Running title: The associations of oxidized lipoprotein lipids with lipoprotein subclass particle concentrations and their lipid compositions. The Cardiovascular Risk in Young Finns Study.

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Abbreviations: $oxHDL_{lipids}$=oxidized HDL lipids, $oxLDL_{lipids}$=oxidized LDL lipids, Apo-B=Apolipoprotein-B, Apo-A1=Apolipoprotein-A1, NMR=Nuclear magnetic resonance, CVD=cardiovascular disease, L=lipids, C=cholesterol, FC= Free cholesterol, CE=Cholesterol esters, PL=phospholipids, TG=triglycerides, HDL=high-density lipoprotein, LDL=low-density lipoprotein, IDL=intermediate-density lipoprotein, VLDL=very low-density lipoprotein.

Abstract

Objective: Oxidation of low-density lipoprotein (LDL) may promote atherosclerosis, whereas the reverse transport of oxidized lipids by high-density lipoprotein (HDL) may contribute to atheroprotection. To provide insights into the associations of lipoprotein lipid oxidation markers with lipoprotein subclasses at the population level, we investigated the associations of oxidized HDL lipids ($oxHDL_{lipids}$) and oxidized LDL lipids ($oxLDL_{lipids}$) with lipoprotein subclasses in a population-based cross-sectional study of 1395 Finnish adults ages 24-39 years.

Methods: The analysis of oxidized lipids was based on the determination of the baseline level of conjugated dienes in lipoprotein lipids. A high-throughput nuclear magnetic resonance (NMR) platform was used to quantify circulating lipoprotein subclass concentrations and analyze their lipid compositions.

Results: $OxHDL_{lipids}$ were mainly not associated with lipoprotein subclass lipid concentrations and lipid composition after adjustment for Apolipoprotein-A1 (Apo-A1), waist circumference and age. $OxLDL_{lipids}$ were associated with several markers of lipoprotein subclass lipid concentrations and composition after adjustment for Apolipoprotein-B (Apo-B), age and waist circumference. Several measures of HDL and LDL subclasses, including phospholipid and triglyceride composition, associated directly with $oxLDL_{lipids.}$ Cholesterol ester and free cholesterol composition in HDL and LDL associated inversely with $oxLDL_{lipids}$.

Conclusion: We conclude that these results do not support the idea that HDL´s particle size or composition would reflect its functional capacity in the reverse transport of oxidized lipids. On the contrary, $oxLDL_{lipids}$ were associated with the entire lipoprotein subclass profile, including numerous associations with the compositional descriptors of the particles. This is in line with the suggested role of LDL oxidation in atherogenesis.

Key words: HDL, LDL, Oxidized lipids, Atherosclerosis

Introduction

Low high-density lipoprotein (HDL) cholesterol levels and high low-density lipoprotein (LDL) levels are risk factors for cardiovascular diseases (CVDs) [1]. The main possible atheroprotective functions of HDL are unclear. HDL has been suggested to play an important role in the reverse cholesterol transport [2]. In addition, it may have anti-inflammatory [3], antioxidant [4,5], and antidiabetic [6] effects. According to the oxidation hypothesis of atherosclerosis, oxidized LDL plays an important role in the pathogenesis of atherosclerosis [7]. Oxidized LDL lipids ($oxLDL_{lipids}$) are associated with several risk factors for atherosclerosis, including hypertension [8], waist circumference [9] and impaired insulin sensitivity [10] and $oxLDL_{lipids}$ are also associated with coronary atherosclerosis [11]. The role of LDL is to transport oxidized lipids toward peripheral tissues. It has been hypothesized that the oxidized lipid transporting capacity of HDL is an independent protective factor for CVDs [12]. Moreover, it has been speculated that the ratio of oxidized HDL lipids ($oxHDL_{lipids}$)/$oxLDL_{lipids}$ could be a marker of HDL´s capacity to transport oxidized lipids [12]. However, it is controversial, whether oxidized HDL or LDL could be used as a risk factor for CVDs [12]. Therefore, there has been recently increased interest to understand the functional properties of HDL in attempt to develop new antiatherogenic therapies [13,14]. Lipoproteins´ main function is to transport lipids in circulation. HDL is a heterogeneous particle with various subpopulations, which differ in density, shape, size, composition, surface lipid and apolipoprotein composition [15,16]. Phospholipids constitute the major lipid class of HDL accounting for 30 % of total HDL mass. Commonly is known that phospholipids cover the surface of HDL together with free cholesterol and apolipoproteins. Cholesteryl esters and triglycerides form the hydrophobic lipid core of HDL. [17] However, cholesterol esters and triglycerides may also locate in the surface of lipoprotein particles [18]. The amount of these “core lipids” that locate in the surface of lipoproteins is higher in the smaller particles [18]. The clinical importance of HDL subpopulations is unclear, but distinct HDL subpopulations show different associations with the risk of CVD [15]. Evidence suggests that large, phospholipid-rich HDL particles promote reverse cholesterol transport [19]. It is moreover suggested that large HDL particles are atheroprotective, because increasing HDL particle size was associated with lower coronary artery disease risk [20]. However, after adjustment for markers

associated with metabolic syndrome, the significant association between HDL particle size and coronary artery disease was diluted [20]. HDL particle profile may therefore play a role in the cardioprotective effects of HDL [21].

To investigate interrelationships between lipoprotein lipid oxidation markers and lipoprotein composition and size, we studied the associations of oxidized HDL and LDL lipids with lipoprotein subclasses measured by nuclear magnetic resonance (NMR) spectroscopy in the Cardiovascular Risk in Young Finns Study. The aim was to gain information of the role of compositional descriptors of lipoprotein particles in the putative reverse transport of oxidized lipids.

Methods

The Cardiovascular Risk in Young Finns Study is a multicenter follow-up study in five cities and their rural surroundings in Finland to evaluate atherosclerotic risk factors from childhood into adulthood. The study began in 1980 and a total of 2284 subjects aged 24-39 years participated in 2001 follow-up. Participants were randomly selected from the national register and they gave written informed consent. The study was approved by local ethics committees. Details of the study design have been presented earlier [22].

