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Post-prandial remodeling of HDL following high saturated fat and high carbohydrate meals.

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Abstract

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Authors' Contributions

MA and TV designed the study. JW, IB, JB, KFS, DC, AH and TV were involved in data collection. MA, KBR, KC, AH, and TV contributed to data analysis and interpretation. MA, KBR, and TV wrote the manuscript. All authors were involved in the revision and approved the final manuscript.

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Background: Humans spend most of the time in the postprandial state, yet most knowledge about high-density lipoproteins (HDL) derives from the fasted state. HDL protein and lipid cargo mediate HDL's antiatherogenic effects, but whether these HDL constituents change in the postprandial state and are affected by dietary macronutrients remain unknown.

Objectives: To assess changes in HDL protein and lipid composition after consumption of a high carbohydrate or high saturated fat (HSF) meal.

Methods: We isolated HDL from plasma collected during a randomized, cross-over study of metabolically healthy subjects. Subjects consumed isocaloric meals consisting predominantly of either carbohydrate or fat. At baseline and at 3 and 6 hours postprandial, we quantified HDL protein and lipid composition by liquid chromatography mass spectrometry.

Results: 15 subjects were included (60% female, ages 34 ± 15 years, BMI 24.1 ± 2.7 kg/m²). Consumption of the HSF meal led to HDL enrichment in total lipid ($P=0.006$), triglyceride ($P=0.02$) and phospholipid ($P=0.008$) content and a corresponding depletion in protein content. Following the HSF meal, 16 of the 25 measured phosphatidylcholine species significantly increased in abundance (P values range 0.027 to <0.001), along with several sphingolipids including ceramides ($P<0.004$), lactosylceramide ($P=0.023$), and sphingomyelin-14 ($P=0.013$). Enrichment in apolipoprotein A-I ($P=0.001$) was the only significant change in HDL protein composition following the HSF meal. The high carbohydrate meal conferred only minimal changes in HDL composition.

Conclusion: Meal macronutrient content acutely affects HDL composition in the postprandial state, with the HSF meal resulting in enrichment of HDL phospholipid content with possible consequences for HDL function.

Keywords

Diet; high density lipoprotein; high density lipoprotein remodeling; high density lipoprotein/ metabolism; lipoproteins; lipidomic analysis; proteomic analysis

Introduction

A cornerstone of clinical cardiovascular (CV) risk assessment is the measurement of plasma lipid concentrations in the fasting state. Serum lipoprotein-associated triglyceride and cholesterol levels have traditionally been measured in the fasting state, in part to avoid the variation introduced by ingestion of meals with variable size and macronutrient content¹. Nonetheless, most individuals spend the majority of time in a non-fasting state, implying that the fed state influences CV risk². Notably, recent clinical data suggest that elevations specifically in non-fasting or postprandial plasma triglyceride levels predict increased risk for CV disease, ischemic stroke, and associated mortality³⁻⁶.

Postprandial lipemia is the physiologic rise in plasma lipids following a meal. Dysregulated postprandial lipemia is a recognized characteristic of many obesity-related disorders, and postprandial hypertriglyceridemia positively correlates with CV risk⁷⁻⁹. The postprandial state is characterized by a rapid remodeling mainly of triglyceride-rich lipoproteins (TRLs), and this remodeling is thought to induce functional changes in HDL¹⁰⁻¹². Elevated

postprandial triglycerides can drive the exchange of cholesterol esters and triglycerides between HDL and TRLs mediated by cholesteryl ester transfer protein (CETP), resulting in enrichment of HDL with triglyceride reducing HDL-C and promoting HDL dysfunction^{13, 14}.

The mechanisms underlying the increased CV risk conferred by postprandial hypertriglyceridemia have not been fully elucidated. Strong evidence supports the generation of proatherogenic remnant lipoprotein particles (RLPs) as one potential mechanism^{5, 15-17}. Additionally, a reduction in HDL-C may also contribute to the increased risk^{13, 14}. However, clinical studies have demonstrated that the association between hypertriglyceridemia and CV risk is independent of HDL-C^{18, 19} and the predictive value of changes in HDL-C for determining CV risk has been challenged by recent clinical findings²⁰⁻²³. Notably, changes in HDL cholesterol content constitute only one facet of the compositional changes in HDL that could occur with postprandial lipemia. HDL comprises a heterogeneous population of particles with a highly complex and malleable protein and lipid content^{24, 25}, and it is primarily the non-cholesterol content of HDL that is thought to determine its functional properties²⁶⁻²⁸. Thus, HDL's protein and phospholipid cargo may directly influence HDL's antiatherogenic effects, and these non-cholesterol constituents may prove better predictors of CV risk than HDL-C^{24, 29-32}. Dynamic remodeling of remnant particle lipoproteins and phospholipids has been described in the postprandial state³³; however, effects of postprandial lipemia on HDL-associated proteins and phospholipids have not been determined.

