ectopic hepatic deposition (18). Hyperplastic adipose tissue is associated with insulin sensitivity independent of body fat in humans (19). Artificial induction of adipogenesis in the subcutaneous adipose tissue of obese mice improves glucose tolerance and HOMA-IR (20). In our study, adipose tissue from MUFA-HFD–fed mice displays a hyperplastic phenotype with reduced M1 adipose tissue macrophages and improved insulin sensitivity. Adipocyte PPARγ and PGC-1α levels were markedly increased after mice were fed the MUFA-HFD. Furthermore, PGC-1α and FOXO-1 levels were amplified in MUFA-HFD SVF compared with SFA-HFD SVF after ex vivo stimulation with adipogenic media. Together, these results are indicative of an increased adipogenic potential of preadipocytes in MUFA-HFD–fed mice. It is well established that IL-1β impedes adipogenesis in 3T3-L1 adipocytes (6). We propose that the hyperplastic adipose morphology may be due to attenuated IL-1β within the microenvironment of adipose tissue from MUFA-HFD–fed mice.

AMPK is a pleiotropic metabolic sensor (21), which also modulates inflammation (22). Inhibition of AMPK by SFA attenuates autophagy and increases mitochondrial dysfunction via reactive oxygen species production, which in turn activate the NLRP3 inflammasome (5,23). MUFA has been shown to activate AMPK in aortic tissue (15). Moreover, oleate prevents palmitate-induced ER stress in skeletal muscle in an AMPK-dependent manner, contributing to improved local insulin sensitivity (24). However, it has not been shown whether OA regulates AMPK in adipose tissue and in turn modulates IL-1β release. Our work has demonstrated that pAMPK levels are equivalent in both chow-fed and MUFA-HFD–fed mouse adipose tissue, and activation is significantly reduced after mice were fed an SFA-HFD. This reduction in pAMPK is associated with the upregulation of mature IL-1β levels in SFA-HFD–fed mouse adipose tissue. We thus postulated that MUFA may signal via the AMPK pathway to disrupt NLRP3 inflammasome activation. In support of our hypothesis, we demonstrate that treatment of LPS-primed macrophages with an AMPK inhibitor can rescue ATP-induced IL-1β secretion in the presence of OA. AMPKα1 knockout experiments corroborate this finding, exhibiting increased IL-1β secretion in the presence of OA. These novel findings suggest that the beneficial effects of a MUFA-HFD may be mediated via preservation of AMPK activity and reduced processing of IL-1β in the obese state. A recent study (14) illustrated that metformin attenuates IL-1β maturation in T2D patients through AMPK activation, which is akin to the MUFA-HFD and OA pretreatment.

However, we cannot overlook the effect of AMPK activation in relation to energy homeostasis. Obese MUFA-HFD–fed animals exhibited greater energy expenditure, with increased VO2 and heat production. This may also be attributable to AMPK activation, which is known to increase fatty acid oxidation and promote mitochondrial biogenesis. Recently, Freigang et al. (25) demonstrated that OA-mediated mitochondrial uncoupling inhibited ATP-induced IL-1β production. However, in this study mRNA markers of mitochondrial biogenesis
were comparable between MUFA-HFD–fed and SFA-HFD–fed groups in adipose, liver, and skeletal muscle tissue. Nevertheless, the difference in adipose morphology, IL-1β levels, and IR remain true when weight matched.

Given the beneficial effects of MUFA on inflammation and insulin sensitivity observed in our animal and cellular models, we examined whether habitual dietary MUFA intake was associated with insulin sensitivity and reduced inflammation in humans. In T2D subjects (CORDIOPREV cohort), high habitual SFA intake, but not high MUFA intake, was associated with IR. Furthermore, individuals with metabolic syndrome (LIPGENE cohort) with high habitual MUFA intake at baseline exhibited lower adipose tissue caspase-1 and Pycard-1 mRNA levels, and demonstrated increased insulin sensitivity after a MUFA dietary intervention compared with habitual high SFA consumers. These studies suggest that individuals with high habitual MUFA intake exhibit a certain degree of flexibility in response to dietary interventions, which was not evident in individuals with habitual high SFA intake.

In summary, we provide the first in vivo evidence that obesigenic diets enriched with MUFA can lower adipose IL-1β secretion, stimulate adipose hyperplasia, and reduce IR that is ordinarily associated with SFA-induced obesity (Fig. 7D). We further demonstrate in vitro that MUFA can block ATP-induced processing of IL-1β in preprimed cells, in an AMPK-dependent manner. These findings indicate that MUFAs may limit SFA-induced IL-1β levels and associated adverse metabolic sequelae in the obese state.

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