Circulating lipoprotein subfractions and their association with aerobic fitness level

Abstract

Aerobic fitness level, measured as maximal oxygen uptake (VO_{2max}), is a strong marker for cardiac health. Based on this, serum profiling of healthy individuals with a large difference in VO_{2max} level may identify circulating biomarkers representing early biomarkers of CVD risk. The aim of this study was to identify circulating lipoprotein subfractions that are differentially expressed in healthy individuals with large differences in VO_{2max} levels. 218 subjects (40-59 years) from the Nord-Trøndelag health study (HUNT3) performed gold-standard VO_{2max}-test and provided a serum sample. The individuals were selected pairwise with one having low and the other high VO_{2max} (selected from top or bottom 15 subjects within each age-year). The individuals were matched on gender, age, physical activity level and fasting status. NMR lipidomics analysis were performed on serum samples, yielding data on 105 lipoprotein subfractions. The results showed significant negative correlations between VO_{2max}-levels and several lipoprotein subfractions, including both LDL and HDL subfractions. The decrease in concentration of LDL subfractions with increasing $VO_{2max.}$ encompassed the largest LDL subfractions (LDL-1, LDL-2 and LDL-3). Particularly the level of triglycerides (TG) in these subfractions negatively correlated with VO_{2max}-levels (LDL1-TG, LDL2-TG and LDL3-TG). In addition, seven of the HDL subfractions were significantly increased in individuals with a low VO_{2max}, including four of the seven subfractions from the HDL-3 group and two of the seven subfractions from the HDL-2 group. Furthermore, 12 VLDL subfractions, particularly the large subfractions, were significantly higher in individuals with low VO_{2max}. Interestingly the lipoprotein profile of participants with low VO_{2max} seems to largely match the profile seen in insulin resistant individuals. This may indicate that the increased CVD risk from both a low VO_{2max} and insulin resistance may be due to the same alterations in lipid profile. In conclusion, we found significant differences in several lipoprotein subfractions when comparing individuals with a large difference in VO_{2max}. The lipoprotein profile of individuals with low VO_{2max} resembles the profile seen in patients with insulin resistance. More studies are needed to test whether some of these lipoprotein subfractions may represent important risk factors for CVD.

Introduction

Aerobic fitness level measured as maximal oxygen uptake (VO_{2max}) is a strong marker of cardiac health. Large-scale epidemiological studies show that low VO_{2max} is the single best predictor of future cardiovascular disease (CVD) mortality both in healthy individuals and in patients with CVD [1-6]. Furthermore, there is an inverse relationship between physical activity and the presence of metabolic markers associated with risk of occlusive CVD, such as atherogenic lipoproteins and levels of inflammation [7]. Based on this, exploring differences in lipoprotein signatures between healthy individuals with large differences in VO_{2max} may provide ~~i~~nformation about processes associated with the early phases of CVD development. Also, the biomarker signature of individuals with low VO_{2max} may include biomarkers that are related to the development of CVD. New biomarkers of CVD may help us identify individuals with risk of CVD at an earlier than today. CVD (which include coronary heart diseases and stroke) is the most common non-communicable disease globally, responsible for 17.8 million deaths in 2017 [8]. In Norway, mortality from CVD peaked in 1970, and has fallen since. From year 2000 to 2017 the mortality of CVD decreased with 48.5% [9]. Anyhow, there is still a considerable high number affected, with as much as 40.000 diagnosed with myocardial infraction (MI) or angina pectoris in 2016 [10]. Risk factors for CVD include tobacco smoking, high blood pressure, high cholesterol, low levels of physical activity, diabetes, overweight, high alcohol consumption, family history, high age, male sex and ethnicity [10]. Lowering the number of risk factors in the population has great importance as patients that have multiple risk factors at the same time, often increase their risk of CVD by far more than the sum of risk factors would imply [11]. Even though much is already known about the development of CVD and its risk factors, there is optimism that the new methods could allow more precise risk prediction. It would be useful to implement prevention strategies at a much earlier time than what current methods of risk prediction allow.