Venous blood samples were collected after fasting. The analysis of oxidized HDL and LDL lipids was based on the determination of the baseline level of conjugated dienes in lipoprotein lipids [23]. The serum HDL fraction ($oxHDL_{lipids}$) was isolated with phosphotungstic acid precipitation [24] and serum LDL fraction ($oxLDL_{lipids}$) with buffered heparin [23]. Lipids were extracted from the isolated lipoproteins by chloroform-methanol (2:1), dried under nitrogen and redissolved in cyclohexane. The amount of oxidized lipids was assessed spectrophotometrically at 234 nm [25] in the supernatant fraction to measure $oxHDL_{lipids}$, and in the precipitate fraction to measure $oxLDL_{lipids}$. The isolation procedures were validated for the purpose and did not affect the level of oxidized lipids [23]. Apolipoproteins A-1 (Apo-A1) and B (Apo-B) were analyzed immunoturbidometrically (Orion Diagnostica, Espoo, Finland). A high-throughput NMR platform was used to quantify serum HDL and LDL subclasses [26]. NMR-based metabolic profiling has been used in in various epidemiological studies [16,27]. Details of the method have been presented earlier [28,29]. Waist circumference was measured at the level of umbilicus to an accuracy of 0.1 cm.

Statistical analyses

The normality assumptions were evaluated by examining histograms and normal probability plots. Variables with skewed distributions were log-transformed. T-test was used to examine differences in main characteristics between sexes. Because of significant characteristic differences between sexes, subsequent analyses were done sex wise (Table 1). Statistical significance was inferred at a two-tailed $P<0.05$. Multivariable mixed models for $oxHDL_{lipids}$ and NMR variables were adjusted for Apo-A1,

waist circumference and age, and models for $oxLDL_{lipids}$ were adjusted for Apo-B, waist circumference and age. Mixed models for $oxHDL_{lipids}$ / $oxLDL_{lipids}$ ratio were adjusted for Apo-A1/Apo-B ratio, waist circumference and age. Covariates in the multivariable mixed models were selected based on the earlier findings regarding the associations of oxidized lipoprotein lipids with risk factors for atherosclerosis [12]. Regression coefficients are reported in standardized units of 1-SD difference in oxidized HDL or LDL lipoprotein lipids per 1-SD difference in outcome variable. Statistical analyses were performed to subjects who had data on $oxHDL_{lipids}$ measured in the 2001 follow-up. Subjects who were pregnant were excluded. A total of 1395 subjects of 2001 follow-up were included in the final analyses. All statistical analyses were performed using Statistical Analysis System (SAS, version 9.4). Multiple testing corrections were performed using the Bonferroni method, with $P < 0.0004$ considered statistically significant.

Results

Main characteristics of the study subjects in 2001 are presented in Table 1. Women had higher levels of $oxHDL_{lipids}$, Apo-A1 and total lipids in all HDL particles except for small HDL particles than in men. Also in women, diameter for HDL was larger than in men. Accordingly, women had lower levels of $oxLDL_{lipids}$, total lipids in all Apo-B containing particles, and smaller VLDL and LDL mean diameters than men. The rest of NMR variable characteristics of the study subjects are presented in the Supplement Table 1.

To gain insights into the associations between lipoprotein oxidation and lipoprotein subclass particle and lipid concentrations and composition, we studied the associations of $oxHDL_{lipids}$ and $oxLDL_{lipids}$ and their ratio ($oxHDL_{lipids}/oxLDL_{lipids}$) with HDL, VLDL, IDL, and LDL subclass particle and lipid concentrations (Figure 1, Supplement Tables 2 and 3), and also with lipid composition of the lipoprotein subclasses (Figure 2, Supplement Tables 4 and 5). The unadjusted results are presented in the Supplemental materials (Figures 1 and 2, Supplement Tables 6, 7, 8 and 9).

The associations of $oxHDL_{lipids}$ with lipoprotein subclass particle and lipid concentrations (Figure 1).

$OxHDL_{lipids}$ were mainly not associated with lipoprotein subclass particle and lipid concentrations (Figure 1 and Supplement Tables 2 and 3).

The associations of $oxLDL_{lipids}$ with lipoprotein subclass particle and lipid concentrations (Figure 1).

Associations with HDL. In both sexes, $oxLDL_{lipids}$ were directly associated with concentration, total lipids, triglycerides, phospholipids and phospholipids/total cholesterol ratio in medium and small HDL particles (Figure 1 and Supplement Tables 2 and 3). Also in both sexes phospholipids in very large HDL particles associated inversely with $oxLDL_{lipids}$. There were additionally several inverse significant associations for $oxLDL_{lipids}$ in men including mean HDL size, total lipids in very large HDL particles, free cholesterol in very large and large HDL particles, and also total HDL cholesterol.

Associations with Apo-B containing particles (VLDL, IDL and LDL). There was a clear tendency that concentrations of VLDL subclass particles; and total lipid, total cholesterol, free cholesterol, cholesterol esters, triglycerides, phospholipids and phospholipid/total cholesterol ratio concentrations were directly associated with $oxLDL_{lipids,}$ except for small and very small VLDL particles. These associations were mainly similar in both sexes. Mean diameter for VLDL associated directly with $oxLDL_{lipids}$ in both sexes. There was additionally a significant inverse association between $oxLDL_{lipids}$ and mean diameter for HDL and LDL in men (Figure 1).

The associations of $oxHDL_{lipids}/oxLDL_{lipids}$ ratio with lipoprotein subclass particle and lipid concentrations (Figure 1).

Associations with HDL. The trend was that concentrations of free cholesterol, cholesterol esters and phospholipids in very large and large HDL particles associated directly with $oxHDL_{lipids}/oxLDL_{lipids}$ ratio (Figure 1 and Supplement Tables 2 and 3). On the contrary, free cholesterol, cholesterol esters and phospholipids in medium and small HDL particles associated inversely with $oxHDL_{lipids}/oxLDL_{lipids}$ ratio. Only concentrations of triglycerides in all HDL particle subclasses associated inversely with $oxHDL_{lipids}/oxLDL_{lipids}$ ratio. These results were similar in both sexes.

