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The HDL lipidome is widely remodeled by fast food vs. Mediterranean diet in four days

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Abbreviations: PL: phospholipid, PC: phosphatidylcholine, PE: phosphatidylethanolamine, LPC: lysophosphatidylcholine, LPE: lysophosphatidylethanolamine, SM: sphingomyelin, Cer: ceramide, CE: cholesteryl ester, FC: free cholesterol, TG: triacylglycerol, DG: diacylglycerol, OCFA: odd chain fatty acid, CVD: cardiovascular disease, T2D: type 2 Diabetes, FF: fast food, Med: Mediterranean, EOD_{18}: equivalent of double bonds per 18 carbons, ACL: average carbon chain length

ABSTRACT

Introduction: HDL is associated with increased longevity and protection from multiple chronic diseases. The major HDL protein ApoA-I has a half-life of about 4 days, however, the effects of diet on the composition of HDL particles at this time scale have not been studied. **Objectives:** The objective of this study is to investigate the short term dietary effect on HDL lipidomic composition. **Methods:** In this randomized order cross-over study, ten healthy subjects consumed a Mediterranean (Med) and a fast food (FF) diet for 4 days, with a 4-day wash-out between treatments. Lipidomic composition was analyzed in isolated HDL fractions by an untargeted LC-MS method with 15 internal standards. **Results:** HDL PE content was increased by FF diet, and 41 out of 170 lipid species were differentially affected by diet. Saturated fatty acids (FA) and odd chain FA were enriched after FF diet, while very-long chain FA and unsaturated FA were enriched after Med diet. The composition of PC, TG and CE were significantly altered to reflect the FA composition of the diet whereas the composition of SM and ceramides were generally unaffected. **Conclusion:** Results from this study indicate that the HDL lipidome is widely remodeled within 4 days of diet change and that certain lipid classes are more sensitive markers of diet whereas other lipid classes are better indicators of non-dietary factors.

Keywords: High-Density Lipoprotein; Lipidomics; Fast Food Diet; Mediterranean Diet

1 INTRODUCTION

Clinical and epidemiologic studies have uniformly demonstrated that cardiovascular disease (CVD) risk is independently associated with both high concentrations of LDL-C and low concentrations of HDL-C (Assmann and Gotto 2004; Barter et al. 2007). Particularly in patients with obesity and/or metabolic syndrome (MetS) low HDL-C is an independent risk factor for CVD (Bays et al. 2013). There is a strong body of evidence indicating that HDL-C concentrations are protective against CVD across populations, however, some recent trials show no benefit or even negative associations with higher HDL-C (reviewed in (März et al. 2017)), and pharmaceutical interventions to increase HDL-C initially failed to demonstrate benefit in cardiovascular endpoints despite raising HDL-C concentrations (Investigators 2011; Schwartz et al. 2012) though subsequent trials with improved drugs showed a benefit (Group et al. 2017). HDL particles are heterogeneous, with multiple subclasses and biologic functions, and undergo significant remodeling in vivo (Asztalos et al. 2011). In fact, chemical, compositional, and structural changes can transform atheroprotective HDL into pro-atherogenic, pro-inflammatory particles (Ansell et al. 2007). Focus has shifted toward assessing the impact of various interventions on the composition and function of HDL particles and not just the absolute concentration of HDL-C in the blood.

Lipidomics studies have been performed to elucidate the molecular basis of HDL and its role in diseases including type 2 diabetes (T2D), dyslipidemia, and CVD (Camont et al. 2013; Pruzanski et al. 2000; Ståhlman et al. 2013). Dietary factors have been shown to affect HDL composition and function; moderate carbohydrate restriction with egg consumption improved HDL cholesterol efflux capacity (Andersen et al. 2013), and fruits and vegetables improved whereas saturated fat and refined carbohydrates reduced HDL anti-inflammatory capacity (reviewed in (Andersen and Fernandez 2013)). Although different HDL protein and lipid components have different turn-over rates, the half-life of ApoA-I, the main protein constituent of HDL, is approximately 4 days (Thompson et al. 1988). The effects of dietary changes on HDL lipidomic composition at this time scale have not been studied. Short-term dietary interventions could potentially be useful as clinical tools to assess individual responsiveness to diet. In addition, if substantial changes occur to the HDL lipidome within this short time frame of several days in response to diet, it is imperative to assess the extent of this influence to inform how frequently and closely diet must be controlled and monitored in longer-term intervention studies to minimize confounding effects.

In this pilot study, we hypothesized that a 4-day dietary intervention period measurably alters HDL lipidomic composition. We investigated the short-term effects of two different dietary patterns. The fast food (FF) diet, or the

Western diet, which is enriched in red meat, simple sugars, fat, saturated fat, and cholesterol, and low in fresh fruits, vegetables and fiber (Bahadoran et al. 2012; Deng et al. 2017; Garcia-Arellano et al. 2015; Nettleton et al. 2006; O'Neil et al. 2015), was compared to the Mediterranean (Med) diet, which is enriched in fresh fruits, vegetables, fiber, monounsaturated and polyunsaturated fat, particularly omega-3 fatty acids (Estruch 2010; Estruch et al. 2013; Martínez-Lapiscina et al. 2013; Salas-Salvadó et al. 2011, 2008; Serra-Majem et al. 2006). HDL lipidomic composition in response to the two dietary patterns was assessed and compared.

2 MATERIALS AND METHODS

2.1 Subjects

Ten healthy human subjects (5 male and 5 female) were recruited from the community in Davis, CA. Subjects were 18-25 years old, non-smokers, with BMI 21.2-32.9 kg/m^2, and currently consuming fast food 3 times per week or less. Subjects with anemia, diabetes, thyroid disease, MetS, cancer, previous cardiovascular events or other disease diagnoses were excluded. Subjects were also excluded if they had extreme dietary or exercise patterns, or were taking prescription medications or other supplements known to alter lipoprotein metabolism such as isoflavones. The study was approved by the University of California Davis Institutional Review Board and the study followed all of the ethical standards of the Helsinki declaration. The study is registered at clinicaltrials.gov under identifier NCT03205254.

2.2 Study design

Subjects were phone screened, consented, and enrolled in the study if they met all inclusion and exclusion criteria. In this randomized, cross-over study, each subject was randomized to intervention order using a randomized block design to either start on the FF or Med diet and cross over to the Med or FF diet, respectively, after the washout period. All treatment periods were 4 days in duration. All study food was provided to the subjects, either by purchasing from a local grocery store (Med) or fast food restaurants (FF). The study dietary plan was designed to match each subject's daily Calorie requirement based on the Harris-Benedict equation. Subjects were asked to keep their normal physical activity during study periods.

Anthropometric measurements, including height, weight, blood pressure, hip and waist circumference were taken at the first and last day of each study arm. A blood sample was collected by a licensed phlebotomist from the antecubital vein after an overnight fast on the first and the day after the last day of each study arm, and plasma or serum was separated within 1 hour of the blood draw. An aliquot of sample was sent to the University of California Davis Medical Center (UCDMC) for a lipid panel test, while the rest of the samples were immediately aliquoted and stored at -80°C before analysis.

2.3 Diet

Breakfast was provided to the subjects on the FF arm, while for lunch and dinner, subjects were instructed on exactly what to purchase from a local fast food restaurant. On the Med arm, ingredients for all three meals were purchased from a local grocery store, portioned by the study team, and picked up by subjects. Subjects were asked to

return to their normal diet during washout. On the FF arm, 1-2 frosted strawberry pop-tarts were given for breakfast, and different hamburgers with or without fries were assigned to subjects for lunch and dinner. The sizes of hamburgers and fries were assigned according to the calorie levels. Subjects also consumed soda ad libitum as part of their meals. On the Med arm, high fiber cereal in 1% milk with one small banana was given for breakfast. Lunch was made of a study salad with dressing and 1-2 servings of canned no salt tuna or chicken, while dinner was made of 1-2 servings of minestrone soup, 1-2 servings of multigrain blend, 1 serving of tomato basil marinara, and extra virgin olive oil (EVOO) adjusted to the calorie level. Almonds and other dried fruits and nuts were provided as snack between meals according to the prescribed Calorie level. The study diet menu for FF and Med at 2000 kcals/day level is shown in **Supplemental Table S1 and S2**.

2.4 HDL isolation

HDL particles were isolated using a 2-step density based sequential flotation ultracentrifugation method modified from a previous study (Krishnan et al. 2017). Potassium Bromide (KBr) solutions of density 1.063 g/mL, 1.210 g/mL, and 1.340 g/mL were freshly prepared and verified using a portable densitometer (Mettler Toledo, Columbus, OH). For each sample 2.0 mL of plasma was adjusted to a density of 1.063 g/mL by adding concentrated KBr solution (d=1.340 g/mL). Adjusted plasma was then underlaid to KBr solution of 1.063 g/mL in an ultracentrifugation tube (OptiSeal, Beckman Coulter), followed by ultracentrifugation at 110,000 rpm for 3 hours and 10 minutes. Ultracentrifugation was performed on a Beckman Optima MAX-TL equipped with a TLA-110 fixed-angle rotor (Beckman Coulter) with a k factor of 13.04. The 2-mL supernatant containing chylomicrons, remnants, VLDL and LDL was removed, and the bottom layer containing the HDL fraction was further adjusted to a density of 1.21 g/mL by adding concentrated KBr solution (d=1.340 g/mL). The adjusted fraction was then underlaid to KBr solution of 1.21 g/mL in two separate ultracentrifugation tubes, followed by ultracentrifugation at 110,000 rpm for 3 hours and 20 minutes. One mL supernatant of HDL fraction from each tube was combined and dialyzed using Amicon Ultra-4, MWCO 10 kDa filter devices twice to remove the KBr. HDL was reconstituted in LC-MS water and kept at -80°C until analysis.

