High-density lipoprotein proteomic composition, and not efflux capacity, reflects differential modulation of reverse cholesterol transport by saturated and monounsaturated fat diets

High-Density Lipoprotein Proteomic Composition, and not Efflux Capacity, Reflects Differential Modulation of Reverse Cholesterol Transport by Saturated and Monounsaturated Fat Diets

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Background—Acute inflammation impairs reverse cholesterol transport (RCT) and reduces high-density lipoprotein (HDL) function in vivo. This study hypothesized that obesity-induced inflammation impedes RCT and alters HDL composition, and investigated if dietary replacement of saturated (SFA) for monounsaturated (MUFA) fatty acids modulates RCT.

Methods and Results—Macrophage-to-feces RCT, HDL efflux capacity, and HDL proteomic profiling was determined in C57BL/6j mice following 24 weeks on SFA- or MUFA-enriched high-fat diets (HFDs) or low-fat diet. The impact of dietary SFA consumption and insulin resistance on HDL efflux function was also assessed in humans. Both HFDs increased plasma \(^3\)H-cholesterol counts during RCT in vivo and ATP-binding cassette, subfamily A, member 1–independent efflux to plasma ex vivo, effects that were attributable to elevated HDL cholesterol. By contrast, ATP-binding cassette, subfamily A, member 1–dependent efflux was reduced after both HFDs, an effect that was also observed with insulin resistance and high SFA consumption in humans. SFA-HFD impaired liver-to-feces RCT, increased hepatic inflammation, and reduced ABC subfamily G member 5/8 and ABC subfamily B member 11 transporter expression in comparison with low-fat diet, whereas liver-to-feces RCT was preserved after MUFA-HFD. HDL particles were enriched with acute-phase proteins (serum amyloid A, haptoglobin, and hemopexin) and depleted of paraoxonase-1 after SFA-HFD in comparison with MUFA-HFD.

Conclusions—Ex vivo efflux assays validated increased macrophage-to-plasma RCT in vivo after both HFDs but failed to capture differential modulation of hepatic cholesterol trafficking. By contrast, proteomics revealed the association of hepatic-derived inflammatory proteins on HDL after SFA-HFD in comparison with MUFA-HFD, which reflected differential hepatic cholesterol trafficking between groups. Acute-phase protein levels on HDL may serve as novel biomarkers of impaired liver-to-feces RCT in vivo. (Circulation. 2016;133:1838-1850. DOI: 10.1161/CIRCULATIONAHA.115.020278.)

Key Words: cholesterol, HDL ■ inflammation ■ lipoproteins ■ obesity ■ proteome ■ reverse cholesterol transport

High-density lipoprotein (HDL) particles play a pivotal role in reverse cholesterol transport (RCT) by facilitating cholesterol efflux from peripheral cells and delivering acquired lipid to the liver for elimination in the feces. Obesity increases the risk of developing cardiovascular disease (CVD); however, little is known about the impact of

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connecting these comorbidities. Dietary saturated fatty acids (SFAs) are metabolic stressors that initiate inflammation. SFAs, but not unsaturated fats, prime pro-interleukin 1β via toll-like receptor 4.3 We therefore hypothesized that replacement of proinflammatory SFAs for inert monounsaturated fatty acids (MUFAs) within obeseogenic high-fat diets (HFDs) would improve cardiometabolic health, in particular, RCT.

Static measurements of high-density lipoprotein cholesterol (HDL-C) fail to reveal the complex function or proteomic composition of HDL particles. Genetically defined low HDL-C has not been associated with CVD.6 Furthermore, raising HDL-C by using cholesteryl ester transfer protein inhibitors failed to have a therapeutic benefit7 despite increasing efflux capacity of serum ex vivo.9 HDL particles carry a large cargo of proteins including acute-phase proteins.10 Alteration in protein composition, in turn, likely modulates HDL atheroprotective functions.11 The HDL proteome is enriched with proinflammatory proteins in CVD.10,12 Little, however, is known about the impact of obesity, would alter HDL function in humans. We also speculated that insulin resistance, a product of HFD-induced obesity, would alter HDL function in humans.

Methods

Materials

Cholesterol [1,2-3H(N)] was purchased from Perkin-Elmer Analytic Sciences (Ireland). Cell culture material was purchased from Lonza (Slough, UK). All other reagents, unless otherwise stated, were from Sigma Aldrich Ltd.

Animals

Male C57BL/6j mice (Harlan, UK) were fed a SFA-HFD (45% kcal from palm oil), MUFA-HFD (45% kcal from trisun sunflower oil), or a micronutrient-matched low-fat diet (LFD; 10% kcal from a combination of oleic acid and palm oil) for up to 24 weeks. All diets were purchased from Research Diets Incorporated, New Brunswick, NJ. Blood was collected via tail vein for measurement of HDL efflux function. After in vivo RCT studies, mice were exsanguinated under anesthesia via the retro-orbital plexus and euthanized by cervical dislocation. Ethical approval was obtained from University College Dublin and St. Vincent’s University Hospital Human Research Ethics committees.

