Atherosclerosis 343 (2022) 51–57

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Atherogenic lipidomics profile in healthy individuals with low cardiorespiratory fitness: The HUNT3 fitness study

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ARTICLE INFO

Keywords:
Biomarkers
Lipidomics
Risk factors
Insulin resistance

ABSTRACT

Background and aims: Low cardiorespiratory fitness is a strong and independent risk factor for cardiovascular disease (CVD). Serum profiling of healthy individuals with large differences in cardiorespiratory fitness may therefore reveal early biomarkers of CVD development. Thus, we aimed to identify circulating lipoprotein subfractions differentially expressed between groups of individuals with large differences in cardiorespiratory fitness, measured as maximal oxygen uptake (VO_{2max}).

Methods: Healthy subjects (40–59 years) were selected from the third wave of the Trøndelag health study (HUNT3) based on having an age-dependent high VO_{2max} (47.1 ± 7.7 mL kg^{-1}·min^{-1}, n = 103) or low VO_{2max} (31.4 ± 4.9 mL kg^{-1}·min^{-1}, n = 108). The individuals were matched on gender, age, physical activity level and fasting status.

Results: 99 lipoprotein subfractions were quantified in serum samples using nuclear magnetic resonance (NMR) lipidomics. Standard clinical analyses showed similar levels of total cholesterol, low-density lipoprotein (LDL)-cholesterol and high-density lipoprotein (HDL)-cholesterol between the groups, and slightly higher levels of triglycerides in participants with low VO_{2max}. Thirteen lipoprotein subfractions were increased in the low VO_{2max} group compared to the high VO_{2max} group ($p < 0.005$), including mainly large very low-density lipoprotein (VLDL) subfractions. In addition, triglyceride levels in small-sized HDL and LDL particles were increased in participants with low VO_{2max}. Correlation analyses between VO_{2max} and lipoproteins subfractions displayed a negative correlation between VO_{2max} and the levels of cholesterol and phospholipids in the small HDL particles. The lipoprotein profile of individuals with low VO_{2max} is similar to the profile of insulin resistant individuals.

Conclusions: Low VO_{2max} was associated with enrichment of large VLDL particles, as well as an increased triglycerides content in the small and dense HDL and LDL particles. This unfavorable lipid profile is likely to be involved in the strong associations between VO_{2max} and CVD.

1. Introduction

Cardiorespiratory fitness, measured as maximal oxygen uptake (VO_{2max}), is a strong marker of cardiac health. Large-scale epidemiological studies have shown 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 VO_{2max} and the presence of metabolic risk factors for CVD, such as atherogenic lipoproteins and levels of inflammation markers [7,8]. However, profiles of serum lipoprotein subfractions associated with low VO_{2max} are still undetermined [9].

Based on this, lipoproteinprofiles of healthy individuals with low VO_{2max} may provide information on processes associated with the initial phases of CVD development, as well as new biomarkers of atherosclerosis.

Limitation in the ability to predict CVD risk have led to increased

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https://doi.org/10.1016/j.atherosclerosis.2022.01.001
Received 20 August 2021; Received in revised form 26 December 2021; Accepted 11 January 2022
Available online 20 January 2022

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 carriers has been largely improved by advances in lipidomics technology [10]. 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 suggests that quantification of lipoprotein subfractions may provide the additional information that is missing in today's evaluation of CVD risk [11].

The aim of this study was to determine differences in lipoprotein subfractions between healthy individuals with high and low VO_{2max}.

2. Materials and methods

2.1. Study design

This is a descriptive study exploring the differences in circulating lipoprotein subfractions between two groups of healthy participants with large differences in cardiorespiratory fitness levels. Serum NMR spectra from these participants have been used in a previous publication that did not extract information on lipoprotein subfractions [12].

2.2. Study population

The participants were recruited from the third wave of the Trøndelag Health Study (HUNT3) in Norway, which was carried out from 2006 to 2008 [13]. Among the 50.821 participants in HUNT3, 4.631 healthy adults attended the Fitness Study, a sub-study designed to measure VO_{2max} [14]. Participants in the Fitness Study reported no history of heart and lung disease. From the Fitness Study, 220 individuals between the age of 40 and 59 were selected pairwise 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. Ranged according to VO_{2max} (quantified as $mL{\cdot}kg^{-1}{\cdot}min^{-1}$) maximum five pairs of subjects were matched from each age-year. All of the selected subjects reached their true VO_{2max} (according to criteria given in Section 2.4). Two of the 220 subjects did not provide a blood sample, one subject was removed as an outlier based on principal component analysis (Supplementary Fig S1 and 6) subjects were later removed due to diabetes diagnosis. The final study population therefore included a total of 211 subjects.

2.3. 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 measured while sitting (Critikon Dinamap 845XT, GE Medical Systems, Little Chalfont, Buckinghamshire, United Kingdom) according to established guidelines [15].

2.4. Quantification of VO_{2max}

An individualized protocol was applied to measure VO_{2max} by treadmill running to exhaustion [16]. 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 was 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 had previously been found valid [17]. 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 $kg^{-1}{\cdot}min^{-1}$ despite increased workload. VO_{2max} was measured as liters of oxygen per minute (L/min), and subsequently calculated as VO_{2max} relative to body mass ($mL{\cdot}kg^{-1}{\cdot}min^{-1}$).

2.5. 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 with the Hexokinase/G-G-PDH methodology (reagent kit 3L82-20/3L82-40 Glucose, Abbot, (Clinical Chemistry, USA). HDL-cholesterol (mmol/L) was analyzed with the Accelerator selective detergent methodology (reagent kit 3K33-20 Ultra HDL, Abbot, Clinical Chemistry, USA). Triglycerides (mmol/L) were analyzed with the Glycerol Phosphate Oxidase methodology. LDL-cholesterol values were calculated using the Friedewald formula. The triglyceride/HDL ratio was calculated to estimate insulin resistance.

