Abstract  We examined the effects of three high-fat diets (HFD), differing in the percentage of total calories from saturated fat (SF) (6%, 12%, and 24%) but identical in total fat (40%), on body composition, macrophage behavior, inflammation, and metabolic dysfunction in mice. Diets were administered for 16 weeks. Body composition and metabolism [glucose, insulin, triglycerides, LDL-cholesterol (LDL-C), HDL-cholesterol (HDL-C), total cholesterol (TC)] were examined monthly. Adipose tissue (AT) expression of marker genes for M1 and M2 macrophages and inflammatory mediators [Toll-like receptor (TLR)-2, TLR-4, MCP-1, tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-10, suppressor of cytokine signaling (SOCS)1, IFN-γ] was measured along with activation of nuclear factor kappa-B (NFκB), c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (MAPK). AT macrophage infiltration was examined using immunohistochemistry. Circulating MCP-1, IL-6, adiponectin, and leptin were also measured. SF content, independent of total fat, can profoundly affect adiposity, macrophage behavior, inflammation, and metabolic dysfunction. In general, the 12%-SF diet, most closely mimicking the standard American diet, led to the greatest adiposity, macrophage infiltration, and insulin resistance (IR), whereas the 6%-SF and 24%-SF diets produced lower levels of these variables, with the 24%-SF diet resulting in the least degree of IR and the highest TC/HDL-C ratio. Macrophage behavior, inflammation, and IR following HFD are heavily influenced by dietary SF content; however, these responses are not necessarily proportional to the SF percentage. According to the World Health Organization, it is estimated that 1.5 billion people worldwide are overweight and at least 500 million are obese (1). Consequently, there has been a rise in obesity-related health problems, including cardiovascular disease, the metabolic syndrome, diabetes, degenerative diseases, and cancer (1, 2). Genetic predispositions, physical inactivity, and consumption of a high-fat diet (HFD) can all lead to the development of obesity. However, given the global acceptance and availability of energy-dense foods, chronic ingestion of diets high in fat is arguably the leading contributor. As a result, there has been a major emphasis on understanding the link between high-fat-diet-induced obesity and chronic disease risk. In this context, low-grade chronic inflammation has emerged as a key pathogenic link (3–5).

It is now widely accepted that high-fat-diet-induced obesity can lead to a chronic state of low-grade inflammation. This is largely mediated through quantitative and functional alterations in white adipose tissue macrophages (ATM) (6, 7). For example, it has been reported that approximately 45–60% of adipose tissue (AT) cells express the macrophage marker EMR1 (F4/80) in obese mice, whereas only 10–15% of cells from lean mice express this marker (6). In addition, ATMs exhibit a pro-inflammatory, classical phenotype (M1) in obese mice, while those from

Supplementary key words  high-fat diet • obesity • adipose tissue • insulin resistance • macrophages

Abbreviations:  4-HNE, 4-hydroxy-2-nonenal; AT, adipose tissue macrophage; DEXA, dual-energy X-ray absorptiometry; H&E, hematoxylin and eosin; HDL-C, HDL-cholesterol; HFD, high-fat diet; HOMA, homeostatic model assessment; IL, interleukin; IR, insulin resistance; JNK, c-Jun N-terminal kinase; LCSFA, long-chain saturated fatty acid; LDL-C, LDL-cholesterol; M1, classically activated macrophage; M2, alternatively activated macrophage; MAPK, mitogen-activated protein kinase; MCF, medium-chain fatty acid; MCP, monocyte chemotactic protein; NFκB, nuclear factor kappa-B; SF, saturated fat; SFA, saturated fatty acid; SOCS, suppressor of cytokine signaling; TC, total cholesterol; TLR, Toll-like receptor; TNF, tumor necrosis factor; USFA, unsaturated fatty acid; WAT, white adipose tissue.

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According to the World Health Organization, it is estimated that 1.5 billion people worldwide are overweight and at least 500 million are obese (1). Consequently, there has been a rise in obesity-related health problems, including cardiovascular disease, the metabolic syndrome, diabetes, degenerative diseases, and cancer (1, 2). Genetic predispositions, physical inactivity, and consumption of a high-fat diet (HFD) can all lead to the development of obesity. However, given the global acceptance and availability of energy-dense foods, chronic ingestion of diets high in fat is arguably the leading contributor. As a result, there has been a major emphasis on understanding the link between high-fat-diet-induced obesity and chronic disease risk. In this context, low-grade chronic inflammation has emerged as a key pathogenic link (3–5).