2.5 HDL lipidomics

HDL complex lipids, including PC (phosphatidylcholine), PE (phosphatidylethanolamine), PG (lysophosphatidylcholine), LPE (lysophosphatidylethanolamine), SM (sphingomyelin), FA (fatty acid), TG (triacylglycerol), DG (diacylglycerol), MG (monoacylglycerol), FC (free cholesterol), and CE (cholesteryl ester)

were measured at the West Coast Metabolomics Center, using the protocol described elsewhere (Cajka et al. 2016). 225 µL of cold methanol containing lipid internal standards (PE(17:0/17:0), PG(17:0/17:0), LPC(17:0), C17 sphingosine, C17 ceramide, SM(d18:1/17:0), palmitic acid (d3), PC(12:0/13:0), cholesterol (d7), TG(17:0/17:1/17:0) d5, DG(12:0/12:0), DG(18:1/2:0), MG(17:0), and LPE(17:1)) were added into 25 µL purified HDL sample, followed by adding 750 µL cold MTBE containing CE(22:1). After shaking at 4 °C for 6 minutes, 188 µL of distilled water was added, and the sample was centrifuged at 14,000 g for 2 minutes. 350 µL supernatant was extracted, dried down, and reconstituted with 65 µL methanol/toluene (9:1, v/v) solution. 3 µL of the reconstituted sample was then injected into a LCMS for analysis. Each sample was injected in parallel into an Agilent 6530 QTOF with positive mode, and an Agilent 6550 QTOF with negative mode, with the purpose of capturing as many complex lipid species as possible. LC separation was done on a Waters UPLC CSH C18 column (1.7 µm, 2.1 mm 100 mm), using a gradient method. A QC (quality control) sample was run every 11th injection. The QC samples all came from the same human plasma pool.

2.6 Lipidomics data processing

MS data was processed using MS-DIAL (Tsugawa et al. 2015). Lipid species were identified through 2 methods. Liquid chromatogram retention time and MS1 m/z was searched against the in house rt-mz library (Cajka et al. 2016). The fragmentation pattern in MS2 was searched against the in silico library LipidBlast (Kind et al. 2013). Lipid species identified using rt-mz library are MSI level 3, and the ones identified through fragmentation pattern searching are MSI level 2 (Sumner et al. 2007). This method is able to identify lipid species in 14 lipid classes, including PC, PE, LPC, LPE, PG, CE, sphingosine, ceramide, SM, FA, FC, TG, DG, and MG. Quantification was done using a single-point, class specific calibration curve using internal standards, as shown below (Cajka et al. 2016).

$$\mathrm{conc}_{i,j}=\frac{\mathrm{int}_{i,j}}{\mathrm{int}_{k,j}} \times \mathrm{conc}_{k}$$

Where the $\mathrm{conc}_{i,j}$ is the concentration of lipid i in the sample j. $\mathrm{int}_{i,j}$ is the intensity of lipid i in sample j. $\mathrm{int}_{i,k}$ is the intensity of internal standard of lipid class k detected in sample j. conc_{k} is the spiked concentration of internal standard for lipid class k.

2.7 Statistical Analysis

The HDL lipidomic data were transformed to a proportion (mg %) for each species.

$$\text{Proportion}_{i,j} = \frac{\text{conc}_{i,j}}{\sum_{i=1}^{n} \text{conc}_{i,j}}$$

The proportion of lipid species i in sample j equals to its concentration divided by the sum of the concentration of all the lipid species in sample j. The proportion of all species in the same lipid class were added up to get the proportion of each lipid class.

Lipidomics data were transformed and summarized to obtain the EOD_{18} (equivalent of double bond per 18 carbons) and ACL (average chain length). The EOD_{18} was calculated using the equation below:

$$EOD_{18} = \frac{\sum_{i=1}^{n} \text{conc}_{i,j} \times \text{ndb}_{i,j}}{\sum_{i=1}^{n} \text{conc}_{i,j} \times \text{nc}_{i,j}} \times 18$$

$\text{conc}_{i,j}$ is the mol concentration of the j^{th} lipid species in i^{th} sample. $\text{ndb}_{i,j}$ is the number of double bonds of this lipid species, while $\text{nc}_{i,j}$ is the number of carbons. The ACL is calculated using the equation below:

$$ACL = \frac{\sum_{i=1}^{n} \text{conc}_{i,j} \times \text{nc}_{i}}{\sum_{i=1}^{n} \text{conc}_{i,j} \times \text{nfa}_{i}}$$

$\text{nfa}_{i,j}$ represents the number of fatty acids for the i^{th} lipid species in the j^{th} sample (for example, a PC or a DG will be 2, and a TG will be 3).

Several lipid class mole ratios were calculated including PC/LPC, CE/FC, SM/PL, and surface/core lipids. Surface lipids are amphipathic lipid classes including PC, PE, SM, Cer, LPC, cholesterol, and DG, while core lipids are hydrophobic lipids CE and TG. PL are total phospholipids, including PC, PE, SM, and LPC. Class specific OCFA (odd chain fatty acids) were calculated by summing up the mole concentration of all lipid species with odd number of carbons in the same lipid class.

A differential abundance test was applied to the normalized HDL lipidomic data using a mixed linear model, with the R package, limma (Ritchie et al. 2015). Multiple test correction was performed on the p-values using the Benjamini-Hochberg method. Data were log2 transformed before differential abundance test and the shapiro.test function in R was used to perform Shapiro-Wilk test of normality. The Pearson's correlation test was applied to find the correlation between different variables. Multiple testing correction was not applied to correlation analysis due to the exploratory nature of this analysis. Compound similarity Tanimoto coefficient was calculated using the R package fmcsR (Wang et al. 2013) and ChemmineR (Cao et al. 2008). The hierarchical clustering method was used to group lipid species that responded similarly across all samples. The hclust function in R's stats package was used,

175 followed by the cutree function setting the argument h equals to 8 to generate 32 clusters. The proportion data was z-
176 score normalized prior to clustering. PCA analysis was performed using the prcomp function in R.

3 RESULTS

3.1 Baseline Characteristics and Dietary Records

The baseline characteristics of the 10 subjects are listed in **Table 1**. All subjects were healthy with normal blood pressure and blood lipids levels. Neither the anthropometric nor the circulating lipid variables were significantly affected by the diets. The BMIs of all subjects were normal to slightly overweight. Summary data from the three-day diet records from baseline and during the two study treatments are listed in **Table 2**. The total calorie intake of each subject was relatively equivalent to their baseline level. The daily calorie intake at baseline was not significantly different from FF ($p = 0.506$) or Med ($p=0.277$). All subjects had significantly higher saturated fat ($p < 0.0001$), trans fat ($p < 0.0001$), protein ($p = 0.0005$) intake, and lower unsaturated fat ($p < 0.0001$) and fiber intake ($p < 0.0001$) on the FF diet arm compared to the Med diet **Table 2**. Carbohydrate intake was not significantly different between the two treatments.

3.2 HDL Lipidome

With the lipidomics method, 170 lipid species in 9 different lipid classes were detected in 78 or more samples, with 167 of them were detected in all samples. **Supplemental Table S3** lists all lipid species with their relative abundance before and after FF and Med, as well as their linear mixed model p-values. According to the quality control samples, the average coefficient of variation (CV) of all the lipid species was 11.43 (± 6.58) %, with 90% of the lipid species having a CV < 20% (**Fig. 1-A&B**). The average composition of isolated HDL from this population across diet treatments was 46.2% CE, 29.4% PC, 9.7% FC, 6.8% TG, 5.2% SM, and 2.2% PE. The rest of the lipid classes including LPC, DG, and Ceramides added up to 0.5% of the total lipids (**Fig. 1-C**). At the lipid class level, HDL PE was significantly ($p = 0.0003$) elevated after FF but not Med (**Fig. 1-D**). All 170 lipid species are presented in the cladogram in **Fig. 1-E**. In the cladogram, lipids were clustered based on their structural similarity using the Tanimoto coefficient, which measures the structural similarity between chemical compounds (Nikolova and Jaworska 2003). The cladogram also shows the change of each lipid species on FF and Med from baseline. It shows that more PC species increased after FF, and more TG species decreased after Med. The linear mixed model shows 65 out of 170 features were significantly and differently affected by FF and Med ($p < 0.05$, unadjusted, **Fig. 1-F**), and the significance remained for 41 lipid species after adjustment for multiple comparisons. The principal component analysis (PCA) and its loading plot shown in **Fig. 1-G&H** were drawn using the change from baseline on FF and Med diet of the 41 species with an adjusted p-value that was significant at $p < 0.05$. The

PCA plot shows the lipidome changed differently on the FF compared with the Med diet, while the loading plots show that different lipid species were enriched on the two diets.