Postprandial HDL remodeling was first investigated over 50 years ago, both *in vitro* using co-culture of HDL and TRLs in the presence of a lipolytic catalyst and *in vivo* after fat ingestion^{34, 35}. These studies consistently demonstrated HDL enrichment in total phospholipid and apolipoprotein A-I (apoA-I) after fat ingestion; however, they were limited by available methods to separate and quantify HDL constituents. Our study was conducted to verify these older findings by testing the hypothesis that HDL composition changes in the postprandial state and is influenced by the macronutrient content of the meal. We predicted that, consistent with prior findings, consumption of a high saturated fat meal would lead to increases in HDL phosphatidylcholine and apoA-I content, and that these findings would be specifically due to lipid ingestion rather than a general postprandial response. We further sought to better characterize the differential changes in HDL phospholipid, sphingolipid, and protein composition that occur in the postprandial state with either fat or carbohydrate as the predominant macronutrient consumed.

Methods

Study subjects and diet intervention

Plasma samples were previously collected through a clinical study conducted to determine the effects of diet composition on plasma ghrelin levels³⁶. In brief, 16 metabolically healthy adults with stable body weight were assigned in a randomized, within-subjects, cross-over study design to consume isocaloric, 500 ml liquid meals. The high carbohydrate meal (80% carbohydrate, 10% fat, 10% protein) and high saturated fat meal (80% fat, 10% carbohydrate, 10% protein) were administered in a cross-over fashion with a wash-out

period (1 week) between the two test meal sessions. Test meals provided 20% of the estimated total energy needs calculated using the Harris-Benedict equation then multiplied by an activity factor (1.5 for women, 1.6 for men). The high carbohydrate meal consisted primarily of glucose, and the high saturated fat meal was composed primarily of whipping cream (see Supplemental Table S1 for meal composition). The meals were prepared by blending ingredients and refrigerating the meals up to 24 hours prior to consumption. Participants presented to the research center before 8am fasted and a fasting blood draw was performed. At 9:00am participants were instructed to consume the entire 500 ml test meal over a 15 minute period, which was counted as the first 15 minutes of the postprandial time period. Participants were not provided with any other meal until completion of the testing that day. Prior to and between test visits, study subjects consumed their typical diet. Analyses were performed with plasma samples collected in the fasted state (premeal) after an overnight fast, and at 3 (± 20 minutes) and 6 (± 20 minutes) hours after initiation of test meal ingestion (12:00pm and 3pm respectively). The plasma samples were collected in 2003, immediately frozen and stored at -80°C until HDL isolation. The postprandial timepoints were selected based on previously published data for the parent study showing that circulating triglyceride levels peak around 3 hours postprandial and return to baseline approximately 6 hours postprandial³⁶. From the 16 subjects in the original study for one subject adequate sample volume was not available and this subject had to be excluded from the present study. Detailed inclusion and exclusion criteria and the comprehensive study design are described elsewhere³⁶. The research protocol and procedures were approved by the University of Washington Institutional Review Board.

Laboratory assessments

Plasma lipids were measured using standard enzymatic methods, and plasma apolipoproteins B (apoB) and apoA-I levels were measured by nephelometry at the University of Washington Nutrition Obesity Research Center (UW NORC) Analytic Core. HDL protein concentration was measured using Bradford assay. HDL lipids for overall HDL composition assessment were measured as follows using well-established assay kits: phospholipids were measured using Phospholipids C Wako kit (Wako, Cat# 997-01801)³⁷; free and total cholesterol were measured using Amplex Red assay kit with and without cholesterol esterase, and esterified cholesterol was calculated as the difference between total and free cholesterol (Invitrogen, Cat# 12216)³⁸; and triglycerides were measured using Triglyceride Quantification Kit (C/F) (BioVision, K622-100) according to manufacturer protocol. All assays were calibrated using manufacturer provided standards.

HDL isolation

HDL ($d = 1.063\text{--}1.210\text{ g/mL}$) was isolated from EDTA-anticoagulated human plasma using sequential density gradient ultracentrifugation following a protocol similar to that previously described³⁹. Briefly, 335 μL of freshly thawed plasma was adjusted to density of 1.21 g/mL with KBr and spun in tabletop ultracentrifuge (Optima XL, Beckman) for 4.5 h at 120,000rpm at 5 C. The top 125 μL was collected, readjusted with saline to density 1.063 g/mL and spun for 2 h at 100,000g. The bottom 125 μL was collected and dialyzed against 20 mM potassium phosphate buffer containing 100 μM diethylenetriaminepentaacetic acid overnight. Quality of the isolated HDL was reported elsewhere⁴⁰. Protein concentration was

determined using the Bradford method with bovine serum albumin as the calibrator (Coomassie Plus, Thermo Fisher Scientific Inc.), and 10 µg protein was aliquoted for proteomic analysis and frozen at –80C until analysis.