Every year patients with few or none modifiable risk factors for developing CAD, suffer from MI [12]. Thus, limitation in the ability to predict CVD risk have led to increased clinical interest in identifying novel risk markers and improving the measurement of traditional risk factors, such as low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C). In the recent years, the possibility to identify and quantify subfractions of lipid components has been largely improved by advances in lipidomics technology [13]. One of the main methods for lipidomics analysis is based on Nuclear Magnetic Resonance (NMR) spectroscopy. NMR lipidomics utilizes differences in lipoprotein composition, size and density to extract information about lipoprotein subclasses. Increasing evidence suggest that quantification of lipoprotein subfractions may provide additional information that is missing in today's evaluation of CVD risk, thus potentially leading to a better outcome [14].

Aims

Overall aims

We intend to determine if there exist a lipidomic difference between healthy individuals with high and low VO_{2max} max levels and to see if there exist a correlation between lipoprotein subfraction composition and VO_{2max}. If such a correlation exists, this may contribute to increase risk prediction in the development of CVD.

Specific aims

The aim of the present study was to investigate lipidomic differences between healthy individuals with high and low VO_{2max}.

Material and methods

Study design

This is a descriptive study exploring the differences in circulating lipoprotein subfractions between two groups of healthy participants with large differences in aerobic fitness levels. Participants in the two groups are matched on age, gender, fasting status and self-reported physical activity level.

Study population

The participants were recruited as part of the third itineration of the Nord-Trøndelag Health Study (HUNT3) in Norway, which was carried out from 2006-2008. Among the 50.821 participants in HUNT3, 4.631 healthy adults attended the Fitness Study [15], a sub-study designed to measure VO_{2max}. Participants in the Fitness Study reported to be free from heart-and lung-disease. From this study, 220 individuals between the age of 40 and 59 were selected pair-wise with one having low and the other high VO_{2max} (selected from top or bottom 15 subjects within each age-year), but otherwise matched for gender, age in years, physical activity index score (within 15% difference) and equal time since last meal. Subjects were ranged according to VO_{2max} reported as $mL/kg^{0.75}/min$, and maximum five pairs of subjects were matched from each age-year. Two subjects did not provide a blood sample, and one subject was removed as an outlier. The study therefore included 217 subjects partitioned as 45 males and 63 females in the low VO_{2max}-group, and 46 males and 63 females in the high VO_{2max}group.

Clinical Measurements

Weight and height were measured on a combined scale (Model DS-102, Arctic Heating AS, Nøtterøy, Norway), and BMI was calculated as weight divided by height squared (kg/m^2). Blood pressure and resting heart rate were both measured while sitting (Critikon Dinamap 845XT, GE Medical Systems, Little Chalfont, Buckinghamshire, United Kingdom) and followed established guidelines [16].

Quantification of VO_{2max}

An individualized protocol was applied to measure VO_{2max} by treadmill running to exhaustion [17]. The VO_{2max}-test was performed using a ramp protocol where the speed was constant, and the incline was increased with 2% every second minute until VO_{2max} was reached. Oxygen uptake kinetics were measured directly by a portable mixing chamber gas-analyzer (Cortex MetaMax II, Cortex, Leipzig, Germany) with the participants wearing a tight face mask (Hans Rudolph, Kansas City, USA) connected to the MetaMax II. The system has previously been found valid [18]. Heart rate was measured by radio telemetry (Polar S610i, Polar Electro Oy, Kempele, Finland). From the warm-up pace, the load was regularly increased when oxygen uptake kinetics flattened. Along with a respiratory quotient of 1.05 or higher, a maximal test was considered achieved when the oxygen uptake did not increase more than 2 $mL/kg^{0.75}/min$ at the highest effort or before the participant disembarked the treadmill [19]. VO_{2max} was measured as liters of oxygen per minute (L/min), and subsequently calculated as VO_{2max} relative to body mass ($mL/kg^{0.75}/min$) and VO_{2max} scaled.

Blood Analysis

All clinical-chemical analyses were performed on fresh venous non-fasting blood samples at Levanger Hospital, Norway. Nonfasting glucose (mmol/L) was analyzed by Hexokinase/G-G-PDH methodology (reagent kit 3L82-20/3L82-40 Glucose, Abbot, (Clinical Chemistry, USA). HDL-cholesterol (mmol/L) was analyzed by Accelerator selective detergent methodology (reagent kit 3K33-20 Ultra HDL, Abbot, Clinical Chemistry, USA). Triglycerides ±(mmol/L) were analyzed by Glycerol Phosphate Oxidase methodology.