Associations with Apo-B containing particles. Concentrations of Apo-B containing particles were mostly inversely associated with $oxHDL_{lipids}/oxLDL_{lipids}$ ratio in both sexes.

The associations of $oxHDL_{lipids}$ with lipid composition of lipoprotein subclass particles (Figure 2).

Associations with HDL. In men, total cholesterol and cholesterol esters in very large HDL particles associated inversely with $oxHDL_{lipids}$, and phospholipids in very large HDL particles associated directly with $oxHDL_{lipids}$. In women, only free cholesterol in very large HDL particles had an inverse association with $oxHDL_{lipids}$.

Associations with Apo-B containing particles. Lipid composition in Apo-B containing particles was not significantly associated with $oxHDL_{lipids}$ in men. In women, triglyceride composition in all LDL particles associated directly, and total cholesterol in all LDL particles, free cholesterol in very small VLDL and IDL, and cholesterol esters in large LDL associated inversely with $oxHDL_{lipids}$.

The associations of $oxLDL_{lipids}$ with lipid composition of lipoprotein subclass particles (Figure 2).

Associations with HDL. There was a trend for inverse association of $oxLDL_{lipids}$ with total cholesterol, free cholesterol and cholesterol esters in HDL particle subclasses. Triglyceride and phospholipid composition in HDL particle subclasses were direct correlates of $oxLDL_{lipids}$. The associations were mostly similar between sexes.

Associations with Apo-B containing particles. There was a trend that $oxLDL_{lipids}$ associated inversely with total cholesterol, free cholesterol and cholesterol esters; and directly with triglycerides in Apo-B containing particles. Phospholipid composition in VLDL subclasses associated inversely and in LDL subclasses directly with $oxLDL_{lipids.}$ These results were mainly similar in both sexes.

The associations of $oxHDL_{lipids}/oxLDL_{lipids}$ ratio with lipid composition of lipoprotein subclass particles (Figure 2).

Associations with HDL. There was a clear tendency in both sexes that total cholesterol and cholesterol ester compositions in HDL particle subclasses associated directly with $oxHDL_{lipids}/oxLDL_{lipids}$ ratio and triglyceride composition associated inversely. In men, large HDL phospholipid composition associated inversely with $oxHDL_{lipids}/oxLDL_{lipids}$ ratio and free cholesterol composition in large HDL particles associated directly with the oxidized lipid ratio.

Associations with Apo-B containing particles. The associations between $oxHDL_{lipids}/oxLDL_{lipids}$ ratio and lipid composition of lipoprotein subclass particles were quite similar between sexes. Trend was that total cholesterol composition was a direct and triglyceride composition was an inverse correlate for oxidized lipid ratio.

Discussion

The possible atheroprotective functions of HDL and the speculative atheroprotective significance of different HDL subpopulations are unknown. We observed in this study that $oxHDL_{lipids}$ are not associated with lipoprotein lipid subclass concentration and composition after adjustment for Apo-A1, age and waist circumference. It seems therefore that oxidized lipids in HDL are associated with HDL particle amount, but not with HDL subclass composition and concentration. Oxidized lipids in LDL particles are however associated with lipoprotein subclasses after adjustment for Apo-B, waist circumference and age.

HDL cholesterol is considered as an independent risk factor for CVD [1,30]. However, therapies raising HDL cholesterol levels have not been beneficial in clinical trials [31,32]. In addition, genetically determined high serum HDL cholesterol levels are not associated with reduced CVD risk [33]. Therefore, the current view is that HDL cholesterol levels *per se* are not causally associated with CVDs. HDL is a heterogeneous particle varying in its density, shape, size, surface and apolipoprotein composition [15]. It may have numerous atheroprotective functions [2–4,6]. Interest has therefore shifted from HDL cholesterol concentration into the functional properties of HDL [13]. The central hypothesis states that HDL is active in the reverse transport of atherogenic oxidized lipids [25], and this capacity is speculated as an important mechanism in atheroprotection [12,25]. Moreover, it has been suggested that the level of $oxHDL_{lipids}$ may be an indicator of HDL´s capacity to remove oxidized lipids from the body [12,34]. Earlier findings indicate that large HDL particles are inversely associated with CVD risk [20]. Therefore, we hypothesized that larger HDL particle size and lipid composition would be associated with higher $oxHDL_{lipids}$ levels. However, we found no evidence for such relations in the present study. Thus, these data do not support the idea that HDL´s particle size would reflect its functional capacity in the reverse transport of oxidized lipids. This may also be partly explained by the fact that HDL cholesterol levels are not causally associated with reduced CVD risk [33]. However, this study was cross-sectional and therefore these results should be taken with caution. A possible functional role for the HDL oxidation was suggested in an earlier intervention study that demonstrated that the intensity of physical exercise was associated with the accumulation of oxidized lipids in HDL [25].

