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Comparison of Nonfasting and Fasting Lipoprotein Subfractions and Size in 15,397 Apparently Healthy Individuals: An Analysis from the VITamin D and OmegA-3 TriaL (VITAL)

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Abstract

Background: Elevated postprandial triglycerides reflect a pro-atherogenic milieu, but underlying mechanisms are unclear.

Objective: We examined differences between fasting and nonfasting profiles of directly measured lipoprotein size and subfractions to assess if postprandial triglycerides reflected

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Contributions

Z.M.F, O.V.D., C.L., N.R.C, H. L-G., R.M.K, J.E.B, J.R.N, S.M. and J.E.M contributed to study design and/or data analysis and manuscript preparation. M.P.C., J.W., K.K and M.C. provided clinical chemistry support and were involved in manuscript preparation. All authors have read and agree to the manuscript as written.

Disclosures

Z M. Farukhi, O V. Demler, N R. Cook, H Luttman-Gibson, J E. Buring and J E. Manson have no disclosures. M Caulfield and J Wohlgemuth are employed by Quest Diagnostic and M Caulfield has a patent with Quest Diagnostics. K Kulkarni and M Cobble are employed by VAP Diagnostics Laboratory. M Cobble has received honoraria for speaking engagements by Amgen, Amarin, Astra Zeneca and Sanofi-Regeneron, a research grant from Janssen Research and has served as consultant for Kowa and Astra Zeneca. JR Nelson has received honoraria for speaking engagements by Bostonheart Diagnostics Laboratory, Amgen, Amarin Boehringer-Ingelheim, Kowa, Sanofi-Aventis and Regeneron. JR Nelson serves on the advisory board for Amgen, Amarin and Kowa. RM Krauss has received a research grant and honororaia for speaking engagements from Quest Diagnostics. S Mora received research grant support from Atherotech Diagnostics, the Molino Family Trust, the NIH and served as consultant to Quest Diagnostics.

increases in very low density and intermediate density lipoproteins (VLDL and IDL) and remnants, or small dense lipid depleted LDL particles (sdLDL).

Methods: We conducted a cross-sectional analysis of 15,397 participants (10,135 fasting; 5262 nonfasting [<8 hours since last meal]) from the VITamin D and OmegA-3 TriaL (VITAL). Baseline cholesterol subfractions were measured by vertical auto profile method and particle subfractions by ion mobility. We performed multivariable linear regression adjusting for cardiovascular risk and lipoprotein modifying factors.

Results: Mean age (SD) was 68.0 years (±7.0), with 50.9% women. Adjusted mean triglyceride concentrations were higher nonfasting by 17.8 ± 1.3%, with higher nonfasting levels of directly-measured VLDL cholesterol (by 3.5 ± 0.6%) and total VLDL particles (by 2.0 ± 0.7 %), specifically large VLDL (by 12.3 ± 1.3%) and medium VLDL particles (by 5.3 ± 0.8%), all $p<0.001$. In contrast, lower concentrations of LDL and IDL cholesterol and particles were noted for nonfasting participants. sdLDL cholesterol levels and particle concentrations showed no statistically significant difference by fasting status (−1.3 ± 2.1% and 0.07 ±0.6%, respectively, $p>0.05$).

Conclusions: Directly measured particle and cholesterol concentrations of VLDL, not sdLDL, were higher nonfasting and may partly contribute to the pro-atherogenicity of postprandial hypertriglyceridemia. These differences, although statistically significant, were small and may not fully explain the increased risk of postprandial hypertriglyceridemia.

Clinical Trial Registration: URL: https://www.clinicaltrials.gov Unique identifier: NCT01169259.

Keywords

Nonfasting lipids; lipoprotein subfractions; postprandial state; triglyceride rich lipoproteins

Introduction

Traditionally, fasting cholesterol profiles have been obtained for cardiovascular disease (CVD) risk assessment with Friedewald calculated low-density lipoprotein cholesterol (LDL-C) and measured high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG). Recently, nonfasting lipid screening has become accepted as a suitable alternative to fasting tests for routine screening [1-3]. Several population studies have shown that nonfasting TG levels are equally or even more strongly associated with CVD endpoints [4-5]. Elevated levels of postprandial TG represent abnormalities in TG-rich lipoprotein (TRLP) metabolism, which may result in a pro-atherogenic milieu that contributes to atherosclerosis and CVD events. However, the exact biological mechanism whereby this occurs is unknown. While delayed plasma clearance of chylomicrons and their lipolytic remnants are likely to be of importance in this regard [6], postprandial increases in liver-derived very low density and intermediate density lipoproteins (VLDL and IDL) may also be implicated [7].

Hypothetically increases in postprandial TGs and resultant catabolism of large sized VLDL, may produce increased numbers of small dense lipid depleted LDL (sdLDL) that contribute

to the enhanced CVD risk associated with hypertriglyceridemia [6-8]. While sdLDL is known to be atherogenic [9] and increased particle numbers likely contribute to the residual risk seen in patients with at goal LDL-C values [10,11], it has yet to be shown that sdLDL increases postprandially or contributes to the postprandial risk of hypertriglyceridemia.

While standard lipids have been compared by fasting state no detailed analysis of cholesterol or lipoprotein subfractions has been performed in a large cohort of individuals to assess potential associations between fasting status and specifically examine postprandial subfraction differences associated with the postprandial TG increase.