Targeted proteomic analysis

Targeted proteomics analysis was performed using Parallel Reaction Monitoring (PRM) liquid chromatography mass spectrometry (LCMS) as previously reported^{39,41}. Seven replicates of a single pooled plasma sample were processed along with the study samples as quality control samples to assess method performance and analyzed interspersed among the study samples.

Protein digestion.—Purified HDL (10 µg protein) was solubilized with 0.1% RapiGest (Waters, MA) in 200 mM ammonium bicarbonate, spiked with 1 µg of ¹⁵N-apoA-I as internal standard, reduced with dithiothreitol, alkylated with iodoacetamide, and digested with two additions of trypsin (1:20, w/w HDL protein; Promega, WI) for 3 h at 37°C, and after the second addition overnight. After acidic hydrolysis of RapiGest, samples were dried and stored at –20°C until analysis. Samples were reconstituted in 5% acetonitrile, 0.1% formic acid to 0.25 µg/µL^{39,41}.

Selection of the peptides and transitions for PRM analysis.—Similar to previously described methods^{39,41} peptides for each protein used in the PRM analysis were selected based on spectral count in a shotgun proteomics experiment, observed frequency in the PeptideAtlas mass spectral database, and screening experiments that evaluated the correlation of peptides from the same protein with one another across clinical study populations. Peptides containing methionine residues were excluded from consideration. The transitions monitored for each peptide were selected based on signal intensity from a screening experiment. List of peptides and acquisition conditions is provided in Supplemental Table S2. Peak areas for each endogenous peptide were normalized to ¹⁵N-apoA-I as internal standard to generate a peak area ratio for each peptide in each sample. Due high correlations between peptides (One peptide was chosen as a representative peptide for the analysis, denoted in supplemental table S1.

Parallel-reaction monitoring LCMS analysis.—Tryptic digests of HDL were desalted on a C18 trapping column (XBridge BEH C18 100 Å, 5 µm, 0.1 × 40 mm, Waters, MA) (trapping flow rate 4 µL/min), and separated on a capillary XBridge BEH C18 analytical column (XBridge BEH C18 100Å, 3.5 µm, 100×0.075 mm, Waters, MA) with a 30 min linear gradient of acetonitrile, 0.1% formic acid (7-35%) in 0.1% formic acid in water at a flow rate of 0.6 µL/min using a NanoAquity UPLC (Waters, MA). The NanoAquity UPLC was connected to a Thermo Orbitrap Fusion trihybrid mass spectrometer with electrospray ionization. PRM acquisition was performed as described before⁴². The instrument was operated in PRM mode selecting precursor ion in the quadrupole analyzer with isolation window 2 Th, fragmented in the HCD cell at normalized collision energy 27%, and the fragment ions were analyzed in the Orbitrap analyzer at 15,000 resolution at m/z 400, AGC target 5×10^4 and maximum ion time 30 ms. For quantification, the 3-5 most intense y-type fragment ions were used. Peak areas were integrated using Skyline software⁴³.

Lipidomic analysis

The HDL lipidome was measured on the same isolated HDL used for proteomics analysis. After isolation the HDL was stored at -70°C prior until lipid extraction. After HDL lipids were extracted, the sphingolipids, ceramides and phosphatidylcholine species were quantified by LCMS. The detailed methodology and method used for evaluation of data are provided in Supplementary Methods. Twenty-five individual species of phosphatidylcholine were detected and quantified in the analysis (Supplemental Table 3). For sphingolipids, we focused the analysis on those that carried a saturated fatty acid acylated to the sphingolipid backbone, including palmitic acid (16:0), stearic acid (18:0), arachidonic acid (20:0), behenic acid (22:0) and lignoceric acid (24:0). In total we measured 13 sphingolipids species that had a coefficient of variation $< 20\%$. These include ceramide (Cer): 22:0 (Cer-22), and two species of ceramides with 24:0 with alternate “d181” and “d182” sphingoid backbones; six sphingomyelins (SM): SM-14, SM-16, SM-18, SM-20, SM-22, SM-24; three glucosyl-ceramides (GluCer): GluCer-16, GluCer-22, and GluCer-24; and one lactosyl-ceramide (LacCer): LacCer-16.