Labeling of Macrophages

HDL Function

J774 macrophages were labeled for 24 hours with 3H-cholesterol (1 μCi/mL) and equilibrated overnight in Dulbecco modified Eagle medium containing 0.2% bovine serum albumin±cAMP (0.3 mmol/L) to drive ABCA1 expression. ApoB-containing lipoproteins were removed from mouse plasma by polyethylene glycol (PEG) precipitation13 or HDL fractions were isolated by fast protein liquid chromatography (FPLC). HDL was isolated from human plasma by using Quantapol A kit (Technoclone, Austria). Ex vivo efflux from labeled macrophages to 2.8% HDL supernatant or 30% vol/vol FPLC fraction in minimal essential media was measured over 4 hours. The difference in efflux from cells stimulated in the presence or absence of cAMP represents ABCA1-dependent efflux. ABCA1-independent efflux was derived from untreated (–cAMP) cells.

RCT Studies

J774 macrophages, incubated for 48 hours in labeling media containing acetylated low-density lipoprotein (LDL; 25 μg/mL; Intracel, USA) and 3H-cholesterol (5 μCi/mL) were washed, equilibrated, and resuspended in minimal essential media before injection.

Rodent In Vivo RCT Studies

After 24 weeks of a HFD, C57BL/6j mice were injected into the intraperitoneal cavity with labeled macrophages (=2×10⁶ cells/4×10⁶ dpm/mouse), were housed individually in metabolic cages (Tecniplast, West Chester, PA) and 3H-cholesterol movement was monitored over 48 hours as described.12 Blood samples were collected from anaesthetized mice at 4, 24, and 48 hours via the retro-orbital plexus. 3H-label counts were measured by liquid scintillation counting.

Human Metabolic Challenge MECHE Study

A cohort of healthy individuals (aged 18–60 y, n=122) were recruited for the Nutrigenomics Investigation of the Body’s Metabolic Response to 2 Different Meal Challenges (MECHE) study as described13 and in the online-only Data Supplement. Following an overnight fast, subjects underwent an oral glucose tolerance test (75 g glucose), and their insulin secretory response was measured by enzyme-linked immunosorbent assay (Mercodia, Sweden). Ethical approval was obtained from University College Dublin and St. Vincent’s University Hospital Human Research Ethics committees.

Laboratory Methods

A description of general laboratory methods including glucose tolerance test, lipoprotein analysis, FPLC, nuclear magnetic resonance spectroscopy, quantitative real-time polymerase chain reaction, histochrometry, proteomics, food frequency questionnaire analysis, stromal vascular cell isolation, and immunoblot analysis are available in the online-only Data Supplement.

Statistical Analysis

For comparison across multiple groups, data were tested for normal Gaussian distribution by the Shapiro-Wilk test and equal variance by the Levene test. Normally distributed raw or log-transformed data (n≥8), with equal variance, were tested by 1-way or 2-way analysis of variance as appropriate and when significant Bonferroni post hoc tests were applied. Data sets with n≤8 or nonnormally distributed data underwent Kruskal-Wallis testing with the Dunn post hoc test. A Welch test with Games-Howell post hoc test was applied to normally distributed data that exhibited significant differences in variance. For comparison of data between 2 groups, an unpaired t test was applied to normally distributed data, whereas a Mann-Whitney U test was applied to nonnormally distributed data. GraphPad Prism 5 (GraphPad Software Inc, San Diego, CA) and SPSS (version 20; IBM) were used for statistical analysis. Data in graphs are presented as mean±standard error of the mean.

Results

Modulation of Macrophage-to-Feces RCT in Obese Mice by Dietary Fatty Acid Manipulation

C57BL/6j mice fed SFA-HFD or MUFA-HFD exhibited higher plasma 3H-cholesterol counts during RCT in comparison with...
LFD (Figure 1A) with counts primarily on larger HDL particles as determined by FPLC (Figure 1B). Increased hepatic \(^3\)H-cholesterol levels were also evident after SFA-HFD but did not translate to increased bile or fecal counts (Figure 1C through 1F). By contrast, MUFA-HFD significantly increased all steps of RCT with elevated plasma, hepatic, and fecal counts in comparison with LFD (Figure 1A through 1F). The ratio of \(^3\)H-cholesterol levels in plasma to liver was unchanged after HFDs, indicating that hepatic uptake of cholesterol from circulation was not modulated (Figure 1G). The ratio of \(^3\)H-cholesterol in liver to feces was significantly increased following SFA-HFD, but not MUFA-HFD, in comparison with LFD, indicating impaired hepatic trafficking (Figure 1G). Normalization of counts to plasma HDL-C revealed that increased HDL-C
was a major contributor to increased plasma counts; however, increased plasma and liver counts were still evident after SFA-HFD relative to LFD, indicating an additional hepatic backlog (Figure IA through ID in the online-only Data Supplement).