In the present study, nested within the recently completed VITamin D and OmegA-3 Trial VITAL (NCT01169259), we hypothesized that increased nonfasting TGs would track with specific plasma VLDL, IDL and/or sdLDL particle subfractions in a large cohort of apparently healthy individuals. Moreover, we have defined the times at which peak plasma concentration levels of each of these subfractions occurs in the postprandial state.

Methods

Study Population

Study participants were enrolled in VITAL, a recently completed randomized, double-blinded, placebo-controlled clinical trial of vitamin D and omega-3 fatty acid supplementation in the primary prevention of cancer and CVD [12]. Men aged ≥50 years and women aged ≥55 years, who were free of self–reported CVD or cancer at study entry (2011-2013) were eligible for the study and were followed up through December, 2017. At the time of enrollment participants provided written informed consent, completed baseline questionnaires and were asked to provide a blood sample as well as time since last meal information at the time of blood draw. Of the 25,871 adults enrolled in VITAL, 16,956 had provided baseline blood samples by the time of this analysis and a subgroup of 15,770 had detailed directly measured lipoprotein subfractions measured by the ion mobility (IM) method (Quest Diagnostics, San Juan Capistrano, CA). After categorizing based on fasting status, the study population comprised of 15,397 individuals. The study was approved by the institutional review boards of the Brigham and Women's Hospital (Boston, MA) and the VITAL study group's Publications and Presentations Committee. The first and senior authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Baseline Plasma Measurements

EDTA blood samples were obtained at the time of enrollment, aliquoted, and stored in vapor phase liquid nitrogen (− 170°C). Aliquoted tubes of plasma were sent to Atherotech Diagnostics (Birmingham, AL) and Quest Diagnostics Nichols Institute (San Juan Capistrano, CA) for lipid and lipoprotein subfraction analysis. 15,397 individuals had lipoprotein subfractions measured by IM and 13,027 of them also had cholesterol levels in lipoprotein subfractions analyzed by the vertical spin density-gradient ultracentrifugation profiling (VAP) method (Atherotech Diagnostics, Birmingham, AL). Due to the dissolution of Atherotech Diagnostics during the study period, for a subset of patients who did not have

VAP lipids (n=2,731), total cholesterol, LDL and HDL cholesterol and triglycerides were determined by Quest Diagnostics Chantilly, VA (Supplemental Methods) with good correlation between the two methods as shown in Supplemental Figure 1. VAP relies on ultracentrifugation with a vertical rotor whereas IM separates particles based on size, independent of their cholesterol composition, via gas-phase electrophoresis. More details about the IM and VAP methods are described in the supplement (Supplemental Methods).Triglycerides were measured enzymatically using the glycerol phosphate oxidase method on the Architect C16200 analyzer, and apolipoproteins B and A-I were measured by immunoturbimetric assays using reagents by Abbott Laboratories (Abbott Park, IL) on the Architect C16200 analyzer (Atherotech Diagnostics).

Definition of Fasting Status

Participants who reported time since last meal as less than 8 hours before their blood draw were defined as nonfasting (n=5262). Those who had not eaten for 8 hours or more were defined as fasting (n=10,135). The study population was also divided into groups based on time since last meal by 2-hour intervals of < 2 (n=1390), 2 to <4 (n=2350), 4 to <6 (n=1122), 6 to <8 (n=400), 8 to <10 (n=949), 10 to <12 (n=2264), 12 to <14 (=4458), 14 to <16 (n=1813), 16-<18 (n=426) and ≥ 18 hours (n=196).

Statistical analysis

Statistical analysis was performed using SAS software version 9.4 (SAS Institute, Inc., Cary, North Carolina). Statistical comparisons of participant characteristics between nonfasting and fasting groups were obtained from Student *t* tests for continuous variables expressed as means and from χ^2 tests for categorical variables. Lipid and lipoprotein levels were skewed, however our large sample size allowed for clinically meaningful mean levels to be determined. Multivariable linear regression was used to estimate absolute difference in mean lipid and lipoprotein subfractions and percent differences (from $\log_e$ transformed outcomes). All models were adjusted for age, race, sex, diabetes, hypertension, cholesterol medication use, postmenopausal hormone use (among women), smoking history and body-mass index (BMI) as a continuous variable. Comparison of LDL pattern (LDL pattern A representing higher prevalence of larger LDL particles and B representing smaller sized LDL particles predominance) between nonfasting and fasting groups was determined using χ^2 tests for categorical variables. To assess if there was a significant impact of the run dates of blood samples on our findings, we additionally adjusted for these, with resultant models demonstrating similar overall results. All *p*-values were 2-tailed using α=0.05 type I error for the main results. Additional statistical tests for interaction between fasting status and race, gender, diabetes, BMI, LDL size, statin use and age were obtained using Bonferroni correction and an α=0.0001 type I error to account for multiple testing. To calculate the Bonferroni corrected threshold, we assumed testing 500 hypotheses (which exceeds the actual number of tests performed) and produced the conservative significance threshold of .0001.

For subgroup analysis, LDL size and age were dichotomized into below and equal to or above median levels. BMI was categorized into three categories; 18.5 to 25, >25 to ≤ 30, and >30 kg/m2. To accommodate for potentially non-linear relationships between levels of lipids

or lipoproteins and time since last meal we used cubic spline regression. Finally, levels of lipids and lipoproteins were examined as a function of time since last meal divided into 2-hour intervals and expressed as medians with 25th and 75th percentile values for each time interval.

Results

Compared with fasting participants, nonfasting participants were slightly older with a greater proportion of whites, lower BMI and less prevalent use of drugs for diabetes, hypertension and lipid lowering as well as postmenopausal hormones. (Table 1) Subsequent analysis adjusted for all parameters in Table 1.