The changes of the 41 lipids species after FF and Med diet from baseline are presented in **Fig. 2-A**. Using the hierarchical clustering method, lipid species that showed similar response across study subjects and treatments clustered together (**Fig. 2-B**). The clustering method was able to form 32 clusters from the 170 lipid species, and 9 clusters were significantly altered after FF versus Med diet ($p < 0.05$, adjusted). PCs with long chain fatty acids (C16-20) and less double bonds were either elevated after FF or decreased after Med, including PC 30:0, PC 32:2, PC 34:0, 34:4, PC 36:2, and PC 36:4 (cluster 6, 8, and 28). PCs with very long chain fatty acids (C20-22) tended to increase after Med but not FF, including PC 40:6 and PC 40:7, which have more double bonds than the PC species that were elevated after FF (cluster 11). Plasmenyl PCs tended to increase after FF rather than Med, including PC 34:1 p, PC 34:2 p, PC 36:2 p, and PC 38:4 p (cluster 12). CE species were elevated after Med but not FF, including CE 18:1, CE 20:4, and CE 22:6 (cluster 20). SM species SM 34:2 and SM 42:3 (cluster 1) were decreased after FF and increased after Med. Several PE and plasmenyl PE species were also elevated after FF but not Med, including PE 36:2 p, PE 34:2 p, and PE 38:4 p (cluster 12).

3.3 Lipidome Characteristics

In order to better understand the HDL lipidome, two descriptive variables, EOD_{18} and ACL, were calculated from the lipidomic data as described above. The overall EOD_{18} of the lipidome was 1.70 double bonds per 18 carbons, and the overall ACL was 18.14. Both the EOD_{18} ($p < 0.001$, adjusted) and ACL ($p = 0.001$, adjusted) of PC significantly decreased after FF and increased after Med diet (**Fig. 3-A&B**). CE had the highest EOD_{18} (2.37) at the baseline level, followed by PE (2.00), and PC (1.44), while TG, SM, Ceramide, and LPC have very small EOD_{18} (0.98, 0.74, 0.50, and 0.61) which suggests saturated fatty acids dominate these lipid classes. Lipid classes with a high EOD_{18}, including PE and CE, were significantly affected by the dietary interventions ($p < 0.001$ & $p = 0.003$, adjusted) as their double bonds decreased after FF and increased after Med. Overall TG, SM, Ceramide, and LPC have small EOD_{18}, and they were not significantly affected by either dietary treatment. The overall EOD_{18} also decreased after FF and increased after Med. PE, PC, CE, and overall EOD_{18} were also significantly correlated with dietary MUFA (PE: $p < 0.001$, $R = 0.796$; PC: $p < 0.001$, $R = 0.651$; CE: $p = 0.017$, $R = 0.431$; Overall: $p = 0.037$, $R = 0.383$. All p-values were unadjusted and all R values are Spearman's correlation coefficients) and PUFA intake (PE: $p < 0.001$, $R = 0.812$; PC: $p < 0.001$, $R = 0.700$; CE: $p = 0.014$, $R = 0.443$; Overall: $p = 0.025$, $R = 0.408$.).

Ceramides had the longest carbon chain length (19.06) at baseline, followed by PE (18.54), SM (18.53), CE (18.50), while DG, TG and LPC had less very long chain lipids (ACL=18.00, 17.35, and 17.12). The ACL of CE, PE, PC, TG and overall ACL were significantly altered by dietary interventions ($p < 0.001$, $p = 0.001$, 0.0012, and 0.0013, adjusted) as the fatty acyl length were decreased after FF and increased after Med.

Four lipid classes were detected with lipid species containing OCFAs (odd chain fatty acid), PC, SM, TG, and Cer. The OCFAs carried by PC and SM were at a similar level, but SM had the highest relative proportion of OCFAs (8.41%), followed by Cer (4.67%), TG (1.95%), while PC (1.55%) had the lowest relative proportion. PC OCFAs were significantly increased after FF and decreased after Med diet ($p < 0.001$, **Fig. 3-C**). OCFAs in SM, TG, and Cer were not significantly affected by the diets (**Fig. 3-D**).

HDL surface lipids include all amphipathic phospholipids, sphingolipids, FC, mono- and di-acylglycerols. PC/LPC ratio was negatively associated with CE/FC ratio (rho = -0.506, $p = 0.001$, **Supplemental Fig. S1-A**). CE/FC ratio had a very high negative correlation with surface to core lipids ratio (rho = -0.846, $p < 0.001$, **Supplemental Fig. S1-B**).

4 DISCUSSION

The Med diet was chosen for this study because it is known to improve cardiometabolic health and decrease disease risk from both epidemiological and intervention studies (De Lorgeril et al. 1999; Esposito et al. 2004; Estruch et al. 2013). The FF diet was chosen because according to the CDC increasing numbers of Americans are consuming convenience and fast foods on a daily basis (Smith et al. 2013), and observational studies have found this type of diet to be associated with increased markers of inflammation and disturbed lipid profiles (Nettleton et al. 2006). Although the term "fast food" can be used to describe many different kinds of foods that are prepared quickly, in this study, the term was used to refer to the most "classic" American fast food meal including a burger, fries, and a soft drink purchased at a fast food restaurant. Although the long-term impact of the two diets on cardiometabolic health and lipid profile are well documented, no research studies reported their short-term effects on a time-scale similar to our study. Only one study directly compared the effects of a fast food style diet to a traditional German diet to a Med diet for 2 weeks in 39 healthy human subjects, and found very little difference in effects on a variety of cardiometabolic health parameters including LDL-C, HDL-C, TG, and homocysteine (Parcina et al. 2015).

A few studies have specifically studied HDL lipidome composition at lipid species level. Weisner et al. developed a LCMS method that provided the pioneering reference to HDL lipidome on FPLC isolated HDL fractions (Wiesner et al. 2009). A study done by Andersen et al reported the PL, CE and free cholesterol proportion to be 46.0, 40.4, and 5.1 % (wt), respectively, in human subjects with MetS (Andersen et al. 2013), and Camont et al reported the three major HDL lipid classes in similar proportions (Camont et al. 2013). A study done by Sawrey-Kubicek et al. found reported more PC (36.3%), TG (13.9%) and SM (8.5%), and less CE (25.9%) in post-menopausal women (Sawrey-Kubicek et al. 2019). HDL lipidome composition was also reviewed by Kontush et al, with PC 33-45%, PE 0.5-1.5%, SM 5-10%, FC 5-10%, and CE 30-40% (Kontush et al. 2013). In our study, the SM (5.2%) and FC (9.7%) contents fall into the range reported by Kontush et al (Kontush et al. 2013). CE (46.2%) and PE (2.2%) contents were higher than reported, while PC (29.4%) was lower (Kontush et al. 2013). HDL lipid composition varies depending on the particle size, and large HDL particles have higher CE and lower PC (Kontush et al. 2013). It is possible that our study population, which included only young, healthy individuals, had more large HDL particles compared to populations from the previously published reports, thus more proportion of core lipids (e.g., CE) and less proportion of surface lipids (e.g., PC) were observed.

This is the first reported study investigating the impact of dietary change on the time scale of 4 days on HDL lipid composition in healthy human subjects. In a study examining the effects of a diet rich in n-3 fatty acids and polyphenols in overweight human subjects with high CVD risk, an increase in PC and TG enriched in n-3 FA was observed in both plasma and HDL after 8 weeks of intervention (Bondia-Pons et al. 2014). In a study that compared the HDL lipidome of T2D patients, T2D patients with dyslipidemia, and healthy subjects, HDL SM was significantly lower in T2D patients with dyslipidemia (Ståhlman et al. 2013). In the current study HDL SM was increased after the Med diet. Several TG species have previously been shown to be significantly higher in T2D patients with or without dyslipidemia compared to healthy controls (Ståhlman et al. 2013), and in our study were decreased after Med but not FF, including TG 48:1, TG 48:2, TG 48:3, and TG 52:1. In another study that looked at the long-term effect of fenofibrate therapy on HDL in CVD patients (Yetukuri et al. 2011), the plasmalogen species PE 40:6 p (plasmalogen) and PE 38:5 p were decreased and SM 32:1 was increased in patients on fenofibrate. In this current study, PE 40:6 p and PE 38:5 p increased after FF, but not Med, and SM 32:1 increased after Med, but not FF.

Since the FF diet was rich in SFAs and the Med diet was rich in MUFAs, FA in several classes, especially PC and PE became shorter and had less double bonds after FF and were longer and had more double bonds after Med. However the ACL and EOD_{18} of SM and ceramides were not affected by either treatment. In addition, although PC and SM carry similar amounts of OCFA, the OCFA content of PC but not SM increased after FF and decreased after Med. These observations suggests that compositional remodeling of phosphoglycerolipid FA is more sensitive to diet compared to phosphosphingolipids.