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lean mice have an alternatively activated, anti-inflammatory phenotype (M2) (7). These changes lead not only to increased inflammation but also to dysregulation of metabolic homeostasis; infiltration and polarisation of macrophages in AT has been linked to lower plasma adiponectin levels as well as insulin and leptin resistance (8–11). While the association between high-fat-diet-induced obesity and macrophage-mediated inflammation has been clearly recognized, there is a fundamental gap in understanding the relative contribution of different types of fatty acids (FA) to these responses.

Saturated fatty acids (SFA) have received the most attention for their ability to influence pro-inflammatory processes in high-fat-diet-induced obesity. These effects are thought to be largely mediated by their capacity to serve as ligands for Toll-like receptor (TLR)-2 and TLR-4; binding of SFAs to TLR-2 and/or TLR-4 on various cell types, in particular macrophages and adipocytes, results in the induction of pro-inflammatory gene transcription via activation of nuclear factor kappa-B (NFkB), the c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (p38 MAPK) signaling cascades (10, 12, 13). Consistent activation of these pathways results in a chronic state of inflammation and subsequent insulin resistance (IR) (10, 14, 15). In addition, SFAs, in general, are more obesogenic than other FAs. Long-chain saturated fatty acids (LC-SFA, > C12:0) are not as efficiently oxidized as unsaturated fatty acids (USFA) and thus are more likely to be stored as AT (16, 17). Given the preponderance of evidence that supports a role of saturated fat (SF) on macrophage-mediated inflammation and metabolic dysfunction in high-fat-diet-induced obesity, it is surprising that there have been no dose response studies to more clearly evaluate their specific role in these processes.

The purpose of this study was to examine the effects of three HFDs, differing in the percentage of total calories from SF (6%, 12%, and 24% of total caloric intake) but identical in total fat (40%), on body composition, macrophage behavior, inflammation, and metabolic dysfunction in mice. We hypothesized that high dietary fat intake would increase adiposity, macrophage infiltration, inflammation, IR, and impair the lipid profile, and that these effects would be augmented as the percentage of SF increased.

METHODS

Animals

Male C57BL/6 mice were bred and cared for in the animal facility at the University of South Carolina. They were housed 4–5 animals per cage, were maintained on a 12:12 h light-dark cycle in a low-stress environment (22°C, 50% humidity, low noise), and were given food and water ad libitum. Principles of laboratory animal care were followed, and the Institutional Animal Care and Usage Committee of the University of South Carolina approved all experiments.

Diets

At four weeks of age, mice were randomly assigned to one of five treatment diets (n = 8–9/group): two control diets (AIN-76A, AIN-76A Mod) and three HFDs (6%-SF, 12%-SF, and 24%-SF) (BioServ, Frenchtown, NJ) (Table 1). The percentage of calories provided by each of the three macronutrients, the ratio of PUFA:MUFA, and the ratio of omega-6:omega-3 FAs were identical for the HFDs and were designed to be similar to the standard American diet (18, 19). The second control diet (AIN-76A Mod) was used to match the PUFA:MUFA and omega-6:omega-3 ratios of the HFDs.

Body weights, food intake, and body composition

Body weight and food intake were monitored weekly. Body composition was assessed every four weeks (weeks 4, 8, 12, 16, and 20). For this procedure, mice were placed under brief anesthesia (isoflurane inhalation) and were assessed for lean mass, fat mass, and body fat percentage via dual-energy X-ray absorptiometry (DEXA) (Lunar PIXImus, Madison, WI).

Metabolism

Plasma was assessed for fasting concentrations of glucose, insulin, total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides at weeks 8, 12, 16, and 20, and for LDL-cholesterol (LDL-C) at weeks 16 and 20. Blood samples were collected from the tip of the tail after a 5 h fast. Blood glucose concentrations were determined in whole blood using a glucometer (Bayer Contour, Michawaka, IN). Collected blood was centrifuged and plasma was aliquoted and stored at −80°C until analysis. Insulin concentrations were determined using an ELISA kit (Mercodia, Uppsala, Sweden), and colorimetric kits were used for plasma triglycerides (Pointe Scientific, Canton, MI), TC, HDL-C, and LDL-C (Genzyme, Kent, UK). Insulin resistance was estimated by the homeostatic model assessment (HOMA) index as follows: insulin resistance index = fasting insulin (µU/ml) × fasting glucose (mmol/l)/22.5 (20).