Table 2 shows the adjusted mean concentrations of lipids, lipoprotein cholesterol and apolipoproteins for nonfasting and fasting participants (with subfractions ordered by size; largest to smallest per subfraction), with percent differences plotted in Figure 1. At baseline less than a quarter of nonfasting participants had TG levels >175 mg/dL with median levels in this group of 219 (interquartile range: 192, 267) mg/dL. Overall adjusted mean triglycerides were significantly higher nonfasting vs. fasting by 26.4 mg/dl or 17.8 (1.2)%. Non-HDL-C, Apo B, and total cholesterol were significantly slightly lower nonfasting by 2.70 (0.42)%, 1.36 (0.39)% and 2.19 (0.33)% respectively. Adjusted mean VLDL-C concentration was higher nonfasting by 3.46 (0.62)%, due to higher nonfasting adjusted mean VLDL-C1+2 (large-medium VLDL), by 7.39 (0.81)%. There was no statistically significant difference seen for the VLDL-C3 (small) subfraction.

Adjusted mean LDL-C by Friedewald calculation and direct VAP measurement was significantly lower nonfasting, as was adjusted mean real LDL cholesterol (LDL-CR) (calculated by LDL-C minus IDL-C and lipoprotein (a) concentration). Similarly all LDL subfractions had lower absolute adjusted mean concentrations nonfasting. Adjusted mean IDL-C was also lower nonfasting by 7.84 (0.74)%.

There was no statistically significant difference between nonfasting and fasting total HDL-C concentrations. The large sized HDL subfraction (HDL-C2) was slightly higher nonfasting by 4.11 (0.83)% and smaller sized HDL-C3 was lower by 2.50 (0.37)%. There was no statistically significant difference in adjusted estimated mean Lp (a)-cholesterol levels based on fasting status.

Table 3 shows the adjusted mean concentrations of size separated lipoprotein particles by fasting status as measured by ion mobility. Adjusted percent differences in nonfasting compared to fasting lipoprotein concentrations are depicted graphically in Figure 2. Similar to findings obtained by VAP for VLDL-C subfractions, the adjusted mean total VLDL particle concentration (VLDL-P) was higher nonfasting by 2.0 (0.69) % with associated higher concentrations in all VLDL-P subfractions except the smallest sized VLDL-P. Large sized VLDL-P was higher by 12.3 (1.3)%, followed by medium sized VLDL-P by 5.3 (0.8)%. Total LDL particle concentration (LDL-P) was statistically significantly lower nonfasting as were all LDL subfractions except for the largest and smallest LDL size subfractions, which did not achieve statistical significance but had a lower absolute trend. Adjusted mean LDL peak size was slightly higher nonfasting (0.18 (0.04)%, $p<0.0001$).

Total, large and small sized IDL-P were all lower nonfasting by 2.66 (0.50)%, 3.57 (0.52)% and 1.82 (0.61)% respectively. There was no significant difference in total HDL particle (HDL-P) concentration by fasting status. Adjusted mean large sized HDL-P was higher nonfasting by 2.02 (0.43)% with slightly lower small sized HDL-P by 0.69 (0.31)%. Finally, total non-HDL particle (non-HDL-P) concentration was lower by 2.33 (0.44)% nonfasting ($p<0.0001$).

To determine the effect of time since last meal intake on selected lipid and lipoprotein concentrations of interest, mean (95% confidence intervals) and median (25-75th percentile) concentrations were plotted over time (Figure 3 and Supplemental Figure 2, respectively). TG concentration peaked during the 4 to 6 hour period after last meal. VLDL-C was consistently higher in the first 8 hours postprandially. Similarly total VLDL-P appeared slightly higher up to 8 hours since last meal, with large sized VLDL-P peaking within the 2 to 4 hour period. IDL-C increased after 6 to 7 hours. LDL-C concentration remained fairly constant over time with a slight gradual increase beyond 8 hours. LDL-P appeared to peak during the 8 to 10 hour period and the smallest LDL-P subfractions levels were fairly constant.

Subgroup Analysis and Effect Modification

In separate subgroup analyses by race, age, LDL size, BMI, statin use and sex, adjusted mean results for TGs, VLDL-C and VLDL-P showed higher nonfasting levels for all subgroups. Compared to nondiabetic participants, those with diabetes had slightly lower VLDL-C and VLDL-P levels (both fasting and nonfasting) as well as blunted postprandial VLDL response (Supplementary Results). Interaction analysis showed no statistically significant effect modification (at $\alpha=0.0001$ to account for multiple testing) by sex, race, diabetes or age on lipid and lipoprotein levels by fasting status. There was significant ($p<0.0001$) effect modification seen by BMI and fasting status on TG levels, LDL size, and large sized HDL-C levels, but not on other lipids or lipoproteins. The was significant effect modification ($p<0.0001$) seen by statin use and fasting status on non-HDL, total LDL, small LDL and total IDL particle concentrations, as well as large and medium sized VLDL-C levels.

Discussion

Our data are the first to report fasting status effect on directly measured lipid and lipoprotein subfraction concentrations in a large contemporary population adjusted for traditional factors that affect their plasma concentrations. One of the earliest proponents of the atherogenic potential of postprandial lipemia, Zilversmit conducted a series of animal studies suggesting that increased levels of and prolonged arterial exposure to TRLPs, specifically chylomicron remnants and VLDL, may be part of the causal process in atherogenesis of the arterial intimal wall [13,14]. Since then various TRLPs and their remnants have been implicated as potentially atherogenic based on small animal and human experimental studies after administration of an oral fat load [8,15]. The current study provides directly measured evidence of higher postprandial concentrations of both VLDL-C and VLDL-P, most notably large and medium sized VLDL particles, associated with the higher nonfasting TG levels.