Large HDL particles containing high amounts of lipids, particularly phospholipids, are shown to enhance scavenger receptor-B1 mediated free cholesterol flux from cells compared with small lipid-poor HDL particles [19]. We observed in this study that in men phospholipid composition in very large HDL particles is associated with higher levels of oxHDLlipids. Higher phospholipid composition in HDL particle results in greater lipid surface area and may consequently explain the direct association between $oxHDL_{lipids}$ and phospholipid composition of HDL particle. It is possible that such HDL composition could promote the ability of HDL to transport oxidized lipids. In addition, large lipid-rich HDL particles are suggested to promote cholesterol efflux via adenosine triphosphate-binding cassette transporter G1 [35]. In line with this, we have earlier observed that higher HDL cholesterol levels are directly associated with higher levels of $oxHDL_{lipids}$ [12]. We additionally found that triglycerides in medium HDL particles are associated with $oxHDL_{lipids}$ in women. In line with this, we have earlier detected a significant association between triglycerides and $oxHDL_{lipids}$ in women [12]. There was additionally a strong direct association between $oxLDL_{lipids}$ and triglyceride composition in all lipoproteins. This may be explained due to the fact that triglycerides contain three fatty acids, which are vulnerable for oxidative modifications. In comparison, cholesterol esters contain only one fatty acid and phospholipids two fatty acids. We have previously shown that $oxLDL_{lipids}$ are indirectly associated with serum polyunsaturated fatty acids and directly with serum saturated fatty acids [36]. High LDL cholesterol is a well-known risk factor for CVDs [1,37] and $oxLDL_{lipids}$ are shown to be associated with an increased risk for atherosclerosis [34,8]. The trend for the associations of $oxLDL_{lipids}$ with the markers of lipoprotein subclasses were apparently different than the associations of $oxHDL_{lipids}$ with the same markers. The associations between $oxLDL_{lipids}$ and lipoprotein subclass variables were mostly significant and $oxHDL_{lipids}$ were not associated with LDL subclasses. The lack of associations between $oxHDL_{lipids}$ and characteristics of LDL particles suggests that there is no close interrelation between these two in vivo. In line with this, it has been shown that in vitro HDL do not protect LDL from oxidation [38]. It seems, however, that $oxLDL_{lipids}$ are associated with lipoprotein particle size and composition. This finding may partly explain why $oxLDL_{lipids}$ are associated with an increased CVD risk. However, based on the present cross-sectional study, we are not able to discuss about the potential role of $oxLDL_{lipids}$ to act as a cardiovascular risk factor. We found additionally

some differences, when results were compared between sexes. For example, men but not women show a consistent relationship between $oxLDL_{lipids}$ and phospholipid concentrations in LDL and IDL subclass particles. It is difficult to explain these results, but it might be related to the fact that women have higher HDL cholesterol levels and lower LDL cholesterol levels than men [12], which may be reflected to the associations between oxidized lipoprotein lipids and lipoprotein subclass particle concentrations.

We have earlier detected that higher levels of $oxHDL_{lipids}$ are associated with higher levels of $oxLDL_{lipids}$ [12]. One possible explanation for this observation is that $oxHDL_{lipids}$ may be an indicator of HDL´s capacity to remove oxidized lipids from LDL particles [12]. This is in accordance with the oxidized lipid removing function of HDL. $OxHDL_{lipids}/oxLDL_{lipids}$ ratio is moreover speculated to be a better marker of HDL´s capacity to transport oxidized lipids than $oxHDL_{lipids}$ alone [12]. In the present study, $oxHDL_{lipids}/oxLDL_{lipids}$ ratio associated widely with lipoprotein subclass concentration and composition markers. It is highly likely that the associations between $oxHDL_{lipids}/oxLDL_{lipids}$ ratio and lipoprotein subclasses are due to effect of $oxLDL_{lipids}$. The effect of $oxHDL_{lipids}$ seems to be non-significant. These findings are in line with the suggestion that Apo-B plays a key role in the chain leading to atherosclerosis [39].

Limitations

Lipoproteins are molecular complexes. Determination of oxidized lipoproteins is challenging due to the heterogeneous nature of the chemistry of HDL and LDL oxidation. Consequently, the results on lipid oxidation should be taken with caution. Important strength of the study is that the baseline diene conjugation method is validated in detail [23]. The conjugated diene measurement provides a general measure of fatty acid oxidation, but exclude analysis of oxysterols not isoprostanes, which may contribute e.g. to inflammation. In addition, some oxidized phospholipids may exert anti-inflammatory properties. Therefore, a global measure of conjugated dienes used here does not comprehensively report on the potential range of functions from oxidized lipids. However, since the present study, to our knowledge, is the first to investigate lipoprotein oxidation and lipoprotein particle concentrations/lipid compositions in a population-based study, a general measure of lipid

oxidation was considered most adequate. With this method, oxidative modifications in all lipid classes, including phospholipids, are measured. This study was cross-sectional. Therefore, this data do not allow assessing the causality of the observed associations. However, complete lack of cross-sectional links between $oxHDL_{lipid}$ levels and HDL´s particle size and composition indicate that these parameters do not reflect HDL´s functional capacity in the reverse transport of oxidized lipids. The Young Finns Study participants are still relatively young. Therefore, we are at this point unable to examine what is the role of the measured lipid markers in predicting atherosclerotic events. However, in the future, it would be important to study the role of lipoprotein oxidation markers with the cardiovascular risk factors in the prospective setting.

Conclusion

Our results in the present study show that higher $oxHDL_{lipid}$ levels are not associated with lipoprotein subclass lipid concentration and composition. These findings do not support the idea that HDL´s particle size or composition would reflect its functional capacity in the reverse transport of oxidized lipids. Lipid oxidation in LDL particles is however associated with lipoprotein subclass lipid composition and concentration.

Conflict of interest

No conflict of interest was declared.