Mice gained less weight, consumed less food, had lower fasting insulin levels, but developed the same degree of glucose intolerance after MUFA-HFD in comparison with SFA-HFD (Figure IIA through IIC in the online-only Data Supplement).

**Figure 2.** Effects of high-fat feeding on ABCA1-independent and ABCA1-dependent efflux to HDL ex vivo. Plasma samples from mice were taken intermittently during high-fat feeding and ApoB particles removed by PEG precipitation. A, HDL-C levels were determined enzymatically \((n=4)\). J774 macrophages, labeled with \(^{3}H\)-cholesterol \((1 \muCi/mL)\), were stimulated ± cAMP \((0.3 \text{ mmol/L})\) to drive ABCA1 protein expression. Percentage of \(^{3}H\)-cholesterol efflux to 2.8% PEG supernatant was monitored over 4 hours. B, ABCA1-independent efflux was measured from unstimulated cells and normalized to HDL-C input \((n=6)\). C, ABCA1-dependent efflux was calculated as the difference in efflux from cells stimulated in the presence and absence of cAMP and was normalized to HDL-C input. After 24 weeks on HFDs, plasma lipoproteins were analyzed by NMR \((n=8; \text{ D})\) and FPLC \((n=6–8; \text{ E}, \text{ F})\). Efflux from labeled macrophages to FPLC fractions in minimal essential media \((30\% \text{ vol/vol})\) was monitored over 4 hours \((n=6–8)\). Protein levels of ApoA1 \((n=4; \text{ G})\) and SAA1 \((n=4; \text{ H})\) in FPLC fractions was determined by immunoblot. Statistical significance is presented as \(\ast P<0.05\), \(\ast\ast P<0.01\), \(\ast\ast\ast P<0.001\) w.r.t. LFD, \(\# P<0.05\) SFA vs MUFA. ABCA1 indicates ATP-binding cassette, subfamily A, member 1; ApoA1, apolipoprotein A-1; FPLC, fast protein liquid chromatography; HDL-C, high-density lipoprotein cholesterol; HFD, high-fat diet; LFD, low-fat diet; MUFA, monounsaturated fatty acid; NMR, nuclear magnetic resonance; PEG, polyethylene glycol; RCT, reverse cholesterol transport; SAA, serum amyloid A; SFA, saturated fatty acid; and w.r.t., with respect to.
Supplement, Table 1, and as described19). Weight-matched RCT data demonstrated a nonsignificant increase in hepatic counts and liver:feed ratio after SFA-HFD in comparison with MUFA-HFD (Figure IE through IH in the online-only Data Supplement), indicating that differences in weight gain are contributing to alterations in RCT between HFD groups.

**Fatty Acid–Enriched Obesogenic Diets Modulate Plasma Lipoprotein Profiles and HDL Functionality**

HDL-C levels increased after both HFDs (Figure 2A), with a specific increase in HDL-associated cholesteryl esters, and not free cholesterol observed after SFA-HFD in comparison with LFD (Figure IIIA in the online-only Data Supplement). HDL-associated phospholipids were increased after both HFDs, whereas triacylglyceride and apolipoprotein A-1 (apoA1) levels were unchanged (Figure IIIB and IIIC in the online-only Data Supplement). Total and ABCA1-independent efflux to HDL was increased after SFA-HFD in comparison with LFD (Figure IID and IIE in the online-only Data Supplement), an effect that was attributable to increased HDL-C levels (Figure 2A and 2B). By contrast, ABCA1-dependent efflux to HDL was reduced after SFA-HFD and MUFA-HFD in comparison with LFD after normalization to HDL-C (Figure 2C) with a similar trend on normalization to ApoA1 (Figure IIIG in the online-only Data Supplement). A significant reduction in large HDL particle concentration and increase in small HDL particle concentration was observed by nuclear magnetic resonance after SFA-HFD in comparison with LFD (Figure 2D), with increased LDL particle concentration observed following both HFDs (Figure IIIH in the online-only Data Supplement). FPLC analysis demonstrated a significant increase of cholesterol on larger HDL particles (Figure 2E). ABCA1-dependent efflux from labeled macrophages to FPLC fractions containing the smallest HDL particles was reduced after SFA-HFD in comparison with MUFA-HFD (nonnormalized data), whereas efflux to larger particles was unaffected (Figure 2F). ApoA1 protein levels were significantly increased in FPLC fraction 32, which contains large HDL particles after SFA-HFD in comparison with LFD, and were unchanged in the fractions containing medium and small HDL particles (34 and 37; Figure 2G). Serum amyloid A (SAA) concentration in FPLC fractions increased significantly after SFA-HFD in comparison with LFD (Figure 2H).