Tissue collection

At 20 weeks of age, mice were sacrificed for tissue collection. Epididymal, mesentery, and retroperitoneal fat pads were removed, weighed, and immediately snap-frozen in liquid nitrogen and stored at −80°C or fixed in 10% formalin until analysis. Blood was collected from the inferior vena cava using heparinized syringes, and then centrifuged at 4,000 rpm for 10 min at 4°C. Plasma was aliquoted and stored at −80°C.

Adipocyte size and F4/80 immunohistochemistry

At sacrifice, a portion of epididymal AT was excised from each mouse, fixed overnight in 10% formalin, dehydrated with harnicized xylenes, and then centrifuged at 4,000 rpm for 10 min at 4°C. Paraffin sections were stained with hematoxylin and eosin (H&E). The surface area of 100 adipocytes was determined (manual trace) and then averaged to represent mean adipocyte size for each mouse using Infinity Analyze software (Lumenera, Ottawa, ON). F4/80 staining was performed in epididymal AT using rat monoclonal antibody (Serotec, Raleigh, NC). Color detection was visualized with a Vectastain avidin-biotinylated enzyme complex detection kit (R and D Systems, Minneapolis, MN), and 3,3′-diaminobenzidine followed by counterstaining with hematoxylin.

Western blots

Epididymal AT was homogenized in radioimmunoprecipitation buffer (Sigma, St. Louis, MO), which included a protease inhibitor cocktail (Sigma, St. Louis, MO), and 1% glyceral phosphate (100X), 0.5% sodium orthovanadate (1 mM), and 1% sodium fluoride (5 mM). The protein concentration was determined by the Bradford method (21). Western blots were performed as previously described using primary antibodies for phosphorylated (Ser536) and total NFkB p65, phosphorylated (Thr183/Tyr185) and total JNK (Cell Signaling, Danvers, MA), and phosphorylated

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Statistical analysis

All data were analyzed using commercial software (SigmaStat, SPSS, Chicago, IL). Body weight, body composition outcomes, metabolic outcomes, the TC:HDL-C ratio, and the HOMA index were analyzed using a repeated measures two-way ANOVA. All other data were analyzed using a one-way ANOVA. Student-Newman-Keuls test was used for all posthoc analyses. Statistical significance was set with an \( P \leq 0.05 \). Data are presented as mean ± SEM.

RESULTS

12%-SF consumption leads to heavier body weights, larger adipocyte size, and greater fat mass than any other diet

Body weights, fat pad weights, and average adipocyte size were determined using commercially available ELISA kits (R and D Systems, Minneapolis, MN).
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Body composition analysis revealed a significant difference among groups ($P \leq 0.05$) (Table 2). Specifically, the 12%-SF-fed mice had a greater body fat percentage compared with control-diet-fed mice (starting at 12 weeks) and 24%-SF-fed mice (16 weeks) ($P \leq 0.05$). However, the 24%-SF and 6%-SF-fed mice did not differ from control-diet-fed mice until week 16. There were no significant differences in body fat percentages across the HFD-fed mice at 20 weeks ($P \leq 0.05$). On the other hand, at week 20, the 12%-SF-fed mice had a greater fat mass than the 24%-SF and 6%-SF-fed mice ($P \leq 0.05$). In general, lean mass increased over time for all groups, and by week 16, the HFD-fed mice exhibited greater lean mass compared with control-diet-fed mice ($P \leq 0.05$).

Body fat percentages across the HFD-fed mice at 20 weeks ($P \leq 0.05$). On the other hand, at week 20, the 12%-SF-fed mice had a greater fat mass than the 24%-SF and 6%-SF-fed mice ($P \leq 0.05$). In general, lean mass increased over time for all groups, and by week 16, the HFD-fed mice exhibited greater lean mass compared with control-diet-fed mice ($P \leq 0.05$).