Interestingly, neither IDL-C nor IDL-P were associated with the postprandial increase in TGs and we found no evidence for increased postprandial concentrations of sdLDL particles in this study population. In addition to lifestyle modifications, targeting VLDL metabolism may represent a potential precision medicine strategy in the future.

Higher mean concentrations of nonfasting TGs by 17.8% was within the expected range as determined by previous investigators [4]. Our findings are consistent with results from a small (n=12) experimental human study that measured plasma samples after an oral fat load and found that VLDL and VLDL remnants were the predominant lipoproteins in postprandial plasma, although the measurement technique could not provide precise particle size information [16]. Due to their rapid metabolism in plasma and measurement via a very specific antibody assay, chylomicrons (CM) and chylomicron remnants (CMR) are not routinely assessed in clinical practice [6]. In our study chylomicron-sized particles are included in the largest VLDL subfraction, measurement and specific testing for CM alone was not possible. These likely contribute to the higher postprandial TG levels observed.

Previous observational data have suggested that large sized VLDL may play an important role in atherogenesis based on their accumulation in hypertriglyceridemic patients with CAD [17]. Interestingly, we found no statistically significant difference in small sized VLDL by VAP and a slightly lower concentration of nonfasting small sized VLDL-P by IM. This may be due a balance between production of these particles and arterial influx rate. Animal studies have demonstrated that the rate of arterial uptake of lipoproteins partly depends on their size, with larger sized particles having a slower rate of influx compared to smaller sized particles [18,19]. If produced in sufficient quantity, higher influx rate of smaller sized particles may lead to the lower concentrations of smaller sized VLDL seen in the first few hours after a meal. At later time points, increased production of small sized VLDL from catabolism of the larger sized VLDL particles (VLDL half-life is about 4 hours) [16] may contribute to higher levels. Kinetic experiments, however, would need to confirm whether either of these factors are related to observed changes in small sized VLDL plasma concentrations over time.

Mechanistic data from animal studies demonstrates that re-bolusing animals with a high fat and cholesterol dietary chow causes continued increases (beyond initial postprandial levels) of TRLPs and delays VLDL catabolism from larger to smaller sized particles [13,14]. If comparable in humans, this has direct implications for clinical practice with patient's lifestyle and dietary choices playing a vital role in the management of residual CVD risk despite optimal medical therapy.

Our findings of lower nonfasting IDL-C and IDL-P concentrations are consistent with recent studies [20]. Similarly lower nonfasting LDL-C is not an unexpected finding. Concerns about misclassification by clinicians regarding CVD risk assessment has been discussed in detail in several papers including the 2016 EAS/EFLM Consensus statement and our data show similar results with respect to nonfasting LDL-C levels as previously shown. Perhaps more interesting was our finding that the smallest LDL subfraction (sdLDL) showed no statistically significant difference based on fasting status overall nor in any subgroup analyses by diabetes, sex, age, LDL size, statin use, BMI or race. The data suggest that, at

least in our relatively healthy population, there is no increase in sdLDL levels postprandially. The lack of an association between sdLDL concentration and the observed VLDL and triglyceride increase suggests that further research should examine the metabolism and uptake of sdLDL particles, especially in a higher risk profile cohort where traditionally lipid metabolism dysfunction is expected.

Normal HDL metabolism is likely responsible for the observed higher levels of nonfasting large sized HDL particles. Larger sized HDL particles are initially involved in exchange of cholesterol and TGs with VLDL and smaller sized HDL particles are subsequently generated over time [21]. Finally in diabetic participants, our findings of slightly lower VLDL-C and VLDL-P levels compared to non-diabetic participants as well as blunted postprandial VLDL response, should be interpreted with caution as this analysis was not a primary objective of this study, and is contrary to would be expected for diabetic patients [22,23]. We cannot exclude that newer diabetic medication use may have contributed to this result [24]. Overall, our exploratory subgroup analysis illustrates the need for further studies designed specifically in the diabetic population.

Strengths and Limitations

Strengths of this study include the large population size and detailed risk factor data enabling comprehensive analysis as well as two complementary measurement methods demonstrating consistent results. The VITAL study population included a high percentage of women (50.9%) and participants of African descent (15.4 %), which improves the generalizability of our findings. One limitation is that nonfasting and fasting samples were not available on the same participants for most individuals, however our large sample size in both fasting categories should be sufficient to draw reproducible conclusions. Furthermore, the study is cross-sectional and findings should be viewed as hypothesis-generating. While we adjusted for multiple cardiovascular risk factors, including age and race, as well as medication use and other potential confounders, there could be other unmeasured confounders. There were no significant interactions by age, sex, race, or diabetes status. Finally, VITAL study participants were found to be healthier on average, than the general population,[12] which may result in a lower than expected change in postprandial lipoprotein concentrations that may not be reflective of findings in a higher risk population. Further studies examining higher risk populations are warranted.