**SFA- and MUFA-Enriched HFDs Differentially Modulate the HDL Proteome**

HDL proteomic analysis was performed on small (fraction 36) and large (fraction 33) HDL-containing FPLC fractions (Tables I and II in the online-only Data Supplement). Increased SAA1 and SAA2 (Figure 3A) were observed on small HDL particles after SFA-HFD in comparison with LFD. ApoA1 and ApoA2 levels were unchanged (Figure 3B). ApoA4, ApoC2, and paraoxonase 1 protein levels were significantly increased on small HDL particles after MUFA-HFD in comparison with SFA-HFD (Figure 3C and 3D). By contrast, small HDL particles were enriched with ApoE, ApoH, haptoglobin, and SAA after SFA-HFD in comparison with MUFA-HFD (Figure 3D). Proteomic profiling of larger HDLs revealed little difference across groups (Table II in the online-only Data Supplement).

**SFA-Driven Obesity Reduces Hepatic Cholesterol Transporter Expression Coincident With Increased Hepatic Inflammation**

Hepatic mRNA and protein expression of ABCG5 and ABCB11 were reduced after SFA-HFD, and to a lesser extent MUFA-HFD, in comparison with LFD (Figure 4A and 4B). Scavenger receptor class B member 1 mRNA levels were reduced by both HFDs, but this did not reach significance at the protein level (Figure 4A and 4B). Hepatic lipid levels and plasma alanine aminotransferase levels were increased after both HFDs, albeit to a greater extent after SFA-HFD (Figure 4C through 4F) in comparison with LFD. Hepatic free cholesterol was increased to a greater extent than cholesteryl ester concentration after both HFDs (Figure 4D). No difference in plasma aspartate aminotransferase levels was observed (Figure 4G). Hepatic expression of SAA was higher after SFA-HFD than after LFD, whereas ApoA1 and liver X receptor α levels remained unchanged (Figure 4B, 4H, and 4I).

**Increased Adipose Tissue Cholesterol Flux During RCT After SFA-HFD**

During in vivo RCT 3H-cholesterol levels were significantly increased within epididymal adipose tissue after SFA-HFD in comparison with LFD, coincident with increased adipose tissue mass (Figure 5A and 5B). ABCA1 mRNA and protein expression was significantly increased in adipose tissue after SFA-HFD in comparison with LFD (Figure 5C and 5D). ABCG1 protein was undetectable as described.20 ABCA1 mRNA levels were higher in the adipocyte fraction than in the stromal vascular fraction (SVF), and a trend toward increased expression was evident after both HFDs (Figure 5E). SFA-SVF, and to a lesser extent MUFA-SVF, exhibited increased efflux to ApoA1 in comparison with LFD (Figure 5F). Adipose tissue M1 macrophage number was increased after SFA-HFD in comparison with MUFA-HFD (Figure 5G) and as described.19 Increased efflux was observed from adipose-derived F4/80+ cells in comparison with F4/80+ cells ex vivo (Figure 5H). Similarly increased efflux from bone marrow...
macrophages was observed in comparison with both pre- and mature 3T3L1 adipocytes in vitro (Figure IV in the online-only Data Supplement). Efflux from F4/80+ cells, normalized to protein concentration, was not altered by HFDs (Figure S5), but the increased number of macrophages present in SFA-SVF may contribute to enhanced efflux.

Figure 3. Modulation of the HDL proteome by fatty acid–enriched diets. Plasma was separated by FPLC after 24 weeks of feeding and protein content in HDL-containing fraction 36 was precipitated for proteomic analysis. Label free quantitative (LFQ) ion intensities were generated by using the default setting of MaxQuant. Levels of SAA (A), ApoA1 and ApoA2 (B), and PON1 (C) on HDL are presented across all groups. D, Proteins that were significantly different on HDL between MUFA-HFD and SFA-HFD groups were determined by using Perseus statistical software and a heat-map was generated (n=4–6, *P<0.05, **P<0.01 w.r.t LFD, †P<0.05 SFA vs MUFA). ApoA1 indicates apolipoprotein A-1; HDL, high-density lipoprotein; HFD, high-fat diet; LFD, low-fat diet; MUFA, monounsaturated fatty acid; PON1, paraoxonase 1; SAA, serum amyloid A; SFA, saturated fatty acid; and w.r.t., with respect to.
Insulin Resistance and High SFA Intake in Humans Is Associated With Loss of ABCA1-Mediated Efflux Capacity of HDL

We next questioned whether there was a relationship between parameters associated with high-fat intake including SFA consumption, insulin resistance, body mass index, chronic inflammation or glucose tolerance, and HDL function in humans. A healthy human cohort (Table 2) was subdivided into tertiles based on the various parameters outlined above. When individuals were grouped into tertiles based on measures of inflammation, glucose tolerance test curves, or body mass index, there was no association to ABCA1-dependent efflux