ATM infiltration is greatest in 12%-SF-fed mice

Expression of F4/80, CD11c (M1), and CD206 (M2) was greater with the consumption of the 12%-SF diet compared with all other diets ($P \leq 0.05$), with the exception of no difference in CD206 between the 12%-SF and 6%-SF-fed mice (Fig. 2A–C). Interestingly, we did not detect a difference in TLR-4 expression across the groups (data not shown), however, TLR-2 expression (Fig. 2D) was increased with

Fig. 1. Effect of consuming HFDs differing in saturated fat composition on (A) weekly mean body weight, (B) retroperitoneal (Rp), mesentery (Mes), epididymal (Epi), and total visceral (Total) fat pad weights, and (C) adipocyte size at sacrifice (n = 8–9). Diets not sharing a common letter differ significantly from one another ($P \leq 0.05$). *Significantly different from AIN-76A (week 11, 12%-SF only; weeks 12–20, 6%-SF, 12%-SF, and 24%-SF diets; $P \leq 0.05$) and significantly different from AIN-76A-Mod (weeks 11 and 12, 12%-SF only; weeks 13–20, 6%-SF, 12%-SF, and 24%-SF diets; $P \leq 0.05$). †Significantly different from 6%-SF (weeks 15 and 17–20, 12%-SF only; $P \leq 0.05$). ‡Significantly different from 24%-SF (weeks 17–20, 12%-SF only; $P \leq 0.05$).
the consumption of the 12%-SF diet compared with all other diets, except the 6%-SF diet (P ≤ 0.05). We next confirmed inflammation and macrophage infiltration in AT via H&E and immunohistochemistry staining of F4/80, respectively. The 12%-SF-fed mice showed increased inflammation (Fig. 2E) and accumulation of macrophages in the AT (Fig. 2F) compared with control-diet-fed mice. Although the 6%-SF and 24%-SF-fed mice also exhibited increased inflammation and macrophage infiltration, these effects were not as pronounced as in 12%-SF.

Adipose tissue inflammation is influenced by the content of dietary fat

There was no difference in the activation of p38 MAPK among any of the diets (Fig. 3A). Alternatively, the activation of JNK, was significantly increased in all HFDs (P ≤ 0.05) (Fig. 3B). However, only 12%-SF exhibited a higher degree of NFκB p65 activation (P ≤ 0.05) (Fig. 3C).

Consumption of all HFDs led to increased expression of MCP-1 in epididymal AT compared with control diets (P ≤ 0.05) (Fig. 4A). TNF-α was increased only in 6%-SF and 12%-SF-fed mice (P ≤ 0.05) (Fig. 4B), but there were no differences in IL-6 (Fig. 4C) or IFN-γ (Fig. 4F) expression among any of the groups. Interestingly, SOCS1 expression was significantly decreased only in the 24%-SF-fed mice (P ≤ 0.05) (Fig. 4E). Regarding the anti-inflammatory cytokine IL-10, we found increased expression in the 12%-SF-fed mice only (P ≤ 0.05) (Fig. 4D).

Circulating leptin is greatest with the 12%-SF diet

For leptin, not only did all HFDs exhibit significantly elevated plasma concentrations compared with control diets but there were also differences among HFDs; the 12%-SF-fed mice had increased leptin levels compared with 6%-SF and 24%-SF-fed mice (P < 0.05) (Fig. 5D). However, there were no significant differences across the groups for circulating levels of IL-6, MCP-1, or adiponectin (Fig. 5A–C).

12%-SF diet leads to the greatest IR, followed by 6%-SF and 24%-SF diets, respectively

Beginning at week 16, all three HFDs produced higher fasting blood glucose concentrations compared with control diets (P ≤ 0.05), but by 20 weeks, only the 6%-SF and 12%-SF-fed mice had elevated fasting blood glucose concentrations (P ≤ 0.05) (Table 3). And in fact, the 12%-SF-fed mice exhibited a higher fasting blood glucose compared with the 24%-SF-fed mice at this time (P ≤ 0.05).

All HFD-fed mice had elevated insulin levels compared with control-diet-fed mice at 16 and 20 weeks (P ≤ 0.05). However, at 16 weeks, the 12%-SF-fed mice had increased levels compared with the 6%-SF and 24%-SF-fed mice (P ≤ 0.05), and at 20 weeks, both the 6%-SF and 12%-SF-fed mice had elevated levels versus the 24%-SF-fed mice (P ≤ 0.05).

Similar to insulin, HFD-fed mice had a greater HOMA index than control-diet-fed mice at 16 weeks (P ≤ 0.05). However, at week 20, not only were the HFD groups different from the control-diet groups, but they were also different from each other; the 12%-SF groups had the greatest HOMA score, followed by 6%-SF and 24%-SF groups (P ≤ 0.05).