Conclusion

To our knowledge, this study represents the first large epidemiologic analysis assessing the differential effect of fasting status on directly measured lipid and lipoprotein subfraction particle and cholesterol concentrations. Concentrations of VLDL particles and cholesterol, but not IDL or sdLDL, were higher nonfasting than fasting suggesting that these particles may partly contribute to the pro-atherogenicity of postprandial hypertriglyceridemia. Whether it is the absolute concentration or the duration of increase in particle concentration or both that is important cannot be determined by our current data, however previous genetic studies would suggest that lifelong high plasma concentrations of TRLPs or their remnants are causally related to an increased risk of coronary heart disease [25]. In addition to

lifestyle modifications, targeting VLDL metabolism [26, 27] may represent a potential precision medicine strategy in the future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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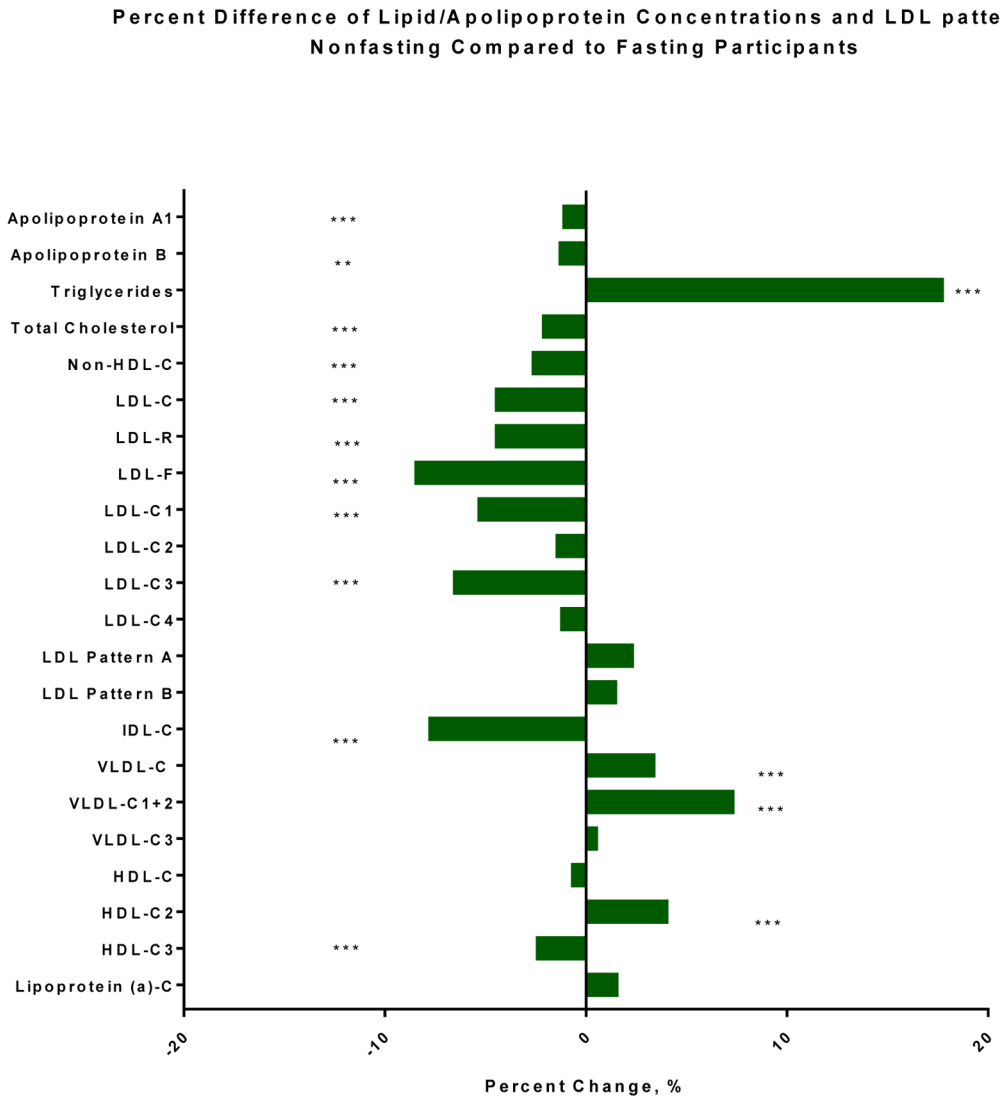

Figure 1.

is the graphical representation of the percent difference in lipid and lipoprotein concentrations (and LDL pattern) in nonfasting vs. fasting participants. *p*-value < 0.0001 represented by ***; *p*-value <0.001 by ** and *p*-value <0.05 by *.