Figure 4. Reduced hepatic cholesterol transporter expression and enhanced hepatic inflammation after SFA-HFD. A, After 24 weeks on HFD, hepatic mRNA expression of cholesterol transporters and bile acid synthesis enzyme CYP7A1 was assessed by real-time PCR (LFD, n=8; SFA and MUFA, n=16). B, Hepatic protein expression of cholesterol transporters and ApoA1 were determined by immunoblot analysis (n=8). Hepatic lipid was monitored by hematoxylin and eosin staining (C) and enzymatic quantitation (D and E) of total cholesterol (TC), free cholesterol (FC), and TAG (n=8). Hepatic cholesteryl esters (CEs) were taken as the difference between total and free cholesterol levels. Plasma levels of ALT (F) and AST (G) were determined enzymatically (n=8). H and I, Hepatic mRNA and protein expression of LXRα and SAA was determined by real-time and immunoblot analysis (n=8). Statistical significance is presented as *P<0.05, **P<0.01, ***P<0.001 w.r.t. LFD, #P<0.05, ###P<0.001 SFA vs MUFA. ABCA1 indicates ATP-binding cassette, subfamily A, member 1; ALT, alanine aminotransferase; ApoA1, apolipoprotein A-1; AST, aspartate aminotransferase; LFD, low-fat diet; LXR, liver X receptor; MUFA, monounsaturated fatty acid; RCT, reverse cholesterol transport; SAA, serum amyloid A; SFA, saturated fatty acid; TAG, triacylglyceride; and w.r.t., with respect to.
or ABCA1-independent efflux (data not shown). When the group is split into tertiles based on their insulin secretory response to an oral glucose tolerance test, we demonstrate an incremental reduction in ABCA1-dependent but not ABCA1-independent efflux (Figure 6A and 6B) with increasing insulin resistance. This effect was independent of HDL-C and ApoA1 concentrations (Figure 6C and 6D). The high insulin secretion...
group was significantly heavier and exhibited impaired glucose tolerance and elevated homeostasis model assessment-estimated insulin resistance (Table 2 and Figure 6E and 6F) in comparison with the low insulin secretion group. It is interesting that the medium insulin secretion group required higher amounts of insulin to maintain glucose homeostasis, despite normal weight and glucose clearance and homeostasis model assessment-estimated insulin resistance equivalent to the low insulin secretion group (Table 2 and Figure 6E and 6F). In terms of the impact of dietary SFA, we demonstrate reduced ABCA1-dependent, but not ABCA1-independent efflux, in high SFA consumers in comparison with low SFA consumers (Figure 6G and 6H). HDL-C levels were similar between high- and low-SFA consumers (Figure VE in the online-only Data Supplement).

**Discussion**

We demonstrate that HFDs enriched with SFA and MUFA increase macrophage-to-plasma RCT in vivo and enhance ABCA1-independent efflux to HDL ex vivo, effects that were driven by increased HDL-C levels. By contrast, ABCA1-dependent efflux to HDL was reduced following both HFDs, an effect that was also evident in insulin-resistant human subjects and in high SFA consumers. Despite similar effects on macrophage-to-plasma RCT, MUFA-HFD and SFA-HFD differentially affected the latter steps of liver-to-feces RCT. Increased hepatic inflammation and reduced $^3$H-cholesterol trafficking from liver to fecal compartments during RCT was observed following SFA-HFD, but not MUFA-HFD, relative to LFD. Furthermore, hepatic-derived acute-phase proteins were enriched on the proteome of smaller HDL particles after SFA-HFD in comparison with MUFA-HFD, likely reflecting differential hepatic inflammation between groups. These novel findings demonstrate that measures of both HDL-C levels and HDL-efflux function are good biomarkers of macrophage-to-plasma RCT, whereas analysis of HDL proteomic quality is a better ex vivo surrogate of the latter steps of liver-to-feces RCT.

Increased plasma levels of $^3$H-cholesterol may reflect increased efflux from injected macrophages (because of increased HDL-C or increased particle function) and reduced clearance from circulation. Both HFDs increased $^3$H-cholesterol and cholesterol mass on larger HDL particles. Indeed, a large proportion of increased plasma counts during RCT were attributable to increased HDL-C, indicative that HDL-C is a major rate-limiting determinant of in vivo RCT. We similarly demonstrate increased ABCA1-independent efflux to HDL after SFA-HFD ex vivo, an effect that was also attributable to increased HDL-C. By contrast,