Changes in lipid profile are influenced by SF content

The 12%-SF-fed mice had elevated TC compared with control-diet-fed mice at week 8 (P ≤ 0.05). By weeks 12 and 16, the 6%-SF and 12%-SF-fed mice had increased levels compared with the AIN-76A-fed mice (week 12) and AIN-76A-Mod-fed mice (week 16), respectively (P ≤ 0.05). All HFD-fed mice had a greater plasma concentration of TC versus the control-diet-fed mice at 20 weeks (P ≤ 0.05), but within the HFD groups, the 12%-SF group had significantly greater TC versus the 24%-SF group (P ≤ 0.05).

In general, the plasma HDL-C concentration tended to be highest with the consumption of the 24%-SF diet. Statistically, mice consuming the 24%-SF diet exhibited significantly
Saturated fat, adiposity, inflammation, and metabolism ultimately leading to poorer health outcomes. It is well known that ATMs play a central role in this relationship (24). However, the extent to which the FA composition of a HFD influences macrophage behavior and inflammation is still poorly understood; most of the available supporting literature is limited by the lack of control for various nutrients (e.g., ratio of MUFA:PUFA; omega-6:omega-3 FAs; protein:carbohydrate:fat, etc.), the utilization of a single ingredient as the sole source of dietary fat (e.g., corn oil, beef tallow, milk fat, etc.), and the absence of dose response studies. We examined the effect of three HFDs, differing in the percentage of total calories from SF (6%, 12%, and 24%) but identical in total fat (40%), on adiposity (absolute fat mass), macrophage phenotype, inflammation, and metabolism utilizing controlled diets consisting of various lipid-rich ingredients. Our findings indicate that manipulating the SF content without elevated HDL-C levels compared with the control-fed mice (week 8), 6%-SF-fed mice (week 16), and 12%-SF-mice (weeks 8, 16, and 20) \((P < 0.05)\). Interestingly, however, there were no significant changes in HDL-C concentrations for any of the groups over time. Both the 6%-SF and 12%-SF-fed mice had a greater TC/HDL-C ratio than all other mice at week 20 \((P < 0.05)\). LDL-C, measured at weeks 16 and 20 only, was elevated in all HFD-fed mice compared with control-diet-fed mice \((P < 0.05)\). Differences in triglycerides across the groups were detected at 20 weeks only; the 24%-SF-fed mice and AIN-76A-Mod-fed mice had the highest and lowest levels of triglycerides, respectively \((P < 0.05)\).

**DISCUSSION**

HFDs are strongly linked with the accumulation of excess body fat, chronic inflammation, and metabolic perturbations, ultimately leading to poorer health outcomes. It is well known that ATMs play a central role in this relationship (24). However, the extent to which the FA composition of a HFD influences macrophage behavior and inflammation is still poorly understood; most of the available supporting literature is limited by the lack of control for various nutrients (e.g., ratio of MUFA:PUFA; omega-6:omega-3 FAs; protein:carbohydrate:fat, etc.), the utilization of a single ingredient as the sole source of dietary fat (e.g., corn oil, beef tallow, milk fat, etc.), and the absence of dose response studies. We examined the effect of three HFDs, differing in the percentage of total calories from SF (6%, 12%, and 24%) but identical in total fat (40%), on adiposity (absolute fat mass), macrophage phenotype, inflammation, and metabolism utilizing controlled diets consisting of various lipid-rich ingredients. Our findings indicate that manipulating the SF content without...
Given that SFAs, in general, are less efficiently oxidized than USFAs, we hypothesized that adiposity would be greatest following consumption of the 24%-SF diet (16, 17). Interestingly, despite the fact that HFD-fed mice consumed similar kilocalories, the 12%-SF diet led to the greatest accumulation of fat and the largest adipocyte size, followed by the 6% and 24%-SF groups, respectively. We also confirmed that plasma leptin was proportional to the degree of fat-mass accumulation as previously reported (9).