Lipidomics

100 µl serum samples were used to perform NMR lipidomics analysis. The analyses were performed by the MR Cancer Group (MR Core Facility, NTNU). Lipoprotein sub classification from the resulting spectra was performed in collaboration with Bruker BioSpin (Germany). Concentrations of cholesterol, free cholesterol, phospholipids, and apolipoprotein-A1, A2 and B in plasma, as well as the amount in each of the lipoproteins (VLDL, IDL, LDL and HDL) were estimated using a regression model developed by Bruker BioSpin. The technique is based on a PLS regression model [20, 21]. Each lipoprotein was further subdivided into subfractions according to their density; VLDL into VLDL1-6,

LDL into LDL1-6, and HDL into HDL1-4, with increasing density, and their concentrations of triglycerides, cholesterol, free cholesterol, phospholipids, apolipoprotein-A1 (A1), A2 and B (AB) were estimated, yielding 117 variables (Appendix 1). Lipidomics analyses were performed in 3-mm NMR tubes. In these tubes a mixture of buffer (100 µl) (0.075 mM Na2HPO4, 5 mM NaN3, 5 mM TSP, pH 7.4) and thawed serum samples (100 µl) were analyzed. In the subsequent procedure, magnetic resonance spectroscopy analysis was performed on Bruker Avance III Ultrashielded Plus 600 MHz spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) armed with a 5mm QCI Cryoprobe. Further trials were fully automated using a SampleJet in union with Icon-NMR on Topspin 3.1 (Bruker BioSpin). 1D 1H Nuclear Overhauser effect spectroscopy (NOESY) and Carr–Purcell–Meiboom–Gill (CPMG) spectra with water presaturation were obtained at 311.4K. The spectra were Fourier transformed to 128 K after 0.3 Hz exponential line broadening.

Statistical analyses

Statistical analyses were performed using SPSS statistics version 26.0 (IBM SPSS, New York, USA) and MatLab R2017a with PLS_Toolbox 8.2.1 (Eigenvector Research, Inc.). Univariate and multivariate statistical tests were used to determine whether participant's fitness level was associated with specific lipoprotein subfractions. The multivariate analyses were performed using principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) using PLS_Toolbox v5.8.3 in Matlab (Eigenvector Research, Manson, WA, USA). The variance structure of the data is explained through linear combinations of the variables called principal components (PCs). The first PCs explaining most of the variance in the data set. In the score plot of the PCs, individuals with a similar lipidomic profile will cluster, while the corresponding loading profile displays the importance of each variable within the PC. PLS-DA is a supervised classification method which uses the class information to detect variables generating maximum separation between the classes (high and low aerobic capacity). Starting with the univariate analysis the Shapiro Wilk test of normality was used to check for normally distributed data. Neither the clinical variables nor the lipoprotein subfractions were normally distributed. Therefore, we executed the statistical analyses using non-parametric tests. Thus, the Mann Whitney U test was used to check for significant differences in concentration of lipoprotein subfractions between the high and low VO_{2max} groups. A significance level of $p<0.005$ was chosen to avoid random errors due the size of the cohort.

Ethics

The study was approved by the Regional Committee for Medical Research Ethics, the Norwegian Data Inspectorate, and by the National Directorate of Health with study ID 2019/612. The study is in conformity with Norwegian laws and the Helsinki declaration, and all participants signed a document of consent.

Results

Clinical characteristics of study population

Clinical characteristics for the patients in the two VO_{2max} groups are shown in **Table 1**. The participants in the two groups were matched for the clinical variables age, gender, fasting time and level of self-reported physical activity. It can be seen from table 1 that the BMI, mean arterial pressure (MAP) and smoking status are significantly different between the two groups.

Table 1: Clinical characteristics of the study population

General	**Low VO_{2max} group**	**High VO_{2max} group**
VO_{2max}	31,4 ± 4,9	47,1 ± 7,7
Age	49,5 ± 5,9	49,5 ± 5,9
Gender (Female/male)	63/45	63/46
SBP	129 ± 6	125 ± 14
DBP	75 ± 10	72 ± 10
MAP	93 ± 11	90 ± 10
BMI	27,5 ± 4,0	24,8 ± 2,6
Smoker (yes)	30.9 % (33)	21.8% (24)
Diabetes (yes)	0.9% (1)	4.5% (5)
Physical activity index score	3.7	3.7
Nonfasting glucose	5,68 ± 1,70	5,17 ± 0,80
HDL	1,39 ± 0,37	1,43 ± 0,34
Triglycerides	1,68 ± 0,89	1,29 ± 0,69

SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MAP: Mean arterial pressure, BMI: Body mass index, HDL: High-density lipoproteins

VO2max and lipoprotein subfractions

Multivariate statistical analyses

To get a visual overview of the distribution of lipoprotein subfractions in the dataset, unsupervised Principal Component Analysis (PCA) was performed on 105 lipoprotein subfractions (Figure 1). The PCA plot identified an outlier (marked in purple in the figure). This participant was considered an outlier and was therefore removed from further analyses.