### Table 2. Baseline Characteristics of MECHE Cohort (n=122)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Low Insulin IAUC (n=40)</th>
<th>Medium Insulin IAUC (n=41)</th>
<th>High Insulin IAUC (n=40)</th>
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<tr>
<td>BMI, kg/m²¶</td>
<td>24.68 (3.64)</td>
<td>24.27 (3.29)</td>
<td>27.49 (7.08)*,§</td>
</tr>
<tr>
<td>Weight, kg#</td>
<td>75.05 (63.08, 85.99)</td>
<td>70.7 (60.8, 80.35)</td>
<td>75.05 (61.64, 94.95)</td>
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<td>Age, y**</td>
<td>31.5 (24.25, 40.75)</td>
<td>30 (24, 36)</td>
<td>29 (23.25, 34.5)</td>
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<tr>
<td>Cholesterol, mmol/L¶</td>
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<td>4.44 (0.93)</td>
<td>4.71 (1.02)*</td>
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<td>Triglycerides, mmol/L††</td>
<td>0.73 (0.5625,1)</td>
<td>0.8 (0.59,1.14)</td>
<td>1.12 (0.85, 1.56)†,</td>
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<tr>
<td>HDL, mmol/L¶</td>
<td>1.41 (0.34)</td>
<td>1.36 (0.31)</td>
<td>1.31 (0.36)</td>
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<td>NEFA, mmol/L**</td>
<td>0.44 (0.27, 0.72)</td>
<td>0.45 (0.285, 0.76)</td>
<td>0.52 (0.42, 0.74)</td>
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<tr>
<td>Insulin, μU/mL††</td>
<td>4.87 (3.20, 7.12)</td>
<td>4.84 (3.14, 6.88)</td>
<td>8.87 (5.89, 12.46)†,</td>
</tr>
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<td>Glucose, mmol/L**</td>
<td>5.12 (4.9, 5.49)</td>
<td>5.14 (4.80, 5.35)</td>
<td>5.21 (4.91, 5.57)</td>
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<tr>
<td>HOMA-IR**</td>
<td>1.19 (0.77, 1.79)</td>
<td>1.08 (0.69,1.58)</td>
<td>1.97 (1.40,3.12)†,</td>
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<td>ApoA1, mg/dL¶</td>
<td>136.05 (28.64)</td>
<td>131.78 (25.84)</td>
<td>131.23 (35.73)</td>
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<td>Apo B, mg/dL¶</td>
<td>69.88 (20.20)</td>
<td>77.24 (23.11)</td>
<td>81.38 (20.53)*</td>
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<td>Apo C2, mg/dL**</td>
<td>3.59 (1.79, 4.33)</td>
<td>3.68 (2.16, 4.74)</td>
<td>4.35 (2.29, 5.53)*</td>
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<td>Apo C3, mg/ dL**</td>
<td>6.25 (4.55, 8.28)</td>
<td>7.29 (5.4, 8.30)</td>
<td>7.83 (6.1, 9.71)*</td>
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<td>Apo E, mg/dL**</td>
<td>2.08 (1.62,2.64)</td>
<td>2.21 (1.67, 2.77)</td>
<td>2.42 (1.87, 2.92)</td>
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<td>hs-CRP, mg/L††</td>
<td>0.7 (0.41, 1.28)</td>
<td>0.75 (0.42, 1.30)</td>
<td>1.27 (0.55, 3.84)†,</td>
</tr>
</tbody>
</table>

A healthy human cohort was challenged with glucose (75 g) and grouped into tertiles based on their insulin secretion response during an OGTT. Data are mean (SD) for normally distributed raw data, or median (25th, 75th percentile) for transformed data and nonnormal data. ANOVA indicates analysis of variance; ApoA1, apolipoprotein A-1; BMI, body mass index; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment-estimated insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IAUC, incremental area under the curve; OGTT, oral glucose tolerance test; and SD, standard deviation.

*P<0.05, †P<0.01, ‡P<0.001 w.r.t low insulin IAUC and ¶P<0.05, and ||P<0.001 medium vs high insulin IAUC

¶Raw data.

#Square root transformed data were analyzed by 1-way ANOVA.

**Nonnormal data were analyzed by Kruskal-Wallis test.

††Log-transformed data were analyzed by 1-way ANOVA.
ABCA1-dependent efflux to HDL was reduced incrementally after MUFA-HFD and SFA-HFD, respectively, after normalization to HDL-C and ApoA1 pools. Similarly, raw efflux to FPLC fractions demonstrated a specific reduction in efflux to small HDL particles after SFA-HFD. Wang et al\textsuperscript{17} previously demonstrated that ABCA1-dependent efflux accounts for \( \approx 30\% \) of plasma \(^3\text{H}\)-cholesterol counts during RCT; hence, the majority of plasma counts are derived from ABCA1-independent pathways. The minor reduction in the efflux function of smaller HDL particles after HFDs was therefore counterbalanced by both increased HDL-C and ABCA1-independent efflux in vivo. Intriguingly the concentration of
large HDL particles was in fact reduced following both SFA-HFD despite elevated HDL-C. These findings suggest that increased HDL-C is attributable to increased particle size, and not particle number, which is corroborated by increased cholesterol esters but not free cholesterol on HFD-derived HDL particles. Increased particle size may also account for the increased efflux observed by ABCA1-independent pathways. Although the majority of increased plasma counts during RCT were attributable to increased HDL-C following HFDs, we still observed a significant increase in plasma 3H-cholesterol levels and liver:feces 3H-cholesterol ratio after normalization to HDL-C following SFA-HFD, which is indicative of additional hepatic backlog.