The FA composition of AT is primarily dependent on the FA composition of the diet (25). Therefore, lipolysis of white adipose tissue (WAT) composed of a greater proportion of SFAs should lead to higher levels of circulating saturated free-fatty acids and subsequent activation of macrophages through binding to TLRs (10, 13). Given this, we expected that a diet higher in SF content would lead to greater macrophage infiltration and more pronounced inflammation. In agreement with previously reported literature, all HFDs increased expression of F4/80 as well as markers for M1 and M2 macrophages; interestingly, however, these reached statistical significance only in the 12%-SF group. The activation of macrophages in AT is thought to be mediated by TLRs; both TLR-2 and TLR-4 have been implicated in HFD-induced macrophage activation and inflammation given their ability to bind saturated free-fatty acids (13, 26, 27). Consistent with the macrophage data, we show a statistically significant increase in TLR-2 expression only in the 12%-SF-fed mice, but surprisingly there was no significant upregulation of TLR-4 in any of the HFD groups. To our knowledge, there have been no reports of an upregulation of WAT TLR-2 expression without significant changes in WAT TLR-4 expression following HFD feedings. These results warrant further investigation into the role that individual TLRs play in HFD-induced inflammation, and conversely, the effect that varying the composition of HFDs has on TLR activation.

Because macrophages are thought to mediate their inflammatory processes through activation of various transcription factors (10, 12, 13), we next examined phosphorylated NFκB, JNK, and p38 MAPK, and we found that all three HFDs increased activation of JNK, whereas only the 12%-SF diet significantly increased NFκB activation. All HFD-fed mice exhibited an increase in mRNA expression of MCP-1 in AT, and the 6%-SF and 12%-SF-fed mice, but not the 24%-SF-fed mice, exhibited an increase in TNF-α mRNA expression. Surprisingly, IFN-γ, which has been shown to be upregulated in WAT and plays a key role in macrophage activation in HFD-induced obesity (28), was not statistically different across diets. This may be due to the specific measurement time point, the composition of these novel diets, or most likely, an increase in factors that can regulate expression of IFN-γ. Additionally, we found no changes in AT IL-6 mRNA expression across groups. Although previous studies have shown AT IL-6 mRNA expression to be upregulated as a result of high-fat feeding (29, 30), others have shown that this is not always the case (31). We also measured IL-10, an anti-inflammatory cytokine, and found it to be upregulated in the 12%-SF mice only, which is

changing the percentage of total calories from fat has a profound effect on these outcomes. The 12%-SF diet, most closely mimicking the standard American diet, led to the greatest adiposity (absolute fat mass), macrophage infiltration, and IR. Although the 24%-SF diet increased adiposity and produced IR, it did not significantly increase macrophage infiltration, it led to a lesser degree of AT inflammation, and it did not raise the TC/HDL-C ratio.
most likely a compensatory response to the increased inflammation (32). Interestingly, SOCS1, a negative regulator of inflammation (33), appeared to be downregulated in all the HFDs, but this reached significance in the 24%-SF group only. Circulating markers of inflammation (MCP-1, IL-6) were measured to determine whether they mirrored the observed changes in WAT as has previously been reported (30, 34). We found no significant increases in plasma MCP-1 or IL-6 for any of the HFDs. The most likely explanation for the discrepancies between our findings and those of others is the differences in the composition of the diets used.

Emerging evidence suggests that pro-inflammatory M1 macrophages may play a role in inducing IR, whereas alternatively activated M2 macrophages may help to maintain insulin sensitivity (29). Our data somewhat supports this hypothesis as the degree of M1 macrophage mRNA expression corresponded well with the level of IR: the

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Fig. 4. Epididymal adipose tissue gene expression of (A) MCP-1, (B) TNF-α, (C) IL-6, (D) IL-10, (E) SOCS1, and (F) IFN-γ after 16 weeks of diet treatment (n = 8–9). Diets not sharing a common letter differ significantly from one another (P ≤ 0.05).

Fig. 5. Plasma concentrations of (A) IL-6, (B) MCP-1, (C) adiponectin, and (D) leptin collected at sacrifice (n = 8–9). Diets not sharing a common letter differ significantly from one another (P ≤ 0.05).
plasma triglycerides, it also resulted in a higher HDL-C level, producing a more favorable TC/HDL-C ratio similar to that of control diets.