The strengths of this study include that all food intake during the study periods was provided and controlled; the consistent changes in specific lipid species across all subjects indicate that compliance with the diet was high; and a complex lipidomics method was applied to quantify each lipid species. A weakness of this study is the relatively small number of subjects. However, the study was a cross-over study and therefore each subject acted as their own control, increasing the power to detect changes in response to diet. Density-based ultracentrifugation is still considered the gold standard for HDL isolation. In this study our ultracentrifugation protocol was optimized to remove ApoB-containing lipoproteins and albumin as far as possible. However, the gel electrophoresis of 4 representative isolated HDL samples from this study (**Supplemental Fig. S2**) shows some albumin and ApoB contamination. A more refined purification strategy including size exclusion chromatography after the

ultracentrifugation step (Holzer et al. 2016), or other, typically more costly depletion methods, could have been used to eliminate the remaining ApoB and albumin.

In conclusion, 4-day dietary interventions feeding the FF diet and the Med diet differentially changed HDL lipidomic composition. Our results suggest that certain HDL lipids could be useful markers of short-term dietary intake (i.e. PC, PE, CE), whereas other HDL lipids (i.e. SM, ceramides) are not as responsive of short-term dietary change and may be more useful indicators of long-term intake as well as non-dietary factors and disease conditions. Our study focused on HDL, hence it is not clear whether a similar pattern would be observed in the plasma lipidome. Future studies investigating the short-term dietary effects on the plasma lipidome would be useful for plasma-based diagnostics. These results have implications for studies comparing the HDL lipidome of individuals with different disease conditions vs. controls, or in response to different treatments. Our findings suggest that certain lipid classes are very sensitive to dietary changes in the short term, making them excellent markers of short-term dietary intake, but potentially not very good candidates for the discovery of biomarkers of disease or other non-diet factors.

5 ACKNOWLEDGEMENTS

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6 ETHICAL STATEMENTS

Conflict of interest: All authors declare that they have no conflict of interest.

Compliance with Ethical Standards: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

Author contribution: AZ conceived and designed the research. LSK, EB, RH and CR conducted the clinical study and participated in the design of the study. CZ and CR processed and analyzed the samples. RS contributed to the HDL isolation method and assisted in conducting the study. CZ and AZ analyzed the data. CZ and AZ wrote the manuscript. All authors read and approved the manuscript.

Informed consent: Informed consent was obtained from all individual participants included in the study.

Data availability statement: The datasets generated during and/or analyzed during the current study are available in the Metabolomics Workbench repository (study ID ST001151).

7 TABLES AND FIGURES

Table 1: Subject baseline characters (n=10)

Variable	**Baseline Value** mean (SD)
Height (m)	1.71 (0.09)
Weight (kg)	69.05 (12.99)
BMI (kg/m^2)	24.39 (3.71)
Age (yrs)	22.10 (2.33)
Systolic Blood Pressure	122.07 (14.27)
Diastolic Blood Pressure	79.00 (13.01)
Waist Circumference (cm)	76.63 (9.83)
Hip Circumference (cm)	99.88 (6.97)
Total Cholesterol (mg/dL)	163.60 (25.01)
HDL Cholesterol (mg/dL)	53.00 (13.56)
LDL Cholesterol (mg/dL)	94.80 (20.36)
Triglycerides (mg/dL)	80.30 (31.82)

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343 **Table 2**: Macronutrient composition of diet at baseline and after dietary treatment.

Nutrient Variable	**unit**	**Baseline** mean (SD)	**FF** mean (SD)	**Med** mean (SD)
Weight	gram	1606.54 (600.52)	1376.21 (627.01)	1777.64 (247.53)
Total Calories	kcal	2525.57 (951.08)	2686.86 (496.50)	2258.85 (360.68)
Protein	gram	102.41 (50.58)	89.93 (10.88)	105.18 (11.27)
Carbohydrates	gram	304.17 (128.84)	292.32 (86.82)	299.00 (40.02)
Fat	kcal	919.62 (531.25)	1190.24 (190.40) *	758.95 (179.11)
	gram	102.22 (59.03)	132.25 (21.16) *	85.44 (20.01)
Saturated Fat	kcal	301.68 (171.12)	402.05 (60.20) *	119.16 (24.77) ***
	gram	33.52 (19.01)	44.67 (6.69) *	13.24 (2.75) ***
Mono-unsaturated Fat	gram	24.07 (22.84)	1.89 (0.60) ***	41.28 (11.74) **
Poly-unsaturated Fat	gram	9.43 (6.04)	3.24 (1.03) ***	15.63 (4.68) **
Trans Fat	kcal	8.00 (9.60)	19.17 (3.93) ***	0.66 (0.10) ***
	gram	0.89 (1.07)	2.13 (0.44) **	0.07 (0.01) *
Cholesterol	mg	389.13 (328.15)	229.95 (28.63)	94.87 (17.53) ***
Sugar	gram	94.67 (36.62)	103.98 (68.00)	95.18 (16.64)
Fiber	gram	31.4 (36.74)	12.9 (2.78) **	58 (8.55) ***

344 * Significantly different from baseline ($p < 0.05$)

345 ** Significantly different from baseline ($p < 0.01$)

346 *** Significantly different from baseline ($p < 0.001$)

347

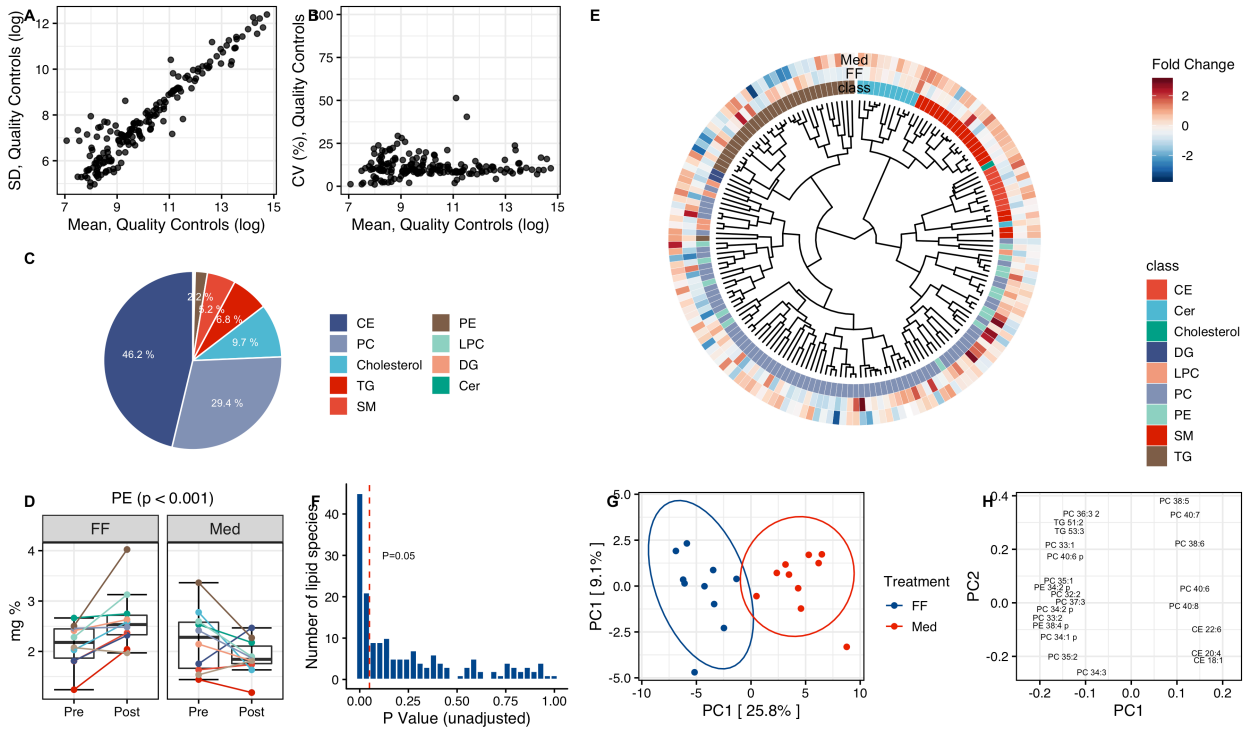

Fig. 1: A&B: The mean versus standard deviation (A), and the mean versus coefficient of variance (B) of lipid species detected in quality control samples. Each point represents a lipid species. **C:** A pie chart of HDL lipid classes (mg %). **D:** Box plots of PE in relative abundance (mg %) for subjects before and after FF and Med diets, with unadjusted P-values. Lines with the same color represent the same subjects. **E:** Cladogram of all 170 lipid species detected. The dendrogram was drawn by calculating the pairwise Tanimoto structural similarity coefficient between any two lipid species. The most inner layer color bar represents the corresponding lipid class of each tree tip. The two outer layers represent the fold change of the corresponding lipid species after FF or Med. **F:** Histogram of p-values (unadjusted) of each lipid species. The p-values were calculated using linear mixed model. **G&H:** PCA and loading plot of 44 selected lipid features with $p < 0.05$ after Benjamini-Hochberg adjustment.