We previously demonstrated that endotoxin-evoked inflammation impaired hepatic cholesterol trafficking.\(^{15}\) Similarly, increased inflammation, reduced transporter expression, and increased free cholesterol and 3H-cholesterol levels were observed in livers after SFA-HFD in comparison with LFD. An intermediate phenotype was observed after MUFA-HFD wherein 3H-cholesterol trafficking was preserved to fecal compartments, and a less profound reduction in hepatic transporter expression was observed as SFA-HFD. Nonetheless, hepatic 3H-cholesterol counts, free cholesterol, and triacylglyceride levels and systemic alanine aminotransferase were elevated after MUFA-HFD in comparison with LFD. These findings demonstrate that dietary fatty acid composition, and not merely quantity, is an important determinant for hepatic cholesterol trafficking in obesity.

Consistent with hepatic inflammation, the proteomic signature of HDL indicated a skewing toward a proinflammatory particle after SFA-HFD, but not after MUFA-HFD. We found dissociation of antioxidant paraoxonase 1 from HDL particles after SFA-HFD in comparison with MUFA-HFD. Reduced paraoxonase 1 activity is associated with increased oxidative stress and CVD in humans\(^ {27}\) and atherogenesis in mice.\(^ {22}\) HDL particles were also enriched with hepatic-derived acute-phase proteins SAA, hemopexin, and haptoglobin after SFA-HFD in comparison with MUFA-HFD. Plasma SAA predicts CVD in humans,\(^ {23}\) whereas haptoglobin and hemopexin are enriched on HDL from hyperlipidemic mice and CVD patients and are associated with reduced anti-inflammatory functions.\(^ {24}\) Enrichment of HDL particles with proinflammatory proteins and loss of anti-inflammatory proteins after SFA-HFD likely affect numerous atheroprotective functions of HDL. Indeed, the antioxidant effects of HDL are reduced in insulin-resistant individuals,\(^ {25}\) whereas particles become proinflammatory in type 2 diabetes mellitus.\(^ {26}\) Our findings suggest that fatty acid composition of obesogenic diets is an important determinant of the HDL proteome that may modulate CVD risk.

Uptake of 3H-cholesterol into adipose tissue increased during RCT following SFA-HFD in comparison with LFD, despite little difference in cholesterol uptake transporter expression. Redistribution of 3H-cholesterol to adipose tissue may neutralize increased CVD risk imposed by impaired hepatic clearance after SFA-HFD. Paradoxically, expression of ABCA1 in whole adipose tissue was upregulated following HFDs with increased cholesterol efflux from SFA-SVF, and, to a lesser extent, MUFA-SVF, to ApoA1 observed ex vivo. Adipose tissue macrophage number was higher after SFA-HFD than after MUFA-HFD and LFD. This may have contributed to enhanced efflux, because SVF cells expressing the macrophage marker F4/80 (SVF-F4/80\(^ +\)) exhibited greater efflux to serum than SVF-F4/80\(^ +\) cells ex vivo. We did not observe a difference in efflux from SVF-F4/80\(^ +\) cells to serum between diet groups, indicative that efflux capacity per cell is not modulated by HFDs. Expression of cholesterol transporters increases during adipogenesis\(^ {29}\) and lipid loading\(^ {27}\) because of activation of peroxisome proliferator-activated receptor γ and liver X receptor,\(^ {27,28}\) and therefore hyperinsulinemia and hypercholesterolemia evident on high-fat feeding may also contribute to increased ABCA1. These findings demonstrate that cholesterol flux in and out of adipose tissue is enhanced on high-fat feeding, and elevated adipose tissue counts are likely attributable to enhanced plasma counts and adipose tissue mass, rather than defective efflux capacity.

The impact of high-fat feeding on HDL efflux capacity was previously investigated in African green monkeys\(^ {29}\) and healthy humans,\(^ {30}\) with no difference in total HDL efflux capacity observed, consistent with our findings. In our study more specific interrogation of ABCA1-dependent versus ABCA1-independent efflux was necessary to establish reduced functionality of smaller HDL particles after HFD in mice. Interestingly, in a human translational setting, we found that hyperinsulinemia, a by-product of high-fat intake, and not inflammatory markers, was associated with reduced ABCA1-mediated efflux capacity of HDL. Reduced ABCA1 efflux function was also evident in those consuming above the recommended daily allowance for SFA in comparison with those consuming low amounts. ABCA1-dependent efflux, but not HDL-C, was recently demonstrated to correlate inversely with pulse wave velocity in healthy humans.\(^ {31}\) These findings suggest that measurements of ABCA1 efflux capacity might be a stronger predictor of CVD risk, but this remains to be verified in large-scale prospective studies.