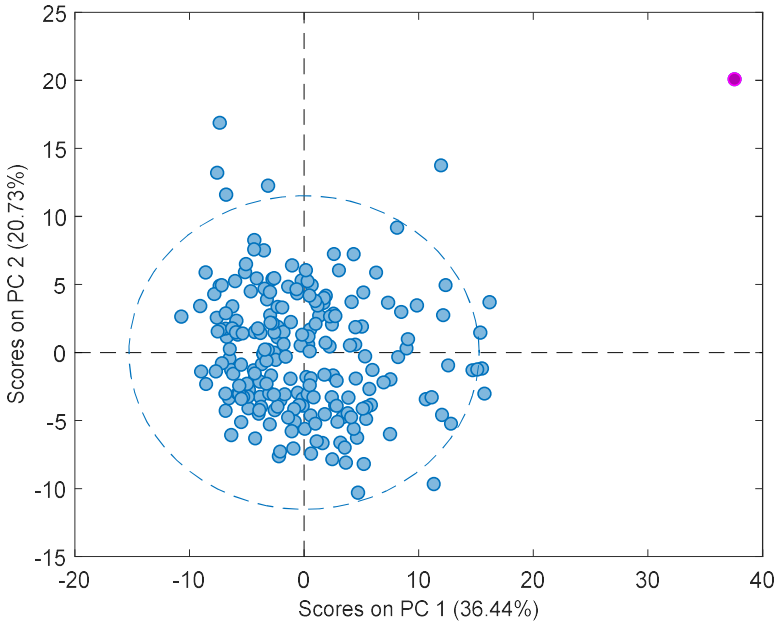

Figure 1: PCA-plot with an outlier.

Principal component analysis (PCA) is a technique used to emphasize variation and bring out strong patterns in a dataset. It's often used to make data easy to explore and visualize. The principal component 1 (PC 1) and PC 2 shown on the x- and y-axis are used to represent the variability in the dataset, with the PC 1 representing the highest variability (36.44%) and the PC 2 the second highest variability in the dataset (20.73%) . In this case we used the PCA-plot to identify and remove and an outlier, marked in purple, from the dataset. The rest of the participants are shown in blue color.

Working out from our hypothesis that there are several significant differences in lipoprotein subfraction concentration, as shown in table 2 and table 3, we tried to separate high and low VO_{2max} individuals based on their lipidomic profile, as illustrated here in figure 2.

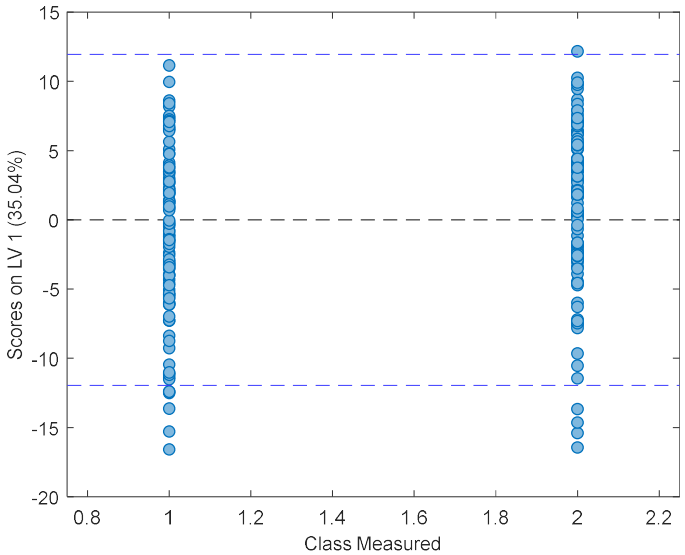

Figure 2: PLSDA-figure displaying differences in lipidomic profiles between the low and high VO_{2max} groups.