Statistical analyses

All plasma lipids, HDL proteins and lipid species were log2 transformed prior to analysis. All modeling was done using mixed effect linear models that included a random intercept per subject to model the within-subject correlation for repeated measures. The postprandial response to test meals at 3 and 6 hours was tested separately for each test meal with time as a fixed effect and subject ID as a random effect (i.e., $y \sim \text{time} + (1|\text{subject ID})$). The outcome variable was level of the HDL composition measure at the given timepoint. It was compared to the fasted state for each diet separately with time as a categorical variable used as a fixed effect and subject ID as a random effect. The difference between test meals in the change from the fasted state was modeled separately at the 3- and 6-hour timepoints with meal and baseline values for each HDL composition measure as fixed effects and subject ID as a random effect (i.e., $d \sim b + \text{diet} + (1|\text{subject ID})$ for each timepoint separately, where d =change from baseline to 3 or 6 hour timepoints and b =baseline). All statistical analyses were completed in R using library nlme. Multiple hypotheses testing was accounted for by adjusting the p-values generated from each analysis group (e.g., plasma lipids, HDL composition, proteome, lipidome) using the Benjamini-Hochberg procedure to control the false discovery rate at 0.05 per analysis group. Only the results/variables passing this p-value criterion are reported in the results section; the supplemental tables contain the complete unadjusted results.

Results

Study participants

The participants were predominately Caucasian, healthy men ($n=6$) and women ($n=9$). The participant demographics are shown in table 2. Participants had normal glucose tolerance (published previously³⁶) and normal fasting plasma lipid and apolipoprotein levels (Table 2). Fasting insulin and glucose levels did not differ within subjects across the study visits³⁶.

Effects of meal consumption on postprandial lipemia.

Fasted and postprandial plasma lipid and apolipoprotein levels are shown in Table 2. Following the high carbohydrate diet, no changes in plasma lipid levels were observed. In contrast, after the high saturated fat meal, a significant increase in plasma triglyceride level was evident at 3 hours postprandial (75.3 ± 34.2 mg/dL fasted vs. 117.9 ± 59.6 mg/dL at 3 hours, $p=0.0008$). The high saturated fat meal also resulted in a decrease in plasma HDL-C level at the 3-hour postprandial timepoint (51.3 ± 12.7 mg/dL fasted vs. 49.4 ± 11.7 mg/dL at 3 hours, $p=0.009$). At 6 hours after the high saturated fat meal, plasma levels of triglycerides and HDL cholesterol were no longer significantly different from baseline. Neither meal altered plasma levels of total or LDL cholesterol, apoA-I, or apoB. At the 3-hour postprandial timepoint, plasma triglyceride levels were significantly increased after the high saturated fat diet compared to the high carbohydrate diet; no other between-meal differences were seen for any of the plasma lipid or apolipoprotein levels at either postprandial timepoint.

Meal macronutrient composition affects overall HDL protein and lipid content.

We then investigated the effects of meal consumption on HDL total protein and lipid content. A significant effect of meal macronutrients was found for HDL relative enrichment in protein, triglyceride, and phospholipid content but not in cholesterol ester or free cholesterol content, when expressed as a % of total HDL mass (Figure 1C-E). Thus, the high saturated fat meal led to relative depletion of HDL protein ($62.1 \pm 4.5\%$ total HDL mass fasted vs. $58.4 \pm 5.7\%$ total HDL mass at 6 hours postprandial; $P=0.006$; Figure 1A) with 25% enrichment in HDL triglyceride content at 6 hours postprandial ($2.4 \pm 1.2\%$ total HDL mass fasted vs. $3.0 \pm 1.5\%$ total HDL mass at 6 hours postprandial, $p=0.02$; Figure 1E). In contrast, the relative abundance of HDL protein and triglyceride remained stable after the high carbohydrate diet. The % content of total HDL phospholipid also increased 6 hours after consumption of the high saturated fat meal ($22.6 \pm 3.8\%$ total HDL mass fasted vs. $26.3 \pm 5.7\%$ total HDL mass at 6 hours postprandial; $p=0.009$; Figure 1F). At the 6-hour postprandial timepoint, HDL phospholipid % content was significantly different between meals ($22.6 \pm 3.8\%$ total HDL mass after the high saturated fat meal vs. $20.7 \pm 2.7\%$ total HDL mass after the high carbohydrate meal, $p=0.006$, Figure 1F). It is notable that all significant changes were seen 6 hours postprandial, which is delayed relative to the postprandial lipemia (Table 2). No other between-meal differences were seen at either timepoint in HDL total protein, neutral lipid, or phospholipid content.