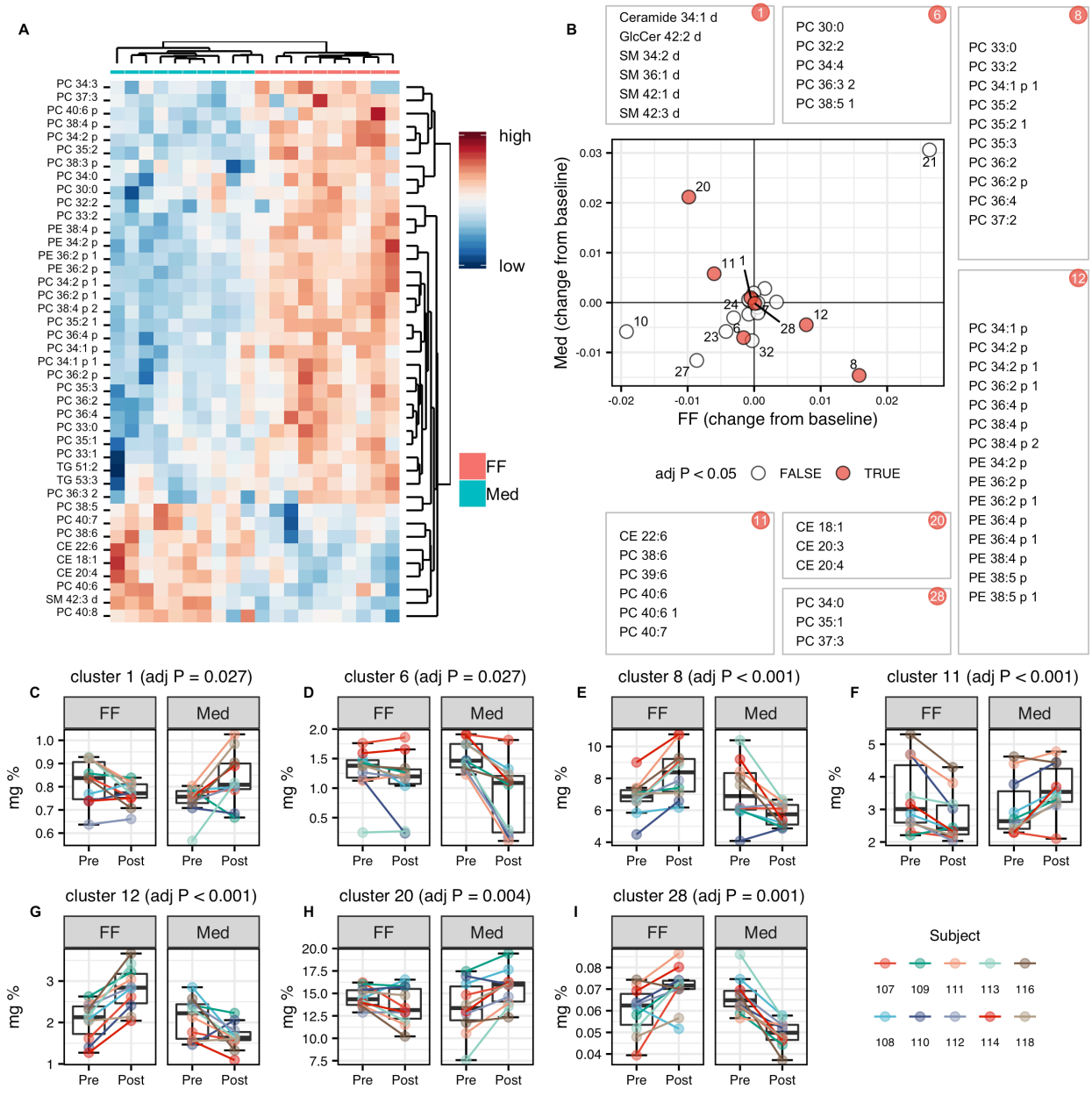

Fig. 2: A: Heatmap of the change (post - pre) of proportions of lipid species after FF and Med from baseline. The p-values of the 44 lipid species are less than 0.05 after Benjamini-Hochberg adjustment. **B:** Scatter plot of the change (post - pre) of lipid species clusters after FF versus Med. The clustering was preformed using hierarchical clustering, and lipid species within each cluster were aggregated to obtain a value for each cluster before and after FF and Med. **C-I:** Box plots of the relative abundance (mg %) of the 7 clusters with $p < 0.05$ after Benjamini-Hochberg adjustment. Lines with the same color represent the same subjects.

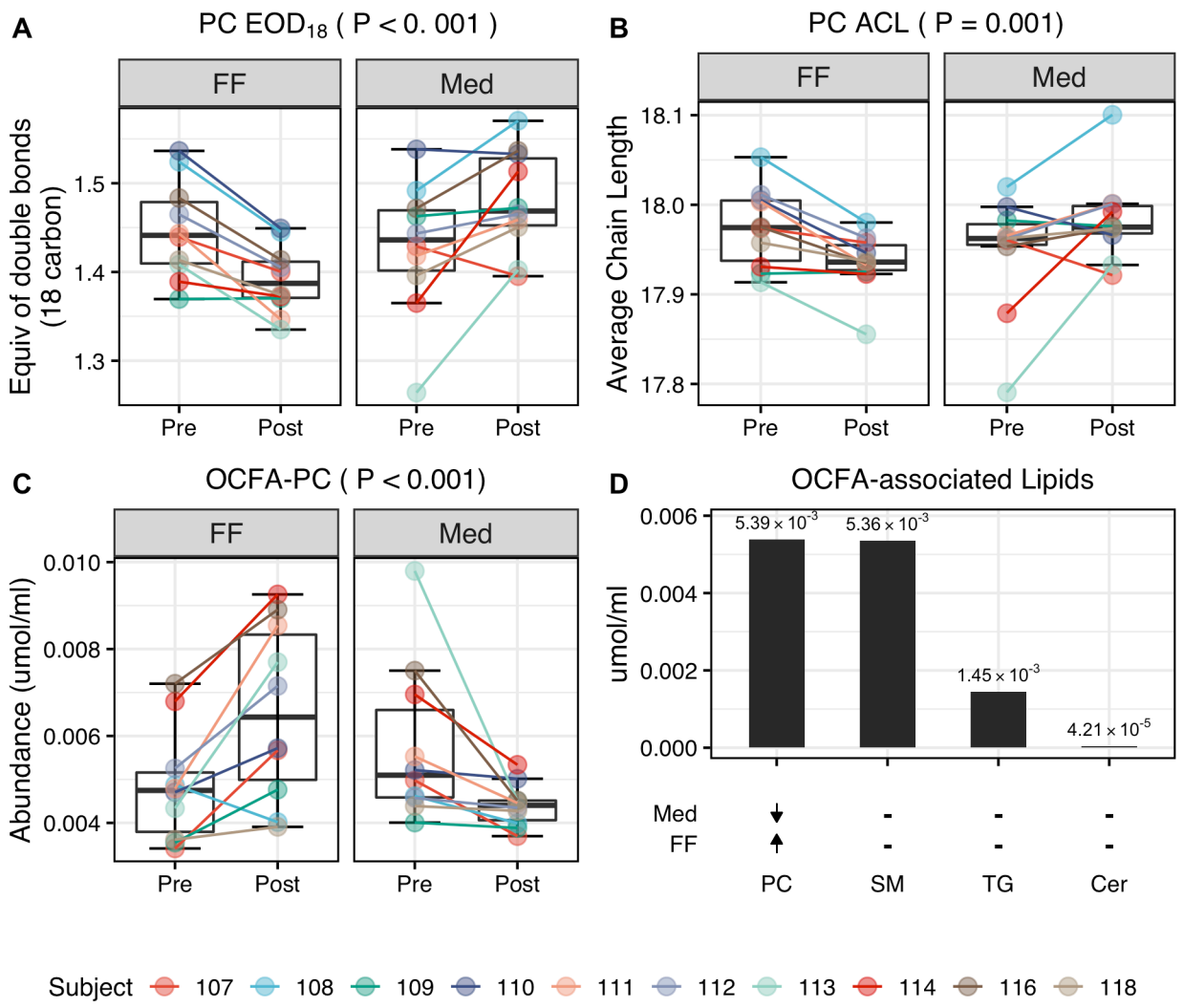

Fig. 3: A-C: Box plots of PC equivalent of double bonds (A), average chain length (B), and the mole concentration of OCFAs in PC (C) before and after FF and Med. **D:** Baseline mole concentration (µmol/ml) of OCFAs in PC, SM, TG, and Cer and how they responded to FF and Med.

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Supplemental Data:

The HDL lipidome is widely remodeled by fast food vs. Mediterranean diet in four days

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Supplemental Table S1: Study diet menu on the FF arm (2000 kcals/day).

Meal	Day 1 Carl's Jr	Day 2 Carl's Jr	Day 3 Carl's Jr	Day 4 Carl's Jrs
Breakfast	Frosted Strawberry Pop-tart	Frosted Strawberry Pop-tart	Frosted Strawberry Pop-tart	Frosted Strawberry Pop-tart
Lunch	Western Bacon Cheeseburger	Famous Star w/ Cheese	Western Bacon Cheeseburger	Famous Star w/ Cheese
Dinner	Teriyaki Burger Med order of fries	Super Bacon Thickburger	Teriyaki Burger Med order of fries	Super Bacon Thickburger

Supplemental Table S2: Study diet menu on the Med arm (2000 kcals/day).