A possible explanation for the discrepancies in adiposity (absolute fat mass), IR, inflammation, and the TC/HDL-C ratio between the 12%-SF-fed and the 24%-SF-fed mice may be the difference in the content of medium-chain fatty acids (MCFA, C8:0–C12:0) (3.6%, and 11.8% of total caloric intake for the 12%-SF and 24%-SF diets, respectively). These variations exist because it was not possible for the composition of the SF in each of the HFDs to be consistent while utilizing various lipid-rich ingredients and simultaneously controlling for the omega-6:omega-3 and MUFA:PUFA ratios. A previous study reported that a diet composed of 12% caprylic (C8:0) and capric acids (C10:0), 12%-SF diet resulted in the most severe IR, followed by the 6%-SF and 24%-SF diets, respectively. However, given that M1 macrophage quantification in this study was limited to mRNA expression of CD11c, this association should be interpreted with caution. Adiponectin, well characterized as an anti-inflammatory adipocytokine known to promote insulin sensitivity, has been shown to be inversely correlated with body fat accumulation (9). Interestingly, there were no changes in the concentration of plasma adiponectin across groups. It is likely that changes in adiponectin would have been observed if it had been measured in the WAT (35); however, our analysis was limited to plasma levels. Concerning lipid metabolism, all three HFDs increased TC and LDL-C levels. Of interest was the finding that although the 24%-SF diet increased LDL-C and elevated plasma triglycerides, it also resulted in a higher HDL-C level, producing a more favorable TC/HDL-C ratio similar to that of control diets.

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A possible explanation for the discrepancies in adiposity (absolute fat mass), IR, inflammation, and the TC/HDL-C ratio between the 12%-SF-fed and the 24%-SF-fed mice may be the difference in the content of medium-chain fatty acids (MCFA, C8:0–C12:0) (3.6%, and 11.8% of total caloric intake for the 12%-SF and 24%-SF diets, respectively). These variations exist because it was not possible for the composition of the SF in each of the HFDs to be consistent while utilizing various lipid-rich ingredients and simultaneously controlling for the omega-6:omega-3 and MUFA:PUFA ratios. A previous study reported that a diet composed of 12% caprylic (C8:0) and capric acids (C10:0),
both MCFAs, augments the rate of fat-mass loss compared with a diet composed of 12% olive oil that contains mostly MUFA (36). Furthermore, there is substantial evidence demonstrating that small doses of MCFAs and HFDs rich in MCFAs can effectively reduce body weight, reduce fat-mass gains, and minimize IR compared with HFDs rich in LCFAs (37–41). Additionally, MCFA-rich diets have been associated with an improved cholesterol profile (42–45) and reduced inflammation (46, 47) compared with other isocaloric diets. The reported reduction in body weight produced by MCFAs is associated with higher energy expenditure and upregulated FA oxidation (48–50). As a result of their shorter chain length, MCFAs can be transported from the intestines directly to the liver where they can be quickly oxidized (37, 51). LCFAs, on the other hand, are first incorporated into chylomicrons before they leave the intestine via the lymphatic system and travel through the blood to extrahepatic tissues to be stored or metabolized. Not only do MCFAs and LCFAs differ in their digestive routes but their propensity to be oxidized is also dissimilar (16). Once inside a cell, MCFAs are less likely to be stored as AT as they can enter the mitochondria to be oxidized independent of carnitine palmitoyltransferase 1, unlike LCFAs.

It is important to point out that the MCFA argument may not be valid when comparing the 6%-SF and 12%-SF diets (0.1% versus 3.6% of total calories from MCFAs, respectively). It is likely that the MCFA-caloric content of the diet would need to be higher to produce similar effects generated by the 24%-SF diet. The differences in adiposity we observed between the 6%-SF and 12%-SF-fed mice may be due to the fact that the 12%-SF diet was composed of more obesogenic LCSFAs (8.4% versus 5.9% of total calories). However, we find this unlikely as the 24%-SF diet had the largest percentage of LCSFAs (12.2% of total calories) and resulted in a similar level of adiposity as in the mice consuming the 6%-SF diet.