High saturated fat and high carbohydrate meals do not substantially alter the HDL proteome in the postprandial state.

A few modest changes in the HDL proteome were seen at 3 and 6 hours after consumption of a high saturated fat versus high carbohydrate meals. Six hours after consumption of the high saturated fat meal, there was a modest increase in the relative abundance of HDL apoA-I relative to baseline ($p=0.001$) (Figure 2, Supplemental Table S4). No other HDL proteins changed significantly from baseline after either meal. However, between-meal differences were evident at 3 hours postprandial in the relative abundance of HDL apoH, apoL1, and

PLTP, all of which showed enrichment after the high saturated fat meal but depletion after the high carbohydrate meal (Figure 2, Supplemental Table S5).

High saturated fat and high carbohydrate meals differentially modulate the HDL lipidome in the postprandial state.

The high carbohydrate meal resulted in minimal changes in phospholipid species compared to baseline, with only Cer-d18:1/24:0 significantly decreased at 6 hours. In contrast, the high saturated fat meal induced significant enrichment of HDL in a number of phospholipid species at both 3- and 6-hour timepoints. At the 3-hour timepoint, the abundance of Cer-d18:1/24:0 ($p=0.0009$) and 3 of the 25 phosphatidylcholine species was increased relative to baseline (Figure 3, Supplemental tables S6 and S7), and at 6 hours, the abundance of SM-14 ($p=0.013$), Cer-d18:1/22:0 ($p=0.0049$), Cer-d18:1/24:0 ($p=0.0036$), Cer-d18:2/24:0 ($p=0.005$), and LacCer-16:0 ($p=0.023$), as well as 16 of the 25 phosphatidylcholine species was significantly increased compared to baseline (Figure 3, Supplemental tables S6 and S7). Significant between-meal differences were evident at 3 hours postprandial in the relative abundance of 7 of the 25 phosphatidylcholine species, and, at 6 hours postprandial, SM-14, Cer-d18:1/24:0, and 11 of the 25 phosphatidylcholines were enriched after the high saturated fat meal relative to the high carbohydrate meal (Figure 3, Supplemental tables S8 and S9).

Discussion

Our findings provide evidence for remodeling of HDL molecular composition in the postprandial state, with specific effects attributable to dietary macronutrient composition. Whereas the high carbohydrate meal conferred minimal changes in HDL composition compared to the fasted state, the consumption of the high saturated fat meal led to relative HDL enrichment in total lipid, triglyceride and phospholipid content and a corresponding relative depletion of protein content. At the molecular level, the high saturated fat meal led to increases in 16 of the 25 phosphatidylcholine species measured as well as several sphingolipids including ceramides, lactosyl-ceramide, and sphingomyelin-14. In contrast, only a single HDL-associated protein was changed from the fasted state by the high saturated fat meal. Notably, whereas plasma triglyceride levels increased significantly at 3 hours after the high saturated fat meal, the changes in HDL appear to lag and were primarily evident at 6 hours postprandial, suggesting that postprandial HDL remodeling is delayed compared to the changes in apoB lipoproteins. Importantly, no common changes in HDL composition were evident for the two meals, and the two diets appear to have divergent effects with significant post-prandial differences in several protein and lipid components, suggesting that macronutrients play a key role in HDL remodeling in the postprandial state. Collectively, our findings demonstrate HDL remodeling in the postprandial state and indicate that dietary macronutrients influence HDL composition and, therefore, may affect HDL antiatherogenic properties.

As expected, a transient increase in plasma triglyceride level was seen after the high saturated fat meal but not the high-carbohydrate meal, confirming the anticipated lipemic effect of dietary fat intake. This increase in plasma triglyceride level further was associated with changes in HDL total lipid and triglyceride content as well as phospholipid species

profile. Thus, an increase in the relative content of total HDL lipid was evident at 3 hours reaching statistical significance at 6 hours postprandial, and HDL was enriched in triglycerides and phospholipids at 6 hours postprandial. In contrast, after the high carbohydrate meal, the relative content of HDL total lipid and protein content remained stable in the absence of a postprandial increase in plasma triglycerides. These findings thus support lipemia-associated HDL remodeling and could have implications for HDL function. For example, postprandial lipemia in subjects with familial combined hyperlipidemia was shown to associate with enhanced capacity of HDL to efflux cholesterol from peripheral cells, but this efflux was followed by preferential transfer of HDL cholesterol ester to TRLs rather than delivery for hepatic uptake⁴⁴. Therefore, despite an increase in HDL efflux capacity, postprandial lipemia in these subjects associated with an apparent reduction in HDL's anti-atherogenic function.