Meal	Day 1	Day 2	Day 3	Day 4
Breakfast	1 serving Kashi GoLean Cereal (180 cal) + 50g Grape-Nuts (180 cal) + 1 cup 1% milk (102 cal) + 1 small banana (90 cal) (Total = 552 cal)	1 serving Kashi GoLean Cereal (180 cal) + 50g Grape-Nuts (180 cal) + 1 cup 1% milk (102 cal) + 1 small banana (90 cal) (Total = 552 cal)	1 serving Kashi GoLean Cereal (180 cal) + 50g Grape-Nuts (180 cal) + 1 cup 1% milk (102 cal) + 1 small banana (90 cal) (Total = 552 cal)	1 serving Kashi GoLean Cereal (180 cal) + 50g Grape-Nuts (180 cal) + 1 cup 1% milk (102 cal) + 1 small banana (90 cal) (Total = 552 cal)
Snack	1 individual packet Trek Mix (Omega) (170 cal) (Total = 170 cal)	1 individual packet Trek Mix (Omega) (170 cal) (Total = 170 cal)	1 individual packet Trek Mix (Omega) (170 cal) (Total = 170 cal)	1 individual packet Trek Mix (Omega) (170 cal) (Total = 170 cal)
Lunch	Study Salad 1 (258 cal) + Study Dressing (133 cal) + 1 serving Canned No Salt Tuna (70 cal) (Total: 461 cal)	Study Salad 1 (258 cal) + Study Dressing (133 cal) + 2 servings Canned No Salt Tuna (140 cal) (Total: 531 cal)	Study Salad 1 (258 cal) + Study Dressing (133 cal) + 1 serving Canned No Salt Tuna (70 cal) (Total: 461 cal)	Study Salad 1 (258 cal) + Study Dressing (133 cal) + 2 servings Canned No Salt Tuna (140 cal) (Total: 531 cal)
Snack	1 individual packet Trek Mix (Simply Almonds) (210 cal) (Total = 210 cal)	1 individual packet Trek Mix (Simply Almonds) (210 cal) (Total = 210 cal)	1 individual packet Trek Mix (Simply Almonds) (210 cal) (Total = 210 cal)	1 individual packet Trek Mix (Simply Almonds) (210 cal) (Total = 210 cal)
Dinner	2 servings Hearty Minestrone Soup (280 cal) + 1 serving Multigrain Blend with Vegetables (180 cal) + 1 tablespoon EVOO (119 cal) + 1 serving Tomato Basil Marinara (90 cal) (Total = 669 cal)	1 serving Whole Wheat Pasta (spaghetti, fusilli, or penne) (210 cal) + 1 serving Tomato Basil Marinara (90 cal) + 1 tablespoon EVOO (119 cal) + 1 serving Grilled Chicken (Balsamic Rosemary, Lemon Pepper, or Plain – 105 cal) + 1 serving Harvest Hodgepodge (30 cal) (Total = 554 cal)	2 servings Hearty Minestrone Soup (280 cal) + 1 serving Multigrain Blend with Vegetables (180 cal) + 1 tablespoon EVOO (119 cal) + 1 serving Tomato Basil Marinara (90 cal) (Total = 669 cal)	1 serving Whole Wheat Pasta (spaghetti, fusilli, or penne) (210 cal) + 1 serving Tomato Basil Marinara (90 cal) + 1 tablespoon EVOO (119 cal) + 1 serving Grilled Chicken (Balsamic Rosemary, Lemon Pepper, or Plain – 105 cal) + 1 serving Harvest Hodgepodge (30 cal) (Total = 554 cal)

Study Salad 1 = 2 cups romaine (11 cal) + ½ cup chopped grape tomatoes (16 cal) + ½ cup quinoa (111) + ½ cup chickpeas (73 cal) + 1 tbsp sunflower seeds (47 cal)
Study Salad 2 = 2 cups romaine + ½ cup chopped grape tomatoes + ½ cup quinoa + ½ cup chickpeas + 2 tbsp sunflower seeds (93 cal)
Study Dressing = 1 tbsp olive oil (119 cal) + 1 tbsp balsamic (14 cal)

Supplemental Table S3: The relative abundance (mg %) of lipid species before and after treatments, and their statistical p values.

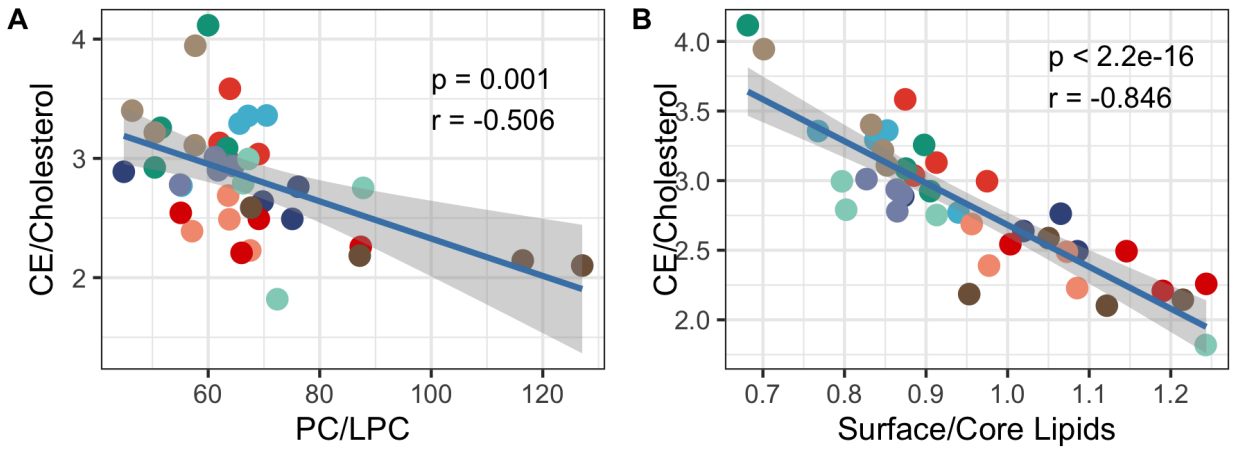
variable	FF_Pre	FF_Post	Med_Pre	Med_Post	pvalue	padj
Ceramide 34:1 d	8.9e-05	8.5e-05	7.9e-05	9.0e-05	0.05 *	0.13
Ceramide 38:1 d	1.4e-05	1.3e-05	1.1e-05	1.2e-05	0.927	0.945
Ceramide 41:1 d	2.9e-05	3.2e-05	2.8e-05	2.7e-05	0.401	0.525
Ceramide 42:1 d	1.1e-04	1.1e-04	1.1e-04	1.0e-04	0.326	0.461
Ceramide 42:2 d	2.3e-05	2.3e-05	1.7e-05	1.3e-05	0.601	0.705
GlcCer 40:1 d	2.0e-05	2.1e-05	1.9e-05	1.9e-05	0.75	0.836
GlcCer 42:1 d	2.0e-05	2.7e-05	2.3e-05	2.7e-05	0.738	0.836
GlcCer 42:2 d	1.9e-05	1.7e-05	1.6e-05	1.8e-05	0.461	0.576
PC 32:2	5.1e-04	5.7e-04	6.6e-04	4.1e-04	0.004 **	0.018 *
PC 34:2	0.023	0.026	0.023	0.023	0.06	0.146
PC 34:3	5.1e-04	6.3e-04	6.5e-04	4.8e-04	0.007 **	0.035 *
PC 35:2	7.5e-04	0.001	7.9e-04	8.0e-04	1.7e-06 ***	2.6e-05 ***
PC 36:3	0.005	0.005	0.005	0.005	0.091	0.202
PC 36:5	0.001	0.001	0.001	0.001	0.934	0.945
PC 38:3	0.002	0.002	0.002	0.002	0.214	0.353
PC 40:5	3.5e-04	3.4e-04	3.7e-04	3.3e-04	0.579	0.683
PC 40:6	0.002	0.001	0.001	0.002	0.011 *	0.045 *
PC 34:1 p	5.0e-04	7.5e-04	4.7e-04	4.2e-04	1.9e-04 ***	0.002 **
PC 34:2 p	0.001	0.002	0.001	1.0e-03	6.7e-07 ***	1.4e-05 ***
PC 36:3 p	0.001	0.001	0.001	0.001	0.262	0.405
PC 38:4 p	2.5e-04	4.2e-04	2.7e-04	2.0e-04	7.8e-07 ***	1.5e-05 ***
PE 36:2	8.9e-04	0.001	9.7e-04	9.9e-04	0.15	0.277
PE 36:4	9.5e-04	8.8e-04	0.001	0.001	0.753	0.836
PE 38:6	6.9e-04	6.2e-04	8.0e-04	0.001	0.171	0.296
PE 34:2 p	9.0e-04	0.001	9.5e-04	6.5e-04	2.6e-05 ***	3.1e-04 ***
PE 36:2 p	0.001	0.002	0.001	8.0e-04	3.9e-09 ***	1.6e-07 ***
PE 36:4 p	0.002	0.003	0.003	0.002	0.035 *	0.105
PE 38:4 p	0.003	0.005	0.004	0.002	1.7e-07 ***	4.1e-06 ***
PE 38:5 p	0.002	0.002	0.002	0.002	0.021 *	0.079
PE 38:6 p	0.001	0.001	0.001	0.001	0.316	0.451
PE 40:6 p	0.001	0.001	9.2e-04	9.2e-04	0.027 *	0.095
SM 33:1 d	0.003	0.002	0.002	0.003	0.286	0.423
SM 40:2 d	0.004	0.004	0.004	0.005	0.028 *	0.096
SM 42:2 d	0.007	0.007	0.008	0.007	0.149	0.277
CE 16:1	0.004	0.003	0.005	0.004	0.412	0.531
CE 18:1	0.047	0.044	0.044	0.051	5.2e-04 ***	0.004 **
CE 18:2	0.286	0.312	0.264	0.294	0.784	0.86
CE 18:3	0.009	0.01	0.009	0.007	0.127	0.255
CE 20:3	0.006	0.005	0.005	0.006	0.131	0.257
CE 20:4	0.093	0.086	0.086	0.099	0.001 **	0.007 **
CE 20:5	0.007	0.005	0.006	0.006	0.379	0.507
CE 22:6	0.012	0.011	0.01	0.013	1.2e-04 ***	0.001 **
Ceramide 40:1 d	4.9e-05	5.6e-05	4.9e-05	5.2e-05	0.57	0.683
Cholesterol	0.097	0.099	0.096	0.098	0.825	0.887
DG 36:2	3.0e-04	2.6e-04	4.0e-04	3.4e-04	0.797	0.866
DG 36:3	4.4e-04	4.3e-04	5.2e-04	5.0e-04	0.924	0.945