A significant limitation of our study is the use of C57BL/6\(^ {\text{j}}\) mice that lack cholesteryl ester transfer protein and exhibit low levels of LDL cholesterol. That withstanding, an increase in LDL particle number was observed by nuclear magnetic resonance after both HFDs, with greater elevation after SFA-HFD than after MUFA-HFD. Briand et al\(^ {32}\) previously demonstrated elevated plasma and hepatic counts, with little difference in fecal counts during RCT in hamsters, which express cholesteryl ester transfer protein, after feeding a diet enriched with cholesterol, saturated fat, and fructose. Our findings go beyond these observations by demonstrating a specific detrimental effect of SFA on the latter stages of RCT, an effect that can be attenuated by replacement of proinflammatory palmitic acid for oleic acid. The use of a simplified mouse model of obesity to reflect human obesity is another study limitation. Human obesity is associated with reduced HDL-C, whereas HFD-induced obesity in mouse models increases HDL-C. That withstanding, saturated-fat diets raise HDL-C in humans,\(^ {33}\) similar to mice, whereas substitution of dietary fat for carbohydrate increases triacylglyceride and reduces HDL-C.\(^ {34,35}\) Thus, although the mouse model of obesity fails to model the complexity of human obesity, it does reflect the effect of high-fat consumption on lipid profiles in humans.
Our findings indicate that high-fat intake increases the rate of macrophage-to-plasma RCT and questions whether fecal elimination is essential to reduce CVD risk. Indeed a large-scale prospective meta-analysis study recently demonstrated no significant relationship between dietary SFA and CVD. Replacement of dietary carbohydrate for fat may in fact normalize lipid profiles in obesity and even increase the rate of efflux from peripheral cells. However, our study highlights the importance of considering fat quality in this approach — dietary enrichment with palmitic acid, the major SFA in the Western diet, impaired hepatic cholesterol clearance and modulated the HDL proteome which may alter other atheroprotective functions. Furthermore, in the human setting, impaired hepatic cholesterol clearance would likely increase LDL cholesterol, and, thus, futile bidirectional cycling of cholesterol into and out of atherosclerotic lesions could be envisaged. By contrast, dietary enrichment with MUFA increased HDL-C and RCT with minimal effects on the HDL proteome.

Conclusions

Our findings demonstrate that HFDs enriched with SFA or MUFA increased macrophage-to-plasma RCT coincident with increased HDL-C. However SFA-HFD impeded hepatic cholesterol clearance and increased association of hepatic-derived acute-phase proteins on HDL particles, in comparison with MUFA-HFD. Ex vivo HDL efflux assays modeled the effects of HFDs on macrophage-to-plasma RCT but did not adequately capture information pertaining to the latter steps of RCT. By contrast proteomics revealed the association of hepatic-derived acute-phase proteins on HDL after SFA-HFD in comparison with MUFA-HFD, which reflected impaired hepatic cholesterol trafficking. Levels of acute-phase proteins on HDL may serve as a novel biomarker of impaired liver-to-fores RCT in vivo.

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Disclosures

None.

References


CLINICAL PERSPECTIVE

Reverse cholesterol transport (RCT) is the process by which cholesterol from peripheral cells is effluxed onto circulating high-density lipoprotein (HDL) particles to be returned to the liver for excretion. Promotion of RCT is thought to reduce cardiovascular disease risk; however, raising high-density lipoprotein cholesterol (HDL-C) levels by using cholesteryl ester transfer protein inhibitors failed to have clinical benefit. HDL particles carry a large cargo of proteins, in particular, immune modulatory proteins that likely alter the atheroprotective functions of HDL. This study demonstrates that saturated fatty acid (SFA)–induced obesity increased HDL-C and macrophage-to-plasma RCT in vivo; however, liver-to-feces RCT was impaired coupled with increased hepatic inflammation and downregulation of hepatic transporter expression in comparison with lean controls. Replacement of SFA with monounsaturated fatty acid within obeseogenic diets increased all steps of macrophage-to-feces RCT with lower levels of hepatic inflammation evident in comparison with SFA–high-fat diet. The proteome of HDL particles was enriched with proinflammatory hepatic-derived acute-phase proteins after SFA–high-fat diet in comparison with monounsaturated fatty acid–high-fat diet reflecting differential modulation of liver-to-feces RCT by SFA- and monounsaturated fatty acid–high-fat diets. Recent meta-analysis has suggested that high-SFA intake is not associated with cardiovascular disease. Furthermore, high carbohydrate consumption lowers HDL-C, whereas high fat consumption raises HDL-C. Although dietary replacement of carbohydrate for fat may normalize lipid profiles in obesity, our study warrants caution over the fat quality used in such an approach. Dietary replacement of carbohydrate with SFA may increase levels of proinflammatory HDL particles and increase cardiovascular disease risk despite elevating HDL-C. By contrast, dietary replacement of carbohydrate for monounsaturated fatty acid may raise HDL-C, preserve the anti-inflammatory proinflammatory composition of HDL particles, and increase RCT.