2.6. NMR lipidomics

Sample preparation and NMR analyses were performed at the MR Core Facility, NTNU, as previously described [12]. Serum samples (150 μl) were mixed with equal amounts of buffer solution (0.075 mM Na2HPO4, 5 mM NaN3, 5 mM TSP, pH 7.4), and transferred to 3 mm NMR tubes. The MR spectra were acquired using a Bruker Avance II (Bruker Biospin, Rheinstetten, Germany) with digital receiver unit (DRU) operating at 600 MHz for proton (1H). The probe was a TCI 1H–13C/15 N/D with z-gradient and automated tuning and matching unit. All spectra were recorded in an automatic fashion using a BACS-60 sample changer and the ICON-NMR software (Bruker Biospin). Proton spectra were obtained at a constant temperature of 310 K using a modified Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with presaturation during the relaxation delay (Bruker: cpmgpr1d) to achieve water suppression and to facilitate the detection of low molecular weight species by avoiding the large, overlapped signals derived from proteins and large molecules. The spectra were collected with 64 scans and 4 dummy scans. The acquisition time was set to 3.067 s, measuring the FID via collection of 36,864 complex data point resulting in a sweep width of 20.0363 ppm. A relaxation delay of 4 s was used, during which a presaturation of 25 Hz was applied. Effective echo time was 80 ms and data acquisition starts at maximum of last echo. An exponential apodization of 1 Hz was applied prior to Fourier transform. Measurement and processing were done in full automation using Bruker standard automation programs controlled by ICON-NMR (along with TopSpin v2.1 patch level 6). Lipoprotein sub-classification from the resulting spectra was performed in collaboration with Bruker BioSpin (Germany) using the Bruker IVDr Lipoprotein Subclass Analysis (B.I.LISA™) [18]. Concentrations of cholesterol, free cholesterol, triglyserides, phospholipids, and apolipoprotein-A1, A2 and B in serum, as well as the amount in each of the lipoproteins (VLDL, IDL, LDL and HDL), were estimated (see Fig. 1). 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, A2 og B were estimated, yielding 99 variables (Supplementary Table S1).

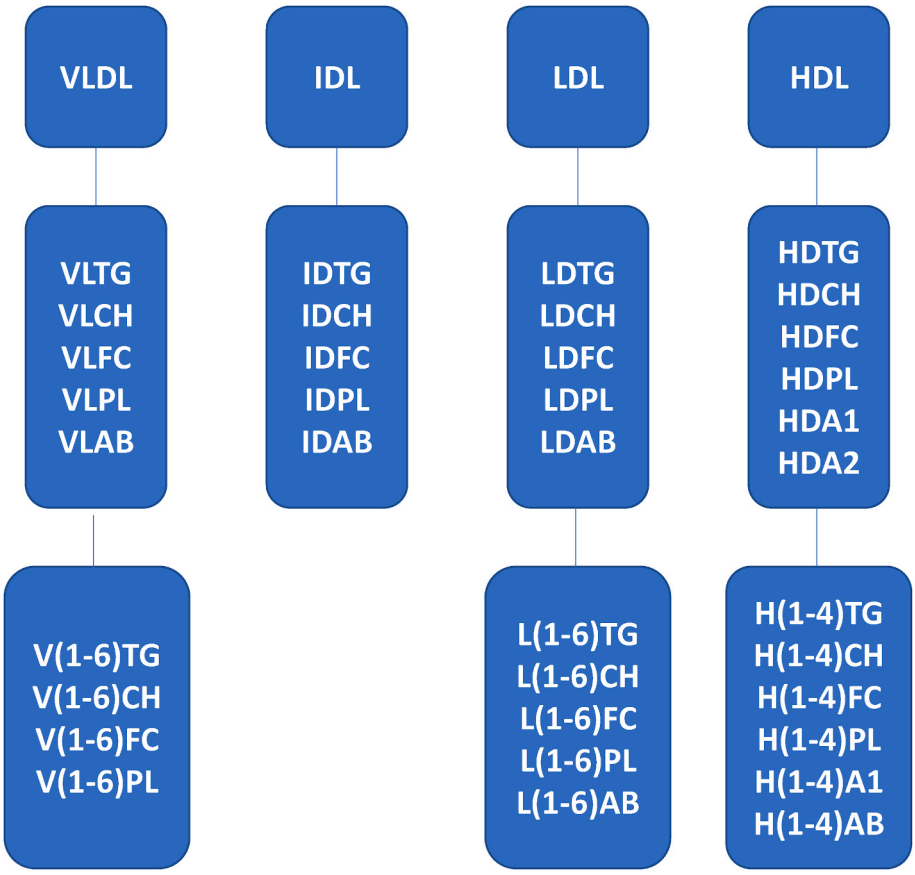

Fig. 1. Overview of the lipoprotein subfractions quantified by NMR lipidomics.
VLDL: very low-density lipoprotein, IDL: intermediate-density lipoprotein, LDL: low-density lipoprotein, HDL: high-density lipoprotein, TG: triglycerides, CH: cholesterol, FC: free cholesterol, PL: phospholipids, AB: apolipoprotein B100, A1: apolipoprotein A1, A2: apolipoprotein A2, NMR: nuclear magnetic resonance.

2.7. 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 analyses were used to determine whether participant's fitness level was associated with specific lipoprotein subfractions. Multivariate analyses were performed using principal component analysis (PCA) in Matlab (Eigenvector Research, Manson, WA, USA). 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, the non