Diet-mediated effects on HDL protein cargo are not well understood. In obese mice, short-term changes in fasted HDL protein composition have been demonstrated consequent to dietary saturated fatty acid consumption⁴⁵. In humans, only a few studies have examined changes in the fasted HDL proteome consequent to dietary interventions^{46, 47}, and no studies to date have examined the effects of dietary macronutrient on HDL protein cargo in the postprandial state. Our findings show that post-prandial lipemia induces only small changes in postprandial HDL protein composition within 6 hours of meal ingestion. HDL apoA-I enrichment after the high saturated fat meal is consistent with previous *in vitro* findings of apoA-I transfer from chylomicrons to HDL⁴⁸. However, our findings are discordant with the prior observation of increases in serum apoA-I concentrations after fat ingestion³⁴, as we found increases specifically in HDL apoA-I but not plasma apoA-I concentration. PLTP is a key protein involved in lipoprotein metabolism that mediates remodeling of triglyceride-rich lipoproteins to HDL⁴⁹, and relative enrichment of HDL PLTP after the high-fat meal may be linked to the greater HDL remodeling observed consequent to fat ingestion. Higher PLTP activity has been identified in populations at increased risk for atherosclerosis, including those with diabetes, obesity and metabolic syndrome⁵⁰ and generally is thought to exert pro-atherogenic effects variably ascribed to increased plasma VLDL and triglyceride levels, decreased plasma HDL-C levels, and changes in the antioxidant and immunomodulatory functions of HDL⁵⁰⁻⁵². HDL apoL1 and apoH both have described roles in host defense against parasite infections, and apoH has also been shown to function as a scavenger of lipopolysaccharide, as well as apoptotic cells, oxidized lipoproteins, and superoxide radicals⁵³. Postprandial differences in these HDL proteins therefore could confer functional changes in HDL particles. Overall, our findings suggest that only small changes in HDL protein composition are evident up to 6 hours after a meal, irrespective of meal macronutrient content.

Several clinical studies have investigated changes in HDL phospholipid and sphingolipid composition consequent to short-term dietary interventions, but these have only reported fasted HDL composition⁵⁴⁻⁵⁶. Postprandial changes in the plasma lipidome were studied after a dairy- or soy-based meal in healthy men and showed markedly different patterns of changes in plasma phospholipids despite similar excursions in plasma triglyceride levels following both meals⁵⁷. Furthermore, previous observations showed that fat ingestion leads to HDL phospholipid enrichment⁵⁸⁻⁶⁰; however, no clinical study to date has examined

postprandial changes in HDL lipid composition with discrete measurement of individual phospholipid and sphingolipid species. In the current study, phosphatidylcholine species were almost uniformly increased after the high saturated fat meal but remained unchanged or decreased after the high carbohydrate meal. In contrast, less pronounced increases in HDL ceramide species were observed, and only one sphingomyelin species was elevated after the high saturated fat meal. Thus, our study is the first to demonstrate that this phospholipid enrichment predominantly reflects increases in phosphatidylcholine species. The implications of these post-lipemia changes in HDL composition for both HDL function and CV risk remain uncertain, but, interestingly, in several studies HDL phosphatidylcholine content has been shown to correlate with reduced CV risk and improved HDL function^{31, 61, 62}. Further suggesting that HDL phosphatidylcholine may play an anti-atherogenic role, reduced HDL phosphatidylcholine content was found among subjects with premature coronary artery disease despite elevated plasma levels of HDL-C³¹. Moreover, a post-lipemia delay in HDL phospholipid enrichment was found in subjects with coronary artery disease, indicating that this HDL remodeling is intrinsic to a normal, healthy response to fat ingestion⁶³. While the underlying mechanisms remain speculative, HDL phosphatidylcholine composition has been shown to influence hepatic uptake of HDL lipid cargo⁶⁴, and HDL enrichment in phosphatidylcholine content promoted scavenger receptor-BI (SR-BI)-mediated cholesterol efflux³², findings consistent with enhanced reverse cholesterol transport.

Collectively, the species-specific increased phospholipid content of HDL together with an absence of change in HDL cholesterol and minimal remodeling of the HDL proteome is consistent with a proposed model of TRL remodeling as a major contributor to HDL biogenesis⁶⁵. Our findings were limited by exclusive measurement of HDL phosphatidylcholine among HDL phospholipids. However, HDL phosphatidylcholine species are the most abundant of HDL lipids and an order of magnitude more abundant than any other HDL phospholipid⁶⁶. Nonetheless, future work also will include measurement of additional HDL phospholipids including phosphatidylserine species, which similarly have shown strong correlations with protective, anti-atherogenic HDL functions^{67,68}. Among HDL sphingolipids, sphingomyelin C14 and ceramides also increased after the high saturated fat meal. HDL sphingomyelin has been implicated in the regulation of HDL membrane fluidity and cholesterol efflux, as well as the regulation of key enzymes involved in HDL remodeling⁶⁹. Thus, observed postprandial changes in the HDL lipidome suggest multiple pathways through which fat ingestion could influence HDL's antiatherogenic functions.