Another possible rationale for the differences in IR and inflammation across the diets may be the disparities in the linoleic content (C18:2), (11.4%, 9.4%, and 5.4% of total caloric intake for the 6%-SF, 12%-SF, and 24%-SF diets, respectively). Even when controlling for various ratios within an isocaloric diet, the manipulation of one macronutrient, or subset of macronutrients, results in an uncontrollable alteration of another. As such, in the current study, alterations in the percentage of SF across diets also resulted in changes in the percentage of unsaturated fat. Thus, although the ratio of omega-6:omega-3 FAs was the same for each of the HFDs, the absolute quantity of linoleic acid in the 6%-SF and 12%-SF diets was greater than in the 24%-SF diet. Linoleic acid serves as a short-chain, parent omega-6 FA necessary for the synthesis of essential omega-6 LCFAs (52) that can play an important role in the promotion of inflammatory processes through the production of various eicosanoids (52–54). In fact, others have shown that a HFD rich in omega-6 FAs can increase inflammation more than a HFD rich in SFAs (55). Further, omega-6 FAs are prone to peroxidation, leading to accumulation of 4-hydroxy-2-nonenal (4-HNE), which has been shown to induce IR (56).

It is also likely that the differences in MCFAs, LCFAs, and linoleic content across the diets can explain some of the other unexpected reported findings. For instance, the discrepancies in MCFA and linoleic content between the 6%-SF and 24%-SF diets may explain the similar adiposity but different levels of IR seen in these groups; previous work has shown that MCFA-rich diets can hinder the development of IR without influencing body weight (57). And it may be that the greater content of linoleic acid in the 6%-SF diet produced significantly more 4-HNE, leading to a greater IR compared with the 24%-SF diet. Also, it is certainly possible that the relatively high content of both linoleic acid and LCSFAs in 12%-SF diet may explain the high degree of adiposity, macrophage infiltration, and IR compared with the 6%-SF diet. Clearly, the disparate findings across the three HFDs do not result from variations of a single group of FAs but instead stem from alterations in the content of several classes of FAs.

It is well established that SFAs play a role in inflammatory signaling (10, 12, 58, 59). However, the degree to which individual FAs activate pro-inflammatory pathways remains somewhat controversial, as there has been at least one study to report that SFAs do not activate TLRs in vitro (60). Further, it is evident from our findings as well as from previous research that varying the composition of dietary SFAs can differentially regulate inflammatory processes in vivo compared with in vitro. For example, Lee and others have shown that lauric acid (C12:0), a MCFA that varies considerably with increasing percentage of SF in our diets, can serve as a potent agonist of TLR signaling in vitro (10, 12). On the contrary, Rivera et. al. (46) reported that a MCFA-rich diet more effectively attenuated nonalcoholic steatohepatitis and reduced hepatic TLR-4 expression versus a PUFA-rich diet. These convergent findings may be explained by the inclination of lauric acid to be oxidized in vivo, thus limiting its ability to serve as a ligand for TLR (16). These findings highlight the importance of additional research to better understand the role of various SFAs on inflammatory-related signaling, and further, to determine whether the effects observed in vitro are actually reflected in vivo.

Additionally, our data and that of others suggest that isocaloric diets with a greater content of omega-6 PUFAs can produce greater IR, result in a poorer TC/HDL-C ratio, and may even increase inflammation (46, 55), more so than an isocaloric diet composed of significantly more SF. Meanwhile, others have shown that high consumption of omega-6 FAs has been associated with reduced inflammation, a more favorable TC/HDL-C ratio (55), and no negative effects on inflammatory markers (61, 62). It should be noted, however, that the majority of these studies were performed in humans, in which it is extremely difficult to control for the nutrient composition of the diet and the activity level of the subjects—two factors known to greatly influence metabolism and inflammation (63, 64). This affirms the need for future research utilizing various controlled diets to better understand the role that omega-6 FAs have on physiological processes.
In summary, we examined the influence of three 40% HFDs, which differed in the percentage of total calories from SF (6%, 12%, and 24%), on body composition, macrophage behavior, inflammation, and metabolic dysfunction in mice. In general, the 12%-SF diet, most closely mimicking the standard American diet, led to the greatest adiposity, macrophage infiltration, and IR, whereas the 24%-SF diet had the lowest levels of these outcomes. In conclusion, our findings support previously published data that ad libitum, high-fat feeding can lead to an increased risk of obesity and obesity-related side effects. However, the extent of excess fat accumulation and adverse health perturbations is not necessarily proportional to the percentage of SF in the diet. Although SFAs and omega-6 FAs have been implicated as pro-inflammatory molecules, future research should examine the degree to which diets differing in SFA and omega-6 FA content impact individual TLR activation, macrophage behavior, inflammatory signaling, and subsequent metabolic dysfunction, as well as the effect that manipulating the absolute quantities of omega-6 and omega-3 FAs, without changing the ratio between these FAs, has on these outcomes.

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