The dataset turned out to give 1 latent variable (LV) with a p-value of 0,017 indicating a difference in lipidomic profile, however, not significant enough to be used as a basis for a classification model. Hence, we were not able to separate individuals into groups of low and high VO_{2max} based on their lipidomic profile.

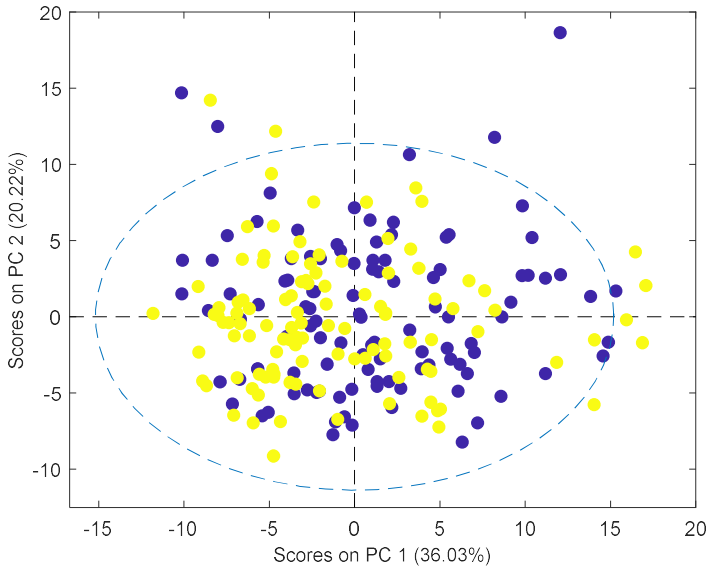

Figure 3: Colored PCA-plot illustrating both low and high VO_{2max}. Low VO_{2max} individuals (purple) and high VO_{2max} (yellow) are presented, the two groups show a scattering of individuals.

We would expect a greater clustering of the two groups in the PCA-plot in figure 3, if there was a significant difference between the two groups. However, the scattering of individuals from the two groups in a seemingly random manner suggest that PCA-plot confirms, what we saw in figure 2, that the lipid profile cannot be used to determine which VO_{2max} class the individuals belong too, and vice-versa.

Univariate statistical analysis

Significant correlations between VO_{2max} scaled and lipoprotein subfractions, shown in **Table 2,** were found by using the Spearman correlation. This analysis resulted in 18 of the 105 lipoprotein subfractions showing significant negative correlations with levels of VO_{2max}, thus decreasing in concentration as the VO_{2max} increased. However, it is important to clarify that these results are not based on a comparison of the two cohort groups, but rather all the participants across both groups.

Table 2: Lipoprotein subfractions correlation with levels of VO_{2max}

Lipoprotein subfraction	Spearman correlation	Significance
H3A1	-0,276	0.000
H3TG	-0,276	0.000
L2TG	-0,269	0.000
LDTG	-0,268	0.000

L1TG	-0,258	0.000
H3A2	-0,243	0.000
H3PL	-0,240	0.000
H2TG	-0,230	0.001
L3TG	-0,226	0.001
H2A2	-0,219	0.001
HDTG	-0,207	0.002
LDAB	-0,202	0.003
TPCH	-0,201	0.003
V5TG	-0,199	0.003
V6TG	-0,199	0.003
TPAB	-0,198	0.003
V6PL	-0,190	0.005
L1AB	-0,190	0.005