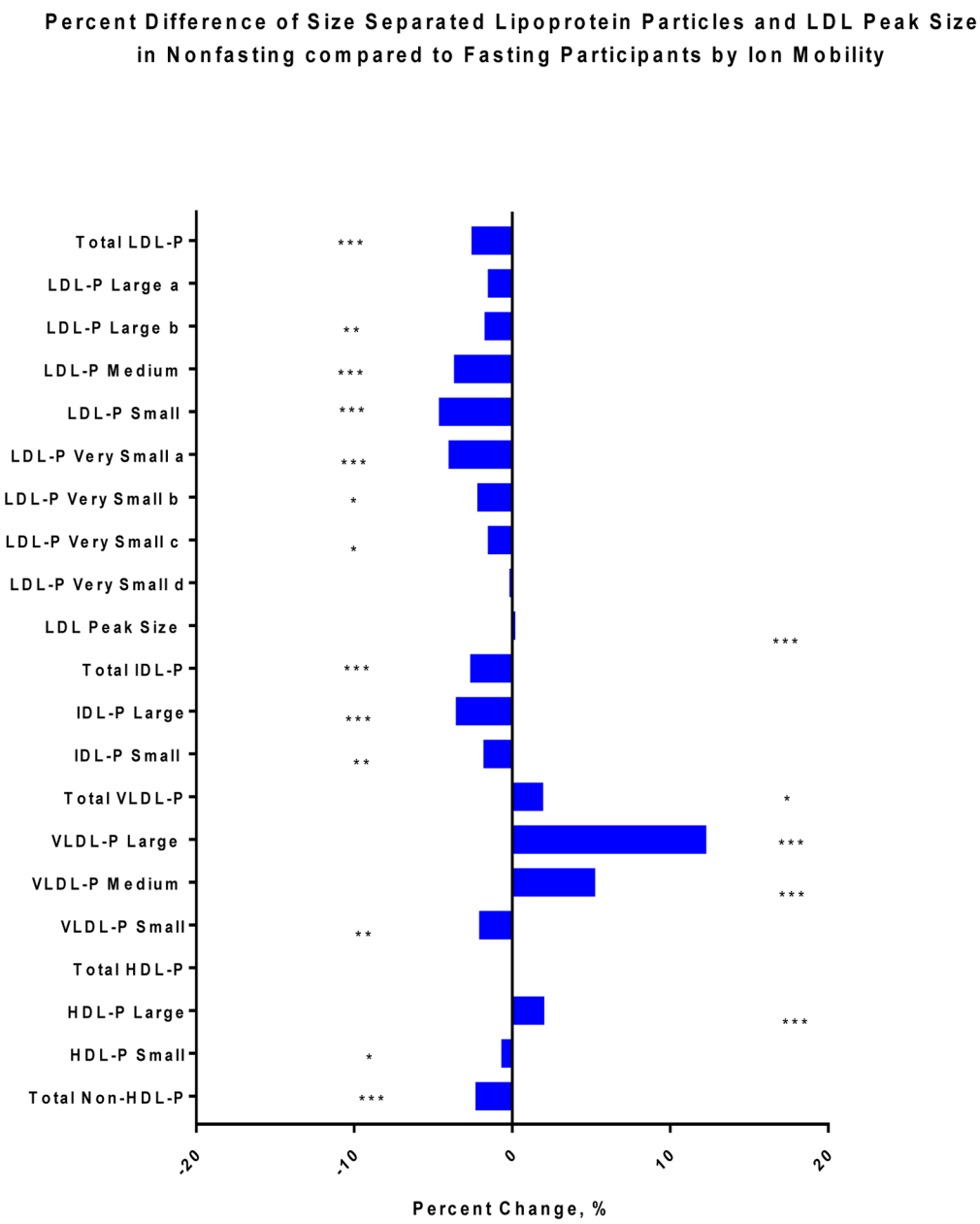

Figure 2.

is the graphical representation of the percent difference in size separated lipoprotein subfractions and LDL peak size by ion mobility in nonfasting vs. fasting participants. *p*-value < 0.0001 represented by ***; *p*-value <0.001 by ** and *p*-value <0.05 by *.

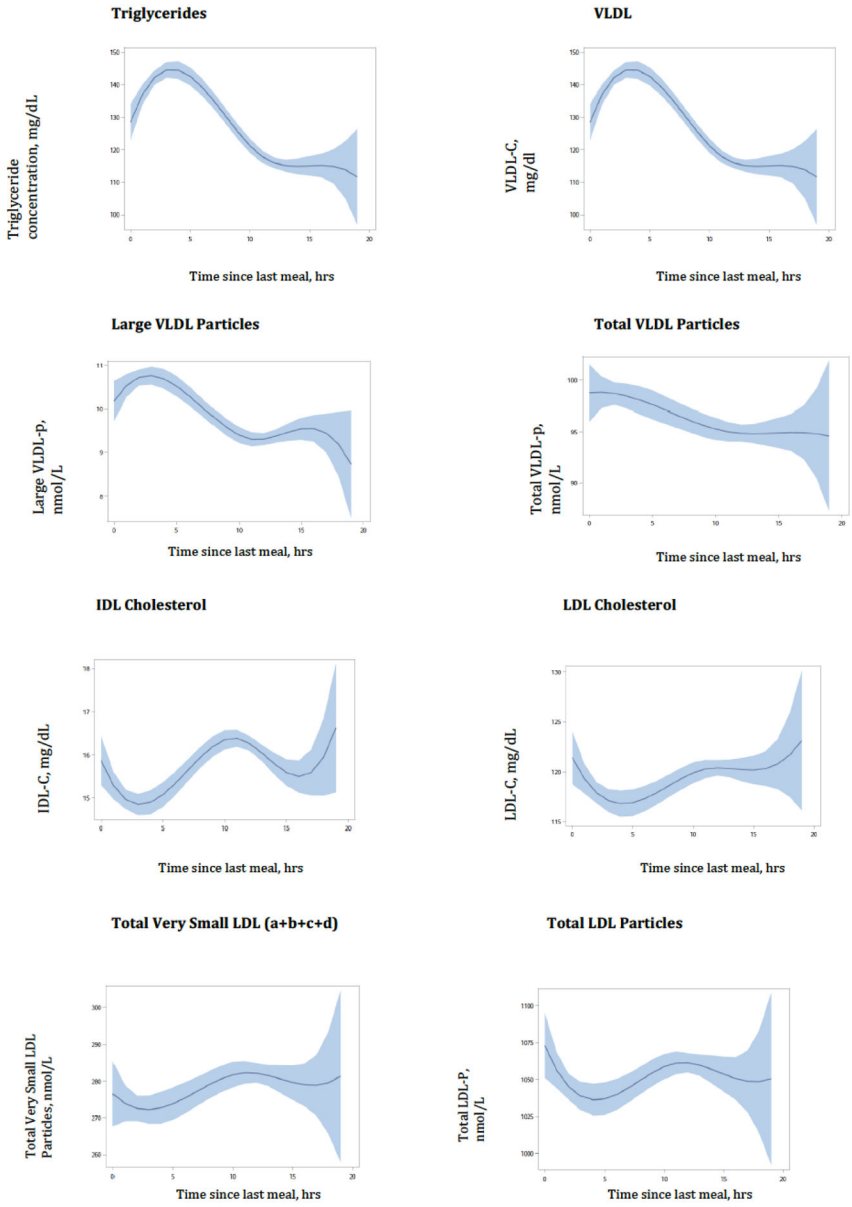

Figure 3.