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References

1. Briel, M. *et al.* Association between change in high density lipoprotein cholesterol and cardiovascular disease morbidity and mortality: Systematic review and meta-regression analysis. *BMJ* (2009) doi:10.1136/bmj.b92.
2. Khera, A. V. *et al.* Cholesterol efflux capacity, high-density lipoprotein function, and atherosclerosis. *N. Engl. J. Med.* (2011) doi:10.1056/NEJMoa1001689.
3. Murphy, A., Chin-Dusting, J. P., Sviridov, D. & Woollard, K. The Anti Inflammatory Effects of High Density Lipoproteins. *Curr. Med. Chem.* (2009) doi:10.2174/092986709787458425.
4. Navab, M. *et al.* Monocyte transmigration induced by modification of low density lipoprotein in cocultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis and is abolished by high density lipoprotein. *J. Clin. Invest.* (1991) doi:10.1172/JCI115532.
5. Brites, F., Martin, M., Guillas, I. & Kontush, A. Antioxidative activity of high-density lipoprotein (HDL): Mechanistic insights into potential clinical benefit. *BBA Clinical* (2017) doi:10.1016/j.bbacli.2017.07.002.
6. Drew, B. G. *et al.* High-density lipoprotein modulates glucose metabolism in patients with type 2 diabetes mellitus. *Circulation* (2009) doi:10.1161/CIRCULATIONAHA.108.843219.
7. Ross, R. The pathogenesis of atherosclerosis: A perspective for the 1990s. *Nature* (1993) doi:10.1038/362801a0.
8. Toikka, J. O. *et al.* Increased arterial intima-media thickness and in vivo LDL oxidation in young men with borderline hypertension. *Hypertension* (2000) doi:10.1161/01.HYP.36.6.929.
9. Vasankari, T. *et al.* Reduced mildly oxidized LDL in young female athletes. *Atherosclerosis* (2000) doi:10.1016/S0021-9150(99)00401-3.
10. Linna, M. S., Ahotupa, M., Kukkonen-Harjula, K., Fogelholm, M. & Vasankari, T. J. Co-existence of insulin resistance and high concentrations of circulating oxidized LDL lipids. *Ann. Med.* (2015) doi:10.3109/07853890.2015.1043939.
11. Vasankari, T. *et al.* Oxidized LDL and thickness of carotid intima-media are associated with coronary atherosclerosis in middle-aged men: Lower levels of oxidized LDL with statin therapy. *Atherosclerosis* (2001) doi:10.1016/S0021-9150(00)00573-6.
12. Kresanov, P. *et al.* The associations of oxidized high-density lipoprotein lipids with risk factors for atherosclerosis: The Cardiovascular Risk in Young Finns Study. *Free Radic. Biol. Med.* (2013) doi:10.1016/j.freeradbiomed.2013.09.023.
13. Asztalos, B. F., Tani, M. & Schaefer, E. J. Metabolic and functional relevance of HDL subspecies. *Curr. Opin. Lipidol.* (2011) doi:10.1097/MOL.0b013e3283468061.
14. Sirtori, C. R. *et al.* HDL therapy today: from atherosclerosis, to stent compatibility to heart failure. *Annals of Medicine* (2019) doi:10.1080/07853890.2019.1694695.
15. Rye, K.-A. & Barter, P. J. Cardioprotective functions of HDLs. *J. Lipid Res.* (2014) doi:10.1194/jlr.r039297.
16. Würtz, P. *et al.* Metabolite profiling and cardiovascular event risk: A prospective study of 3 population-based cohorts. *Circulation* (2015) doi:10.1161/CIRCULATIONAHA.114.013116.
17. Toth, P. P. *et al.* High-density lipoproteins: A consensus statement from the National Lipid Association. *J. Clin. Lipidol.* (2013) doi:10.1016/j.jacl.2013.08.001.
18. Kumpula, L. S. *et al.* Reconsideration of hydrophobic lipid distributions in lipoprotein particles. *Chem. Phys. Lipids* (2008) doi:10.1016/j.chemphyslip.2008.06.003.
19. Yancey, P. G. *et al.* High density lipoprotein phospholipid composition is a major determinant of the bi-directional flux and net movement of cellular free cholesterol mediated by scavenger receptor BI. *J. Biol. Chem.* (2000) doi:10.1074/jbc.M006924200.
20. El Harchaoui, K. *et al.* High-density lipoprotein particle size and concentration and coronary risk. *Ann. Intern. Med.* (2009) doi:10.7326/0003-4819-150-2-200901200-00006.
21. Heinecke, J. W. The HDL proteome: A marker-and perhaps mediator-of coronary artery disease. *Journal of Lipid Research* (2009) doi:10.1194/jlr.R800097-JLR200.
22. Raitakari, O. T. *et al.* Cohort profile: The cardiovascular risk in young Finns study. *Int. J.*

Epidemiol. (2008) doi:10.1093/ije/dym225.
23. Ahotupa, M. *et al.* Baseline diene conjugation in LDL lipids as a direct measure of in vivo LDL oxidation. *Clin. Biochem.* (1998) doi:10.1016/S0009-9120(98)00018-6.
24. Väisänen, S., Gävert, J., Julkunen, A., Ainen, E. V. & Penttilä, I. Contents of apolipoprotein a-i, a-II and b of the human serum fractions for high-density and low-density lipoproteins prepared by common precipitation methods. *Scand. J. Clin. Lab. Invest.* (1992) doi:10.3109/00365519209088391.
25. Ahotupa, M., Suomela, J. P., Vuorimaa, T. & Vasankari, T. Lipoprotein-specific transport of circulating lipid peroxides. *Ann. Med.* (2010) doi:10.3109/07853890.2010.510932.
26. Soininen, P. *et al.* High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. *Analyst* (2009) doi:10.1039/b910205a.
27. Würtz, P. *et al.* Metabolic signatures of insulin resistance in 7,098 young adults. *Diabetes* (2012) doi:10.2337/db11-1355.
28. Soininen, P., Kangas, A. J., Würtz, P., Suna, T. & Ala-Korpela, M. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circ. Cardiovasc. Genet.* (2015) doi:10.1161/CIRCGENETICS.114.000216.
29. Würtz, P. *et al.* Quantitative Serum Nuclear Magnetic Resonance Metabolomics in Large-Scale Epidemiology: A Primer on -Omic Technologies. *Am. J. Epidemiol.* (2017) doi:10.1093/aje/kwx016.
30. Di Angelantonio, E., Sarwar, N. & Perry, P. Emerging risk factors collaboration: Major Lipids, Apolipoproteins, and Risk of Vascular Disease. *JAMA-J Am Med Assoc.* (2009) doi:10.1016/s0749-4041(10)79320-9.
31. Barter, P. J. *et al.* Effects of torcetrapib in patients at high risk for coronary events. *N. Engl. J. Med.* (2007) doi:10.1056/NEJMoa0706628.
32. Soran, H., Schofield, J. D. & Durrington, P. N. Antioxidant properties of HDL. *Front. Pharmacol.* (2015) doi:10.3389/fphar.2015.00222.
33. Voight, B. F. *et al.* Plasma HDL cholesterol and risk of myocardial infarction: A mendelian randomisation study. *Lancet* (2012) doi:10.1016/S0140-6736(12)60312-2.
34. Ahotupa, M. Oxidized lipoprotein lipids and atherosclerosis. *Free Radical Research* (2017) doi:10.1080/10715762.2017.1319944.
35. Favari, E. *et al.* Small discoidal pre-β1 HDL particles are efficient acceptors of cell cholesterol via ABCA1 and ABCG1. *Biochemistry* (2009) doi:10.1021/bi901564g.
36. Kaikkonen, J. E. *et al.* High serum n6 fatty acid proportion is associated with lowered LDL oxidation and inflammation: The Cardiovascular Risk in Young Finns Study. *Free Radic. Res.* (2014) doi:10.3109/10715762.2014.883071.
37. Gordon, T., Castelli, W. P., Hjortland, M. C., Kannel, W. B. & Dawber, T. R. High density lipoprotein as a protective factor against coronary heart disease. The Framingham study. *Am. J. Med.* (1977) doi:10.1016/0002-9343(77)90874-9.
38. Solakivi, T. *et al.* HDL enhances oxidation of LDL in vitro in both men and women. *Lipids Health Dis.* (2005) doi:10.1186/1476-511X-4-25.
39. Ala-Korpela, M. The culprit is the carrier, not the loads: Cholesterol, triglycerides and apolipoprotein B in atherosclerosis and coronary heart disease. *International Journal of Epidemiology* (2019) doi:10.1093/ije/dyz068.