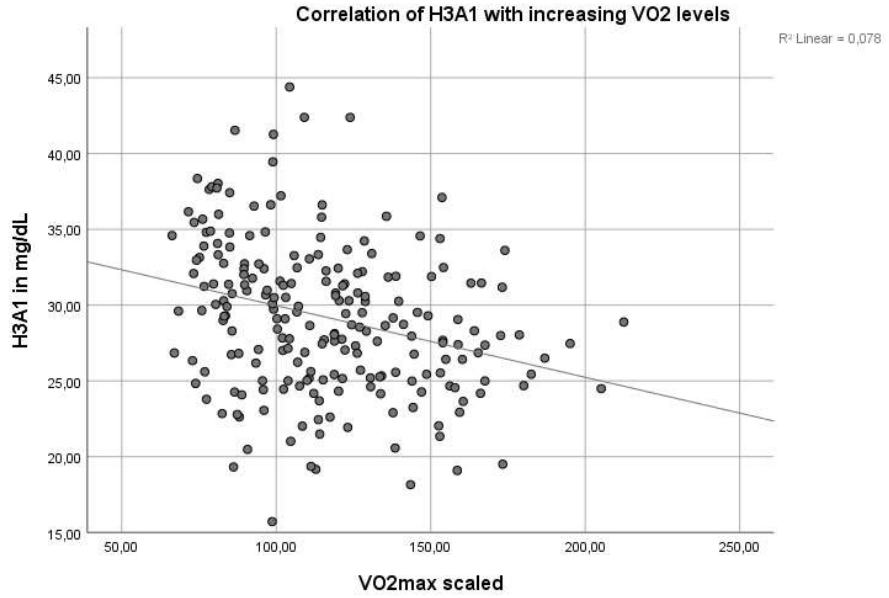

Figure 4: The figure illustrates a declining concentration of the HDL-3-ApoA1 subfraction (H3A1) in relation to the increasing aerobic fitness (VO_2) levels.

The steady decline in lipoprotein subfraction concentration as the VO_{2max} increases, as shown in figure 4, is something that all of the 18-correlating lipoprotein subfractions in table 2 have in common. These result show that aerobic fitness has a clear effect of on the composition of lipoprotein groups.

We know that other factors, apart from aerobic fitness, play a pivotal role in the development of CVD. We decided to look at overall differences in lipoprotein subfraction concentrations between the two groups as seen in table 3, using the Mann-Whitney U test. We found that a total of fifteen lipoprotein subfractions differed significantly between the two groups. The results showed that 13 of the 15 values were subfractions of VLDL, one was H3TG and last one was a measure of total plasma triglycerides (TPTG).

Table 3: Overview of the fifteen lipoprotein subfractions that were significantly different in concentration between the high VO_{2max} and the low VO_{2max} group.

Lipoprotein subfraction	**Description**	**Low VO_{2max} group (mean + SD)**	**High VO_{2max} group (mean + SD)**	**P-value**
VLTG	Triglycerides in VLDL (mg/dL)	110,8±58,0	88,9±51,6	0.001
VLAB	Apo-B in VLDL (mg/dL)	7,9 ± 3,0	6,9 ± 3,1	0.001
V1TG	Triglycerides in VLDL-1 (mg/dL)	52,0 ± 41,7	37,2 ± 35,2	0.001
V1FC	Free cholesterol in VLDL-1 (mg/dL)	3,0 ± 2,7	2,0 ± 2,4	0.001
V1PL	Phospholipids in VLDL-1 (mg/dL)	7,3 ± 6,1	5,0 ± 5,4	0.001
TPTG	Total plasma triglycerides (mg/dL)	159,3±78,2	130,7±68,5	0.002
VLPL	Phospholipids in VLDL (mg/dL)	25,1 ± 11,7	20,6 ± 11,2	0.002
H3TG	Triglycerides in HDL-3 (mg/dL)	1,98 ± 0,76	1,68 ± 0,7	0.002
V3FC	Free cholesterol in VLDL-3 (mg/dL)	2,3 ± 1,4	1,8 ± 1,3	0.003
V3PL	Phospholipids in VLDL-3 (mg/dL)	5,2 ± 2,3	4,4 ± 2,4	0.003
VLFC	Free cholesterol in VLDL (mg/dL)	12,61 ± 5,7	10,6 ± 5,4	0.004
V1CH	Cholesterol in VLDL-1 (mg/dL)	9,1 ± 8,2	6,3 ± 6,6	0.004
V3CH	Cholesterol in VLDL-3 (mg/dL)	4,9 ± 2,7	4,05 ± 3,0	0.004
V4TG	Triglycerides in VLDL-4 (mg/dL)	12,7 ± 3,7	11,44 ± 4,3	0.004
V6PL	Phospholipids in VLDL-6 (mg/dL)	0,28 ± 0,02	0,28 ± 0,02	0.005