Gal-Gal-Cer 34:1 d	2.1e-04	2.2e-04	2.3e-04	2.2e-04	0.452	0.569
GlcCer 40:1 d 1	4.9e-05	5.3e-05	4.8e-05	4.5e-05	0.256	0.399
GlcCer 42:1 d 1	5.9e-05	6.3e-05	6.4e-05	5.9e-05	0.405	0.526
LPC 16:0	0.001	0.001	0.001	0.001	0.24	0.38
LPC 18:0	6.7e-04	6.4e-04	6.5e-04	5.7e-04	0.354	0.49
LPC 18:1	4.0e-04	3.3e-04	3.8e-04	4.1e-04	0.013 *	0.054
LPC 18:2	5.5e-04	6.3e-04	5.1e-04	5.2e-04	0.375	0.507
LPC 20:4	7.9e-05	6.7e-05	8.5e-05	7.0e-05	0.869	0.912
PC 30:0	6.1e-04	6.5e-04	9.9e-04	5.3e-04	0.002 **	0.009 **
PC 32:0	0.001	0.001	0.001	0.001	0.531	0.657
PC 32:1	0.003	0.002	0.004	0.003	0.153	0.277
PC 33:0	5.1e-05	1.0e-04	8.1e-05	4.9e-05	1.2e-04 ***	0.001 **
PC 33:1	3.1e-04	3.6e-04	3.9e-04	2.7e-04	0.003 **	0.015 *
PC 33:2	5.1e-04	6.5e-04	5.4e-04	4.6e-04	1.2e-06 ***	2.0e-05 ***
PC 34:0	2.2e-04	2.4e-04	2.4e-04	2.0e-04	0.003 **	0.018 *
PC 34:1	0.04	0.032	0.045	0.036	0.867	0.912
PC 34:3 1	9.2e-04	8.5e-04	8.3e-04	9.1e-04	0.117	0.241
PC 34:4	1.5e-04	1.2e-04	1.9e-04	1.3e-04	0.175	0.301
PC 35:1	2.9e-04	3.5e-04	3.2e-04	2.5e-04	0.001 **	0.007 **
PC 35:2 1	9.9e-04	0.002	0.001	9.4e-04	1.8e-09 ***	1.0e-07 ***
PC 35:3	2.9e-04	4.2e-04	3.2e-04	2.8e-04	2.4e-04 ***	0.002 **
PC 35:4	1.4e-04	1.4e-04	1.5e-04	1.4e-04	0.998	0.998
PC 36:1	0.004	0.003	0.004	0.003	0.163	0.286
PC 36:2	0.06	0.072	0.063	0.05	5.5e-05 ***	6.3e-04 ***
PC 36:3 1	0.016	0.016	0.017	0.019	0.289	0.423
PC 36:3 2	0.01	0.009	0.012	0.007	0.011 *	0.045 *
PC 36:4	0.004	0.006	0.005	0.004	2.8e-04 ***	0.002 **
PC 36:4 1	0.039	0.029	0.043	0.04	0.033 *	0.104
PC 36:5 1	1.2e-04	2.0e-04	1.1e-04	1.1e-04	0.119	0.241
PC 37:2	1.1e-04	1.3e-04	1.2e-04	1.2e-04	0.059	0.145
PC 37:3	8.6e-05	1.1e-04	1.2e-04	4.7e-05	0.001 **	0.008 **
PC 37:4	3.4e-04	3.8e-04	3.6e-04	3.3e-04	0.024 *	0.087
PC 38:2	2.7e-04	2.4e-04	3.0e-04	2.4e-04	0.208	0.347
PC 38:3 1	0.003	0.003	0.004	0.003	0.27	0.409
PC 38:4	0.029	0.024	0.029	0.027	0.137	0.265
PC 38:5	0.007	0.005	0.007	0.007	0.01 *	0.045 *
PC 38:5 1	0.001	9.5e-04	0.001	8.3e-04	0.689	0.786
PC 38:6	0.016	0.012	0.015	0.017	1.7e-04 ***	0.002 **
PC 39:6	1.7e-04	1.8e-04	1.5e-04	1.6e-04	0.83	0.887
PC 40:4	2.4e-04	2.1e-04	2.6e-04	2.3e-04	0.866	0.912
PC 40:5 1	0.001	0.001	0.002	0.001	0.634	0.733
PC 40:5 2	2.5e-04	1.8e-04	2.5e-04	2.3e-04	0.227	0.367
PC 40:6 1	0.003	0.002	0.002	0.002	0.035 *	0.105
PC 40:7	0.002	0.001	0.002	0.002	0.001 **	0.008 **
PC 40:8	1.3e-04	1.1e-04	1.2e-04	1.4e-04	0.004 **	0.018 *
PC 32:0 o	2.2e-04	2.3e-04	2.1e-04	2.1e-04	0.387	0.51
PC 32:0 p	2.0e-04	2.3e-04	2.0e-04	2.0e-04	0.048 *	0.13
PC 34:0 p	5.4e-04	5.0e-04	5.2e-04	5.5e-04	0.014 *	0.054
PC 34:1 p 1	7.7e-04	0.001	8.5e-04	6.4e-04	1.2e-05 ***	1.7e-04 ***
PC 34:1 p 2	4.0e-04	4.3e-04	4.0e-04	4.0e-04	0.112	0.239