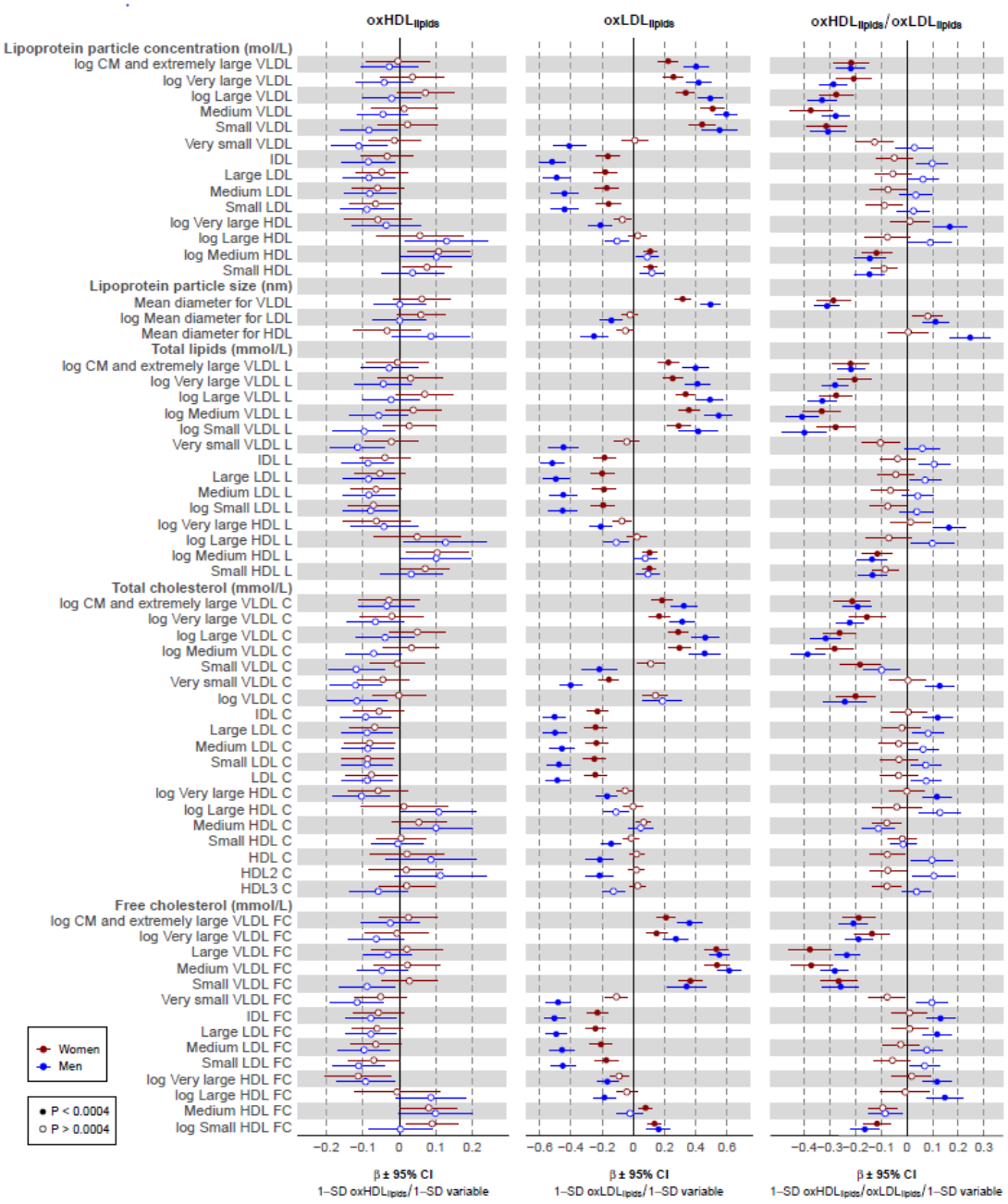
Table 1. Main characteristics of the study subjects.