The mechanisms underlying these observed changes in HDL composition are unknown. Among the primary limitations of our study are the small sample size and collection of data at only 2 postprandial timepoints, especially considering the marked variation in postprandial lipid metabolism. Our study population included only apparently healthy, predominately Caucasian, subjects and thus future work will need to include participants with underlying cardiometabolic disease as well as those representing broader diversity in race/ethnicity to determine how these parameters might alter postprandial HDL. Previously subjects with familial combined hyperlipidemia were found to exhibit enhanced postprandial flux of cholesterol esters from HDL to TRLs relative to normolipidemic controls¹⁰ and

circulating HDL-C and triglyceride levels were shown to vary as a function of race/ethnicity^{70,71}.

Variation in postprandial lipid metabolism is determined in part by variation in activity of lipolytic enzymes (lipoprotein, hepatic, or endothelial lipases) as well as lipoprotein remodeling enzymes (LCAT, PLTP, CETP) that play a key role in linking compositional changes in TRLs and HDL. Although we measured protein levels of several of these enzymes, their activity may not be directly reflected by protein levels, and direct measurement of their enzymatic activity would be beneficial in future studies. Additional work will be required to establish role of these enzymes as well as the potential roles of insulin, incretins, and other signals released in the postprandial state in the regulation of HDL composition. Finally, because HDL comprises a heterogeneous mixture of particles, either subfractionation of HDL into HDL2 and HDL3 or direct measurement of HDL particle subpopulations by NMR or differential ion mobility analysis may provide further information about HDL particle remodeling during postprandial lipemia.

The high fat meal was predominantly composed of saturated fatty acids, and it is unknown what changes in HDL composition are related to the general lipemic state or specific to the fatty acid composition of the test meal. Prior research suggests that polyunsaturated fatty acids, such as omega-3 fatty acids, may influence HDL remodeling⁷², and it is plausible that a different mixture of fatty acids could elicit different changes from those observed in the current study. Future studies also will need to address the possible effects of dietary protein and polysaccharide on postprandial HDL remodeling, as these have been shown to influence HDL composition in rodent studies⁶⁹. The current findings demonstrate compositional changes in HDL after ingestion of a single meal, and the effects of repeated meals mimicking normal daily food intake also warrant future exploration. This 'second meal effect' results in progressive increments in plasma triglyceride levels over the course of the day, and, the macronutrient content of an initial meal has been shown to influence postprandial glucose and triglyceride clearance after subsequent meals⁷³. Finally, the meals used in this study do not reflect typical dietary patterns, as they were chosen to elicit robust responses specific to carbohydrate or saturated fat intake. Thus, further work is critical to determine the impact of macronutrients and other dietary constituents on HDL composition and function over the course of repeated meal ingestion and using meals representative of typical dietary patterns.

In summary, our findings indicate that meal macronutrient content acutely regulates HDL composition in the postprandial state. After a high saturated fat but not high carbohydrate meal, HDL enrichment in phospholipids is evident whereas HDL protein cargo is only minimally affected. These findings therefore suggest that the macronutrient content of meals could play a direct role in influencing HDL function and thereby may influence atherogenesis and CV risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1: Meal Macronutrient composition changes the relative composition of HDL particles. Relative proportions (% total HDL particle mass) of protein (a), total lipid (b), free (c) and esterified cholesterol (d), triglycerides (e) and phospholipids (f) in HDL particles were determined in the fasted state and 3 and 6 hours after a high saturated fat or high carbohydrate test meal. Open circles (O) represent the high saturated fat meal, and the closed squares (■) represent the high carbohydrate meal. Values are shown as mean $\pm$ SD. * $P < 0.05$ for comparison of postprandial values compared to fasted values for the specific diet using linear mixed effects model with subject ID as a random factor and time as the fixed factor, each diet analyzed separately. # $P < 0.05$ for comparison of the change from baseline to specific timepoint between test meals using linear mixed effects model with subject as a random factor, diet and baseline values as fixed factors, each time point analyzed separately. All significance shown is at 0.05 FDR.

Figure 2: Significant protein changes after test meals.