Graph of mean lipid and lipoprotein levels as a function of time since last meal using cubic spline regression with associated 95 percent confidence intervals. Total Very Small LDL-P is the sum of all the very small LDL particle subfractions: a+b+c+d.

Table 1.

Baseline Characteristics for Nonfasting and Fasting Participants

Baseline Characteristics	Nonfasting (n=5262)	Fasting (n=10 135)	P-Value
Age, years	68.2 (7.1)	67.9 (6.9)	0.003
Women, %	51.6	50.5	0.1926
Time Since Last Meal, hours	2.7 (1.7)	12.2 (2.2)	<0.0001
Race [a], %			
White	85.7	80.2	
Black	11.9	16.9	<0.0001
Other	2.4	3.0	
Smoking, %			
Never	51.5	52.0	
Past	43.0	41.8	0.2028
Current	5.5	6.2	
Body Mass Index, kg/m^2	27.2 (5.2)	28.2 (5.7)	<0.0001
Hypertension, %	49.4	55.0	<0.0001
Diabetes, %	10.8	14.5	<0.0001
Postmenopausal Hormone Use, %			
Current	13.5	11.6	0.0027
Past	48.4	47.1	0.0027
None	37.7	41.3	
Cholesterol Medication Use [b], %	34.3	42.2	<0.0001

Values displayed represent mean with standard deviation (SD) for continuous variables or percentage (%) for categorical variables. Statistical significance was assessed by t-tests for continuous variables and chi-squared analysis for categorical variables.

[a] Other race includes American Indian/Alaskan native, Pacific islander/Hawaiian native, Asian and more than one race or other designation.

[b] 94% of participants on cholesterol lowering medication reported a statin as the cholesterol lowering drug. 6% were on non-statin agents.

Table 2.

Adjusted Mean Concentrations of Lipids and Apolipoproteins in Nonfasting and Fasting Participants

Lipid /Apolipoprotein (mg/dL)	Nonfasting, Mean (SE) N=5262	Fasting, Mean (SE) N=10135	Absolute Difference, Nonfasting minus Fasting (SE) [b]	Percent Difference, Nonfasting minus fasting [c]
Apolipoproteins				
Apo AI	164.81 (1.46)	166.78 (1.43)	−1.97 (0.48) ***	−1.17 (0.28) ***
ApoB	90.35 (1.12)	91.81 (1.10)	−1.46 (0.37) ***	−1.36 (0.39) **
Total Cholesterol	194.86 (2.08)	199.67 (2.04)	−4.82 (0.68) ***	−2.19 (0.33) ***
Triglycerides	157.38 (3.87)	130.94 (3.79)	+26.44 (1.27) ***	+17.80 (1.25) ***
Non-HDL-C	136.44 (1.87)	140.59 (1.83)	−4.15 (0.61) ***	−2.70 (0.42) ***
LDL-C (Friedewald)	104.98 (1.89)	114.41 (1.85)	−9.44 (0.62) ***	−8.53 (0.55) ***
Lipids By VAP [a]				
LDL-C	109.82 (1.86)	115.54 (1.83)	−5.72 (0.61) ***	−4.53 (0.51) ***
LDL-CR	86.70 (1.63)	91.23 (1.59)	−4.53 (0.54) ***	−4.53 (0.58) ***
LDL-C1 (Largest)	19.48 (0.54)	20.57 (0.53)	−1.09 (0.18) ***	−5.39 (0.82) ***
LDL-C2	28.04 (0.89)	28.88 (0.87)	−0.84 (0.29) **	−1.52 (1.27)
LDL-C3	30.49 (0.90)	32.79 (0.88)	−2.29 (0.30) ***	−6.62 (0.88) ***
LDL-C4 (Smallest)	8.71 (0.38)	9.02 (0.37)	−0.31 (0.13) *	−1.28 (2.11)
LDL Pattern [d], %			***	
A	82.27	80.30	+1.97	+2.39
A/B	5.44	7.60	−2.16	−39.7
B	12.29	12.10	+0.19	+1.55
IDL-C	14.47 (0.39)	15.74 (0.38)	−1.27 (0.13) ***	−7.84 (0.74) ***
VLDL-C	23.98 (0.47)	23.32 (0.46)	+0.67 (0.16) ***	+3.46 (0.62) ***
VLDL-C1+2	10.53 (0.26)	9.85 (0.25)	+0.68 (0.09) ***	+7.39 (0.81) ***
VLDL-C3	13.45 (0.23)	13.47 (0.22)	−0.02 (0.08)	+0.60 (0.53)
HDL-C	61.94 (0.94)	62.38 (0.92)	−0.44 (0.31)	−0.74 (0.47)
HDL-C2	18.81 (0.48)	18.14 (0.47)	+0.67 (0.16) ***	+4.11 (0.83) **
HDL-C3	43.14 (0.52)	44.25 (0.51)	−1.11 (0.17) ***	−2.50 (0.37) ***
Lipoprotein (a)-C	8.75 (0.27)	8.68 (0.26)	+0.07 (0.09)	+1.61 (0.94)