	Women N=766		Men N=629	
Variables	Mean	SD	Mean	SD
Age (yrs)	31.5	5.0	31.6	5.1
Waist circumference (cm)*	78.6	10.9	89.7	10.7
oxHDLlipids (µmol/L)*	29.763	7.855	27.921	7.424
oxLDLlipids (µmol/L)*	24.629	8.396	28.962	11.333
Apo-A1 (g/L)*	1.557	0.259	1.404	0.206
Apo-B (g/L)*	0.985	0.226	1.139	0.273
Lipoprotein total lipids (mmol/L)				
CM and extremely large VLDL*	0.019	0.020	0.036	0.038
Very large VLDL*	0.044	0.053	0.089	0.096
Large VLDL*	0.184	0.179	0.338	0.300
Medium VLDL*	0.476	0.270	0.722	0.419
Small VLDL*	0.547	0.209	0.715	0.260
Very small VLDL*	0.538	0.145	0.588	0.161
IDL*	1.257	0.301	1.303	0.341
Large LDL*	1.447	0.364	1.536	0.415
Medium LDL*	0.806	0.222	0.883	0.257
Small LDL*	0.506	0.135	0.555	0.156
HDL*	3.854	0.889	3.115	0.690
Very large HDL*	0.485	0.245	0.287	0.207
Large HDL*	1.002	0.408	0.590	0.353
Medium HDL*	1.136	0.279	1.007	0.217
Small HDL	1.231	0.196	1.231	0.168
Lipoprotein total cholesterol (mmol/L)				
CM and extremely large VLDL*	0.004	0.004	0.007	0.007
Very large VLDL*	0.010	0.011	0.020	0.020
Large VLDL*	0.047	0.042	0.084	0.069
Medium VLDL*	0.154	0.073	0.213	0.107
Small VLDL*	0.225	0.080	0.280	0.095
Very small VLDL*	0.272	0.079	0.292	0.091
VLDL*	0.713	0.252	0.896	0.328
IDL*	0.801	0.205	0.837	0.231
Large LDL*	0.980	0.266	1.054	0.305
Medium LDL*	0.539	0.167	0.601	0.195
Small LDL*	0.321	0.100	0.359	0.116
LDL*	1.842	0.527	2.014	0.613
Very large HDL*	0.210	0.117	0.140	0.105
Large HDL*	0.473	0.210	0.262	0.180
Medium HDL*	0.567	0.148	0.497	0.123
Small HDL	0.520	0.100	0.529	0.106
HDL*	1.775	0.415	1.439	0.341
HDL2*	1.256	0.383	0.929	0.319

HDL3*	0.519	0.045	0.510	0.043
Lipoprotein size (nm)				
VLDL*	35.926	1.139	35.791	1.455
LDL*	23.653	0.145	23.584	0.153
HDL*	10.048	0.235	10.798	0.235

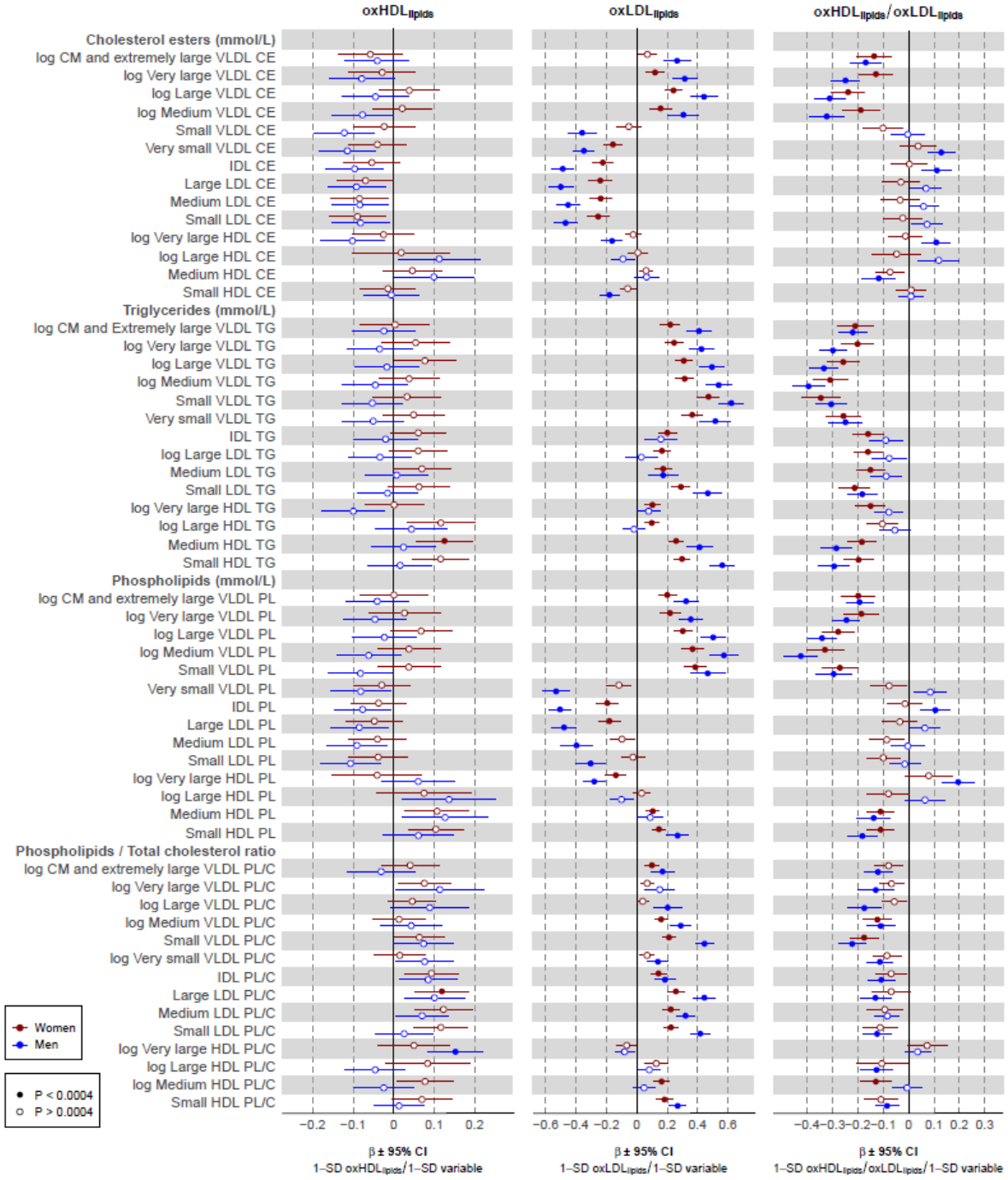
*P<0.05 between sexes. SD=standard deviation, HDL=high-density lipoprotein, CM=chylomicron, VLDL=very low-density lipoprotein, IDL=intermediate-density lipoprotein, LDL=low-density lipoprotein, oxLDLlipids=oxidized LDL lipids, oxHDLlipids=oxidized HDL lipids, Apo-B= apolipoprotein-B, Apo-A1=apolipoprotein-A1.

Figure 1. The adjusted associations of oxidized lipoprotein lipids with lipoprotein subclass particle and lipid concentrations.