Percent change from fasted levels for all proteins that show significant changes in the linear mixed effects model. Apolipoprotein A-I (apoA-I) significantly changed from baseline to 6 hours after the high saturated fat meal. Apolipoprotein H (apoH), apolipoprotein L1 (apoL1), and phospholipid transfer protein (PLTP) all showed significant differences between diets 3 hours following the test meal.

Open bars represent the high carbohydrate test meal and dark bars represent the high saturated fat test meal, at 3 hours and 6 hours after each test meal, respectively. %Change is calculated as the difference between the postprandial time point and fasted, divided by the fasted values (E.g. $[(3\text{hr}-0\text{hr})/0\text{hr}]*100$). Boxplots show median, interquartile range, and minimum/maximum values. * $P<0.05$ for comparison of postprandial values to the fasted values for the specified diet using linear mixed effects model with subject ID as a random factor and time as the fixed factor, each diet analyzed separately. # $P<0.05$ for comparison of the change from baseline to specific timepoint between meals using linear mixed effects model with subject as a random factor, diet and baseline values as fixed factors, each time point analyzed separately. All significance shown is at 0.05 FDR.

A. Phosphatidylcholines

B. Ceramides and Sphingomyelins

Figure 3: Effects of high saturated fat meal on postprandial HDL phosphatidylcholine, sphingomyelin, ceramide, and lactosylceramide levels.

Data show the percent change from fasted in HDL-associated lipid species measured in isolated HDL, with significant changes in a linear mixed effects model examining the effect of time on a) phosphatidylcholine species and b) sphingolipids. Cer = ceramide, SM=sphingomyelin, LacCer= lactosyl-ceramide. Open bars - high carbohydrate meal, black bars – high saturated fat meal. %Change is calculated as the difference between the postprandial time point and fasted, divided by the fasted values (E.g. [(3hr-0hr)/0hr]*100). Boxplots show median, interquartile range, and minimum/maximum values. * $P < 0.05$ for the comparison of postprandial values to the fasted values for the specified diet using linear mixed effects model with subject ID as a random factor and time as the fixed factor, each diet analyzed separately. # $P < 0.05$ comparison of the change from baseline to specific timepoint between meals using linear mixed effects model with subject as a random factor,

diet and baseline values as fixed factors, each time point analyzed separately. All significance shown is at 0.05 FDR.

Table 1.**Participant Demographics**

Age (Mean $\pm$ SD)	34 $\pm$ 15
BMI kg/m ² (Mean $\pm$ SD)	24.1 $\pm$ 2.7
20% TEE kcals (Mean $\pm$ SD)	476 $\pm$ 71
Gender	
Male (N, %)	6 (40%)
Female (N, %)	9 (60%)
Race/Ethnicity	
Caucasian (N, %)	12 (80%)
African American (N, %)	2 (13%)
Hispanic (N, %)	1 (7%)

BMI: Body Mass Index, TEE: Total Energy Expenditure

Table 2:

Fasted and postprandial plasma lipid and apolipoprotein levels.

Plasma Lipid	High Carbohydrate Meal			High Saturated Fat Meal		
	Fasted	3 hours	6 hours	Fasted	3 hours	6 hours
Triglycerides (mg/dL)	70 (51-89)	59 (33-86)[#]	78 (52-103)	68 (50-85)	104(73-134)^{##}	75 (54-97)
Total Cholesterol (md/dL)	158 (140-175)	156 (139-174)	155 (138-172)	157 (140-174)	156 (139-174)	159 (141-176)
LDL-cholesterol (mg/dL)	88 (72-104)	87 (72-103)	87 (72-101)	87 (71-103)	86 (70-102)	88 (72-104)
HDL-cholesterol (mg/dL)	51 (46-56)	51 (46-55)	49 (44-54)	50 (43-56)	48 (43-55)[*]	49 (43-55)
ApoA-I (mg/dL)	151 (140-162)	149 (138-159)	148 (136-159)	146 (133-158)	144 (131-157)	144 (132-157)
ApoB (mg/dL)	72 (61-82)	71 (59-82)	71 (60-81)	70 (59-81)	72 (60-84)	70 (59-81)

Data shown as geometric mean $\pm$ 95% CI; 3 and 6 hours refer to the time after the start of the meal. Significant values are in bold.

^{*} $P < 0.05$ comparison of postprandial values compared to fasted values for the specified diet, linear mixed effects model with subject ID as a random factor and time as the fixed factor, each diet run separately.

[#] $P < 0.05$ comparison of the change from baseline between meals at the specified time, linear mixed effects model with subject as a random factor, diet and baseline values as fixed factors, each time point run separately.

All significance shown is at 0.05 FDR.