Discussion

In this study, we performed lipidomic analyses using NMR spectroscopy to quantify the differences in lipoprotein subfractions between individuals split into two groups, of either high or low aerobic fitness level. We observed significant negative correlation between VO_{2max}-level and several lipoprotein subfractions. Interestingly this negative correlation encompasses both LDL subfractions and HDL subfractions.
The decrease in concentration of LDL subfractions with increasing VO_{2max} encompassed the largest LDL subfractions (LDL-1), but also the second and third largest LDL subfractions, LDL-2 and LDL-3, respectively. Particularly the level of triglycerides (TG) in these subfractions correlated with VO_{2max}-levels (LDL1-TG, LDL2-TG and LDL3-TG). A possible explanation for the higher concentration of TG in LDL is the increased BMI of the participants in the low VO_{2max}-group. Enrichment of TG in LDL has been observed in visceral obesity [22], which is correlated to an increase in BMI and waist/hip ratio [23, 24]. Furthermore, increases in levels of LDL-TG in individuals with low VO_{2max} may also signify impairment of lipoprotein-lipase activity, reducing the efficiency of VLDL conversion to LDL (via hydrolysis of TGs by LPL,) as is observed with insulin resistance and type 2 diabetes [25, 26]. These results show a clear lipidomic distinction between the two test groups. Nevertheless we would have expected a significant negative correlation with small LDL particles, as they are also associated with CVD [27].

In addition, results showed that some of the HDL subfractions were significantly increased in individuals with a low VO_{2max}, including five of the seven subfractions from the HDL-3 group and two of the seven subfractions from the HDL-2 group (se Appendix 1 for an overview of all quantified subfractions). Earlier research has touted HDL as solely cardioprotective, but more recent studies on HDL subfractions have shown that those with high CAD risk often have lower levels of large HDL2 and higher levels of small HDL 3 than do healthy individuals, and that certain HDL3 and HDL2 groups were inversely associated with CAD severity [28-30]. This coincides with our results, shown in table 2. It also supports our hypothesis that there is an altered ratio between lipoprotein subfractions that reflect the increased risk of CAD that individuals with low aerobic fitness have compared to those with a high aerobic fitness.

Furthermore, when comparing the high VO_{2max}-group to the low VO_{2max}-group, as shown in table 3, we found 14 lipoprotein subfractions with significantly higher concentration in participants with low VO_{2max}, 12 of them being subfractions of VLDL. Interestingly the lipoprotein profile of participants with low VO_{2max} seems to largely match the profile seen in insulin resistant individuals, with increases in large VLDL particle concentrations, an increase in VLDL size and a decrease in LDL and a decrease in HDL size as a result of depletion of large HDL particles size [30]. These results, except for a decrease in HDL size, coincide with the result from our study. Furthermore, previous studies have shown that a low VO_{2max} is associated with IR [31]. The convergence in lipoprotein profile of participants with low VO_{2max} and IR may indicate that the increased CVD risk from both a low VO_{2max} and increased IR may be due to the same alterations in lipid profile. Considering these similarities in lipid profile it is possible that the participants with low VO_{2max} have increased insulin resistance, although they have quite similar characteristics considering risk factors of IR as the participants with high VO_{2max}. To assess the degree of IR in participants with low VO_{2max} we calculated the triglyceride/HDL-ratio, which has been proven to correlate strongly with HBA1c and results from oral glucose tolerance tests [32, 33]. No data were available on insulin levels, fasting glucose or HBA1C from the HUNT databank. The low VO_{2max}-group had an average TG/HDL-ratio of 1.42 and the high VO_{2max}-group had a ratio of 1.01 which equals a 41% difference. Although no standard reference range is established for the TG/HDL ratio, some sources point to having a score lower than 1.7 as being low-risk, while a higher score would be intermediate/high risk, indication that lower scores are better [34]. Other studies show that TG/HDL has an incremental

association to IR, but also that its efficacy as a tool for assessing IR varies by ethnicity [35, 36]. Nevertheless, our cohort consisting of Norwegians fit the ethnic group where TG/HDL has proven itself as a good tool for IR assessment.

Considering the traditional risk markers of CVD, we would expect higher levels of HDL in the high VO_{2max}-group [37], but on the contrary we observe as certain subfractions of HDL-2 and HDL-3 decrease in concentration as VO_{2max} increases. Looking at earlier studies we did not expected that LDL levels would be significantly correlated with VO_{2max}, as earlier studies have not found any correlation [38]. However we did find a negative correlation between VO_{2max} and the large LDL-1 subfractions, which is contrary to what we would expect and to what has been found in earlier studies on VO_{2max} [39]. This finding may be explained by the low VO_{2max} group having a higher omega 3 fatty acid intake which has been shown to increase large subfractions of LDL [40]. The data we have on dietary habits supports this, showing 50,5% in the low VO_{2max} group supplementing with omega 3 fatty acids either "every day" or "every now and then" versus 41,5% in the high VO_{2max} group.