[a]VAP is the vertical autoprofile method; 4402 nonfasting and 8349 fasting participants had VAP measurements performed. LDL-C denotes low density lipoprotein cholesterol, LDL-C1 to LDL-C4 represent subfractions of low density lipoprotein in decreasing size, LDL-CR represents real LDL cholesterol values calculated as LDL-C minus IDL-C minus Lp (a)-C IDL-C denotes intermediate low density lipoprotein cholesterol, VLDL-C; very low density lipoprotein cholesterol, VLDL-C1+2 represents cholesterol concentration of large and medium sized VLDL and includes chylomicrons (CM) and CM remnants, VLDL-C3 is the cholesterol concentration of the smallest subfraction of VLDL measured by VAP and HDL-C represents high density lipoprotein cholesterol. HDL-C2 and HDL-C3 are subfractions of HDL in order of decreasing size. Non-HDL-C represents non-HDL cholesterol and lipoprotein (a)-C, lipoprotein (a) cholesterol.

*[b]*p*-value <0.05

** *p*-value <0.001

*** *p*-value<0.0001.

[c] Percent difference was calculated with log transformed lipid/apolipoprotein data.

[d] $\chi 2$ analysis was performed for all 3 patterns.

All models were adjusted for age, race, gender, diabetes, hypertension, cholesterol medication use, smoking history, postmenopausal hormone use and body mass index.

Table 3.

Adjusted Mean Concentrations of Size Separated Lipoprotein Particles by Ion Mobility in Nonfasting and Fasting Participants

Particle Concentrations (nmol/L) by IM	Nonfasting (Mean) (SE) N=5262	Fasting (Mean) (SE) N=10,135	Absolute Difference, Nonfasting minus Fasting (SE) [a]	Percent Difference, Nonfasting minus Fasting [b]
LDL Particles				
Total LDL-P	993.74 (15.10)	1021.93 (14.78)	−28.20 (4.97) ***	−2.57 (0.46) ***
LDL-P Large a	218.77 (4.72)	220.86 (4.62)	−2.09 (1.55)	−1.54 (0.87)
LDL-P Large b	146.69 (3.01)	149.73 (2.95)	−3.04 (0.99) **	−1.74 (0.62) **
LDL-P Medium	197.33 (4.21)	205.66 (4.12)	−8.32 (1.38) ***	−3.68 (0.62) ***
LDL-P Small	155.69 (4.26)	164.03 (4.17)	−8.34 (1.40) ***	−4.63 (0.74) ***
LDL-P Very Small a	66.18 (2.23)	69.38 (2.18)	−3.21 (0.73) ***	−4.03 (0.82) ***
LDL-P Very Small b	71.36 (2.19)	73.24 (2.14)	−1.87 (0.72) **	−2.20 (0.72) **
LDL-P Very Small c	72.80 (1.51)	74.02 (1.48)	−1.21 (0.50) *	−1.54 (0.60) *
LDL-P Very Small d	64.51 (1.09)	64.62 (1.07)	−0.11 (0.38)	0.070 (0.56)
Low Density Lipoprotein Peak Size (Angstroms)	219.95 (0.29)	219.56 (0.28)	+0.38 (0.09) ***	+0.18 (0.04) ***
IDL Particles				
Total IDL-p	257.06 (4.41)	264.24 (4.31)	−7.18 (1.45) ***	−2.66 (0.50) ***
IDL-P Large	113.96 (2.00)	118.41 (1.96)	−4.45 (0.66) ***	−3.57 (0.52) ***
IDL-P Small	132.92 (2.90)	135.67 (2.83)	−2.75 (1.01) **	−1.82 (0.61) **
VLDL Particles				
Total VLDL-P	94.02 (1.91)	92.62 (1.87)	+1.40 (0.63) *	+1.95 (0.69) **
VLDL-P Large	10.08 (0.32)	9.06 (0.31)	1.02 (0.11) ***	+12.28 (1.32) ***
VLDL-P Medium	35.64 (0.80)	34.12 (0.79)	+1.52 (0.26) ***	+5.25 (0.82) ***
VLDL-P Small	48.31 (0.92)	49.45 (0.90)	−1.14 (0.30) **	−2.09 (0.59) **
HDL Particles				
Total HDL-P	24847 (235)	24843 (230)	+3.56 (77)	0.00 (0.31)
HDL-P Large	6457 (83)	6329 (82)	+127 (27) ***	+2.02 (0.43) ***
HDL-P Small	18390 (177)	18514 (173)	−124 (58) *	−0.69 (0.31) *
Non-HDL Particles				
Total Non-HDL-P	1355 (22)	1390 (22)	−36 (7) ***	−2.33 (0.44) ***

VLDL-P represents very low density lipoprotein particle concentration, IDL-P; intermediate low density lipoprotein particle concentration, LDL-P denotes low density lipoprotein particle concentration and HDL-P represents high density lipoprotein particle concentration. Non-HDL-P represents non-HDL particle concentration. All lipoprotein subfractions are presented in order of decreasing size.

*[a] *p*-value <0.05

** *p*-value <0.001

*** *p*-value<0.0001.

[b]Percent difference was calculated with log transformed lipid/lipoprotein data. All models adjusted for age, race, gender, diabetes, hypertension, cholesterol medication use, smoking history, postmenopausal hormone use and body mass index.