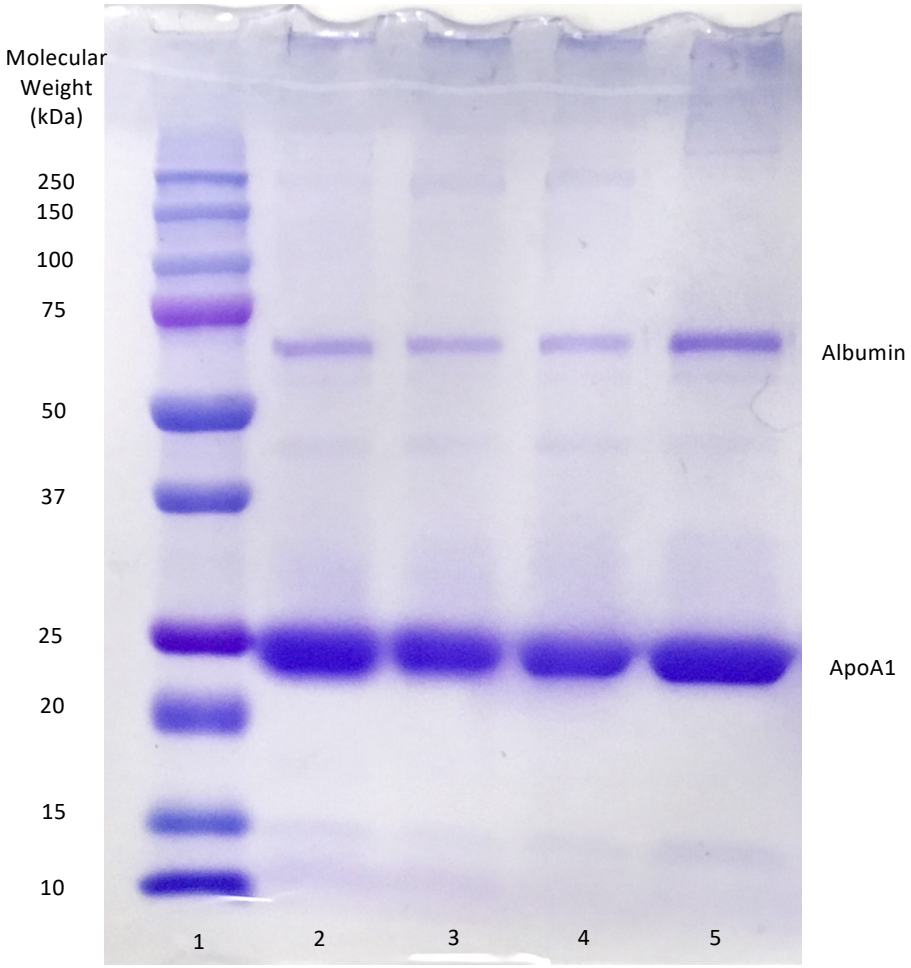
PC 34:2 p 1	0.001	0.002	0.001	0.001	5.4e-08 ***	1.5e-06 ***
PC 36:1 p	1.6e-04	1.6e-04	1.5e-04	1.6e-04	0.575	0.683
PC 36:2 p	4.2e-04	5.1e-04	4.1e-04	3.6e-04	4.3e-04 ***	0.003 **
PC 36:3 p 1	0.002	0.002	0.002	0.002	0.115	0.241
PC 36:4 p	0.002	0.002	0.002	0.002	2.0e-05 ***	2.6e-04 ***
PC 38:2 p	7.1e-05	8.0e-05	6.2e-05	6.3e-05	0.571	0.683
PC 38:3 p	6.7e-04	7.1e-04	7.3e-04	6.7e-04	0.003 **	0.014 *
PC 38:4 p 1	0.002	0.002	0.002	0.002	0.283	0.422
PC 38:4 p 2	3.3e-04	4.7e-04	3.4e-04	3.1e-04	5.7e-10 ***	9.7e-08 ***
PC 38:5 p	4.0e-04	4.6e-04	4.2e-04	4.2e-04	0.043 *	0.119
PC 40:3 p	1.0e-04	7.5e-05	9.7e-05	9.1e-05	0.193	0.328
PC 40:4 p	1.7e-04	1.7e-04	1.7e-04	1.7e-04	0.634	0.733
PC 40:5 p	1.1e-04	1.1e-04	1.0e-04	1.2e-04	0.376	0.507
PC 40:6 p	1.7e-04	2.0e-04	1.7e-04	1.5e-04	0.001 **	0.007 **
PC 42:4 p	1.4e-04	1.3e-04	1.3e-04	1.3e-04	0.132	0.257
PC 42:5 p	7.5e-05	7.8e-05	7.3e-05	7.8e-05	0.899	0.932
PC 44:4 p	1.8e-04	1.8e-04	1.7e-04	1.8e-04	0.153	0.277
PC 38:4 1	7.2e-04	6.4e-04	8.3e-04	6.2e-04	0.057	0.142
PE 38:4	0.001	9.3e-04	9.3e-04	0.001	0.382	0.507
PE 38:6 1	7.2e-04	7.0e-04	8.2e-04	0.001	0.2	0.337
PE 36:2 p 1	9.3e-04	0.002	9.5e-04	6.1e-04	4.1e-08 ***	1.4e-06 ***
PE 36:4 p 1	0.002	0.002	0.002	0.002	0.05 *	0.13
PE 38:5 p 1	0.002	0.002	0.002	0.002	0.082	0.187
PC 36:2 p 1	2.6e-04	5.2e-04	2.7e-04	2.2e-04	1.3e-09 ***	1.0e-07 ***
PC 38:6 p	3.9e-04	4.9e-04	3.6e-04	3.9e-04	0.088	0.197
SM 32:1 d	0.001	9.8e-04	9.3e-04	9.8e-04	0.241	0.38
SM 32:2 d	1.2e-04	1.0e-04	1.3e-04	9.0e-05	0.43	0.549
SM 34:0 d	5.2e-04	4.9e-04	5.0e-04	5.4e-04	0.031 *	0.099
SM 34:1 d	0.014	0.014	0.014	0.014	0.078	0.179
SM 34:2 d	0.002	0.002	0.002	0.002	0.026 *	0.094
SM 36:0 d	1.5e-04	1.5e-04	1.3e-04	1.2e-04	0.928	0.945
SM 36:1 d	0.002	0.002	0.002	0.002	0.272	0.409
SM 36:2 d	0.007	0.007	0.007	0.007	0.222	0.363
SM 38:1 d	0.001	0.001	0.001	0.001	0.159	0.281
SM 38:2 d	8.1e-04	8.3e-04	7.8e-04	7.8e-04	0.751	0.836
SM 39:1 d	4.4e-04	4.4e-04	4.2e-04	4.6e-04	0.339	0.477
SM 40:1 d	0.002	0.002	0.002	0.002	0.309	0.445
SM 40:2 d 1	0.002	0.002	0.002	0.002	0.678	0.779
SM 41:1 d	8.6e-04	8.5e-04	8.0e-04	9.2e-04	0.098	0.215
SM 41:2 d	6.1e-04	5.8e-04	5.8e-04	6.5e-04	0.063	0.15
SM 42:1 d	0.001	0.001	0.001	0.001	0.051	0.131
SM 42:3 d	0.003	0.002	0.002	0.003	6.1e-05 ***	6.5e-04 ***
SM 43:2 d	1.1e-04	1.6e-04	1.2e-04	1.3e-04	0.146	0.277
TG 46:0	1.5e-04	1.8e-04	1.9e-04	1.4e-04	0.037 *	0.109
TG 46:1	1.0e-04	1.0e-04	1.8e-04	8.7e-05	0.052	0.133
TG 48:1	7.1e-04	7.6e-04	0.001	6.6e-04	0.05 *	0.13
TG 48:2	4.0e-04	4.2e-04	8.1e-04	3.7e-04	0.039 *	0.113
TG 48:3	9.9e-05	9.0e-05	1.8e-04	6.8e-05	0.035 *	0.105
TG 49:2	7.8e-05	8.6e-05	1.2e-04	6.1e-05	0.03 *	0.098
TG 50:1	0.003	0.003	0.005	0.003	0.065	0.153

TG 50:2	0.004	0.003	0.006	0.004	0.158	0.281
TG 50:3	0.001	0.001	0.002	0.001	0.296	0.43
TG 50:4	2.3e-04	2.3e-04	3.0e-04	2.0e-04	0.23	0.368
TG 51:2	3.0e-04	3.9e-04	5.0e-04	2.9e-04	0.009 **	0.04 *
TG 51:3	2.5e-04	3.0e-04	3.3e-04	2.3e-04	0.029 *	0.096
TG 52:1	8.5e-04	9.4e-04	0.001	6.3e-04	0.016 *	0.062
TG 52:2	0.014	0.011	0.02	0.015	0.548	0.665
TG 52:3	0.018	0.016	0.021	0.018	0.768	0.847
TG 52:4	0.005	0.006	0.006	0.006	0.8	0.866
TG 52:5	7.4e-04	8.3e-04	8.2e-04	7.5e-04	0.438	0.556
TG 52:6	7.5e-05	7.4e-05	8.5e-05	7.0e-05	0.538	0.657
TG 53:2	1.0e-04	1.2e-04	2.1e-04	9.4e-05	0.139	0.265
TG 53:3	2.8e-04	3.9e-04	3.8e-04	2.6e-04	0.008 **	0.037 *
TG 53:4	1.0e-04	1.6e-04	1.1e-04	9.7e-05	0.041 *	0.117
TG 54:2	0.001	0.001	0.001	9.3e-04	0.111	0.239
TG 54:3	0.004	0.004	0.006	0.005	0.536	0.657
TG 54:4	0.005	0.004	0.005	0.006	0.119	0.241
TG 54:5	0.002	0.002	0.002	0.003	0.271	0.409
TG 54:5 1	7.0e-04	5.2e-04	8.3e-04	6.4e-04	0.98	0.985
TG 54:6	4.0e-04	3.4e-04	4.0e-04	3.5e-04	0.885	0.923
TG 56:4	9.4e-05	8.1e-05	1.2e-04	1.2e-04	0.354	0.49
TG 56:5	1.8e-04	1.5e-04	1.7e-04	1.8e-04	0.017 *	0.065
TG 56:7	2.9e-04	2.5e-04	2.6e-04	3.1e-04	0.072	0.168
TG 56:8	1.2e-04	1.3e-04	1.2e-04	1.6e-04	0.369	0.506

* P value < 0.05
** P value < 0.01
*** P value < 0.001



Supplemental Figure S1: scatter plot between PC to LPC ratio versus CE to free cholesterol ratio (A), and surface to core lipids ratio versus CE to free cholesterol ratio (B).



Supplemental Figure S2: Gel electrophoresis with Coomassie blue of isolated HDL fractions. Column 1 is molecular weight marker in kDa. Column 2-5 are ultracentrifugation-isolated HDL from two of the study subjects.