In addition, the levels of apolipoprotein-B (ApoB) decreased significantly in the, LDAB and L1AB subfractions as VO_{2max} goes up. This may be clinically significant as Apo-B may be measured to find information about the concentration of circulation atherogenic lipoproteins [41], and it also and influences the apoB/apoA1 ratio which has been shown to be a very good measure of CVD risk [42]. As such we see a little difference in ApoB/ApoA1 ratio between the groups with the low VO_{2max}-group having a ratio of 0.531 and the high VO_{2max}-group having a ratio of 0.470 signifying a higher concentration of atherogenic lipoproteins in the low VO_{2max}-group.

Limitations of the study

Unfortunately, information on statin therapy in theses participants was not available from the HUNT database. The main effect of statin therapy lowering the LDL-cholesterol [43] and possibly increasing the amount of HDL cholesterol [44]. Unfortunately knowledge about which subclasses of lipoproteins that are decreased or increased by statins is lacking, but as statins are estimated to reduce the risk of CVD by 25% [45]. Patients in our studies may have altered lipoprotein subfractions as a result of their statin use, but we see this as highly unlikely due their age and to the criteria of inclusion in the study which specifies that the participants must be free of heart disease.

Conclusion

In this study we found significant differences in several lipoprotein subfractions when comparing individuals with a large difference in VO_{2max}. The lipoprotein profile of individuals with low VO_{2max} resembles the profile seen in patients with insulin resistance. Additional studies are needed to test whether some of these lipoprotein subfractions may represent important risk factors for CVD.

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Appendix 1

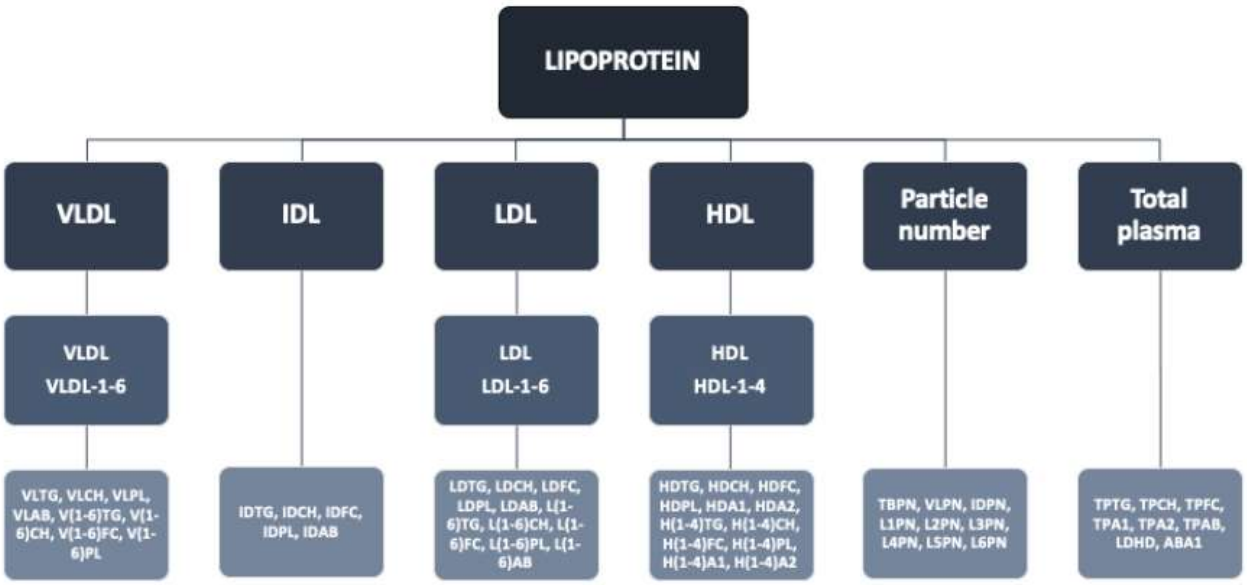

Figure A1: Overview of the main lipoproteins, lipoprotein subfractions and lipoprotein particles that are quantified by NMR lipidomics