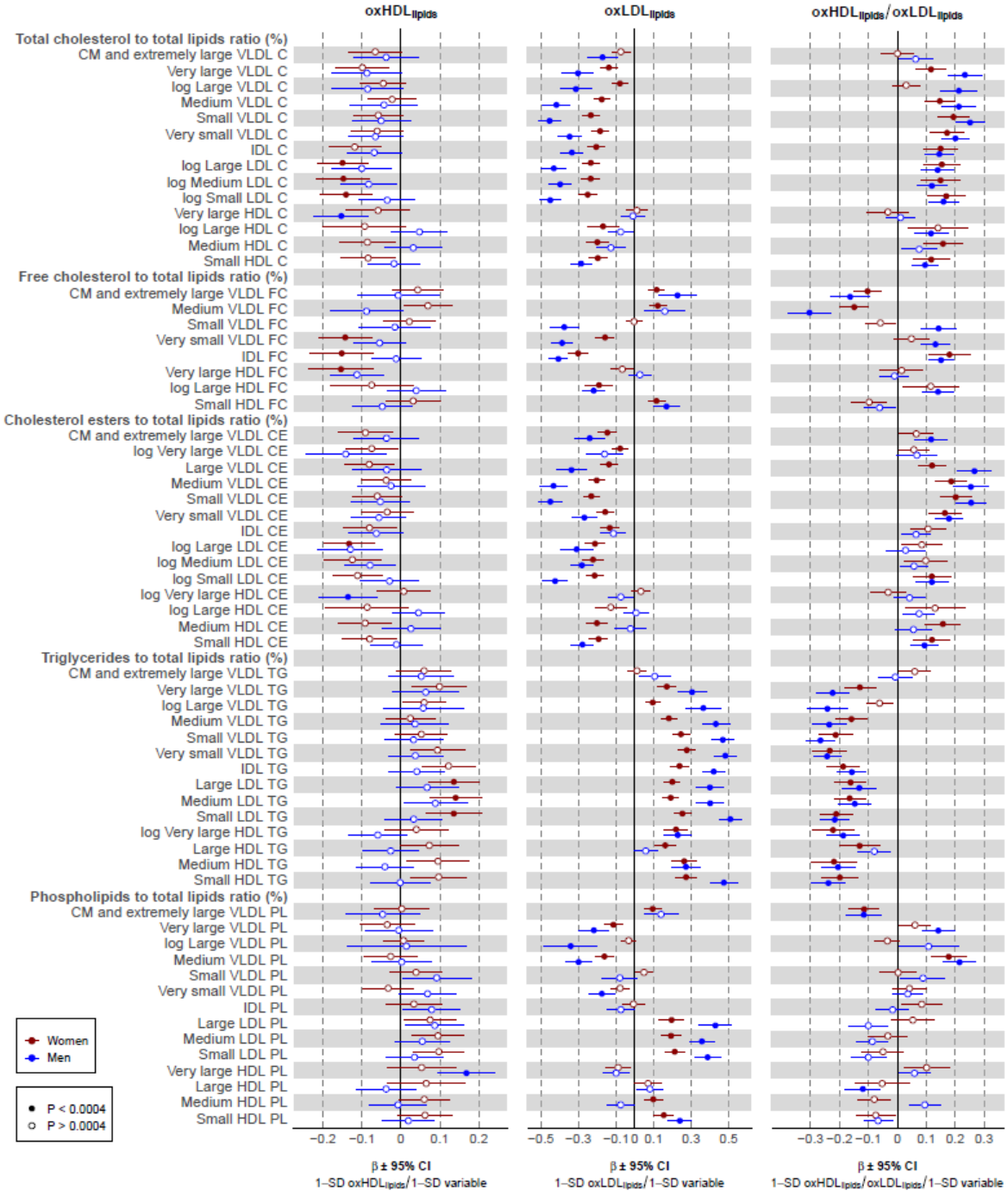
Abbreviations as in Table 1. N=766 in women and N=629 in men. L, lipids; C, cholesterol; FC, Free cholesterol. Linear regression models for oxHDL$_{lipids}$ were adjusted with Apo-A1, waist circumference and age, and models for oxLDL$_{lipids}$ were adjusted for Apo-B, waist circumference and age. Multivariable models for oxHDL$_{lipids}$ / oxLDL$_{lipids}$ ratio were adjusted for Apo-A1/Apo-B ratio, waist circumference and age. Regression coefficients are reported in standardized units of 1-SD difference in oxidized HDL or LDL lipoprotein lipids per 1-SD difference in outcome variable.

Figure 1 (continued). The adjusted associations of oxidized lipoprotein lipids with lipoprotein subclass particle and lipid concentrations.



Abbreviations as in Table 1. N=766 in women and N=629 in men. CE, Cholesterol esters; PL, phospholipids; TG, triglycerides. Linear regression models for $oxHDL_{lipids}$ were adjusted with Apo-A1, waist circumference and age, and models for $oxLDL_{lipids}$ were adjusted for Apo-B, waist circumference and age. Multivariable models for $oxHDL_{lipids}$ / $oxLDL_{lipids}$ ratio were adjusted for Apo-A1/Apo-B ratio, waist circumference and age. Regression coefficients are reported in standardized units of 1-SD difference in oxidized HDL or LDL lipoprotein lipids per 1-SD difference in outcome variable.

Figure 2. The adjusted associations of oxidized lipoprotein lipids with lipid composition of lipoprotein subclass particles.



Abbreviations as in Table 1 and in Figures 1a and 1b. N=766 in women and N=629 in men. Linear regression models for oxHDL$_{lipids}$ were adjusted with Apo-A1, waist circumference and age, and models for oxLDL$_{lipids}$ were adjusted for Apo-B, waist circumference and age. Multivariable models for oxHDL$_{lipids}$ / oxLDL$_{lipids}$ ratio were adjusted for Apo-A1/Apo-B ratio, waist circumference and age. Regression coefficients are reported in standardized units of 1-SD difference in oxidized HDL or LDL lipoprotein lipids per 1-SD difference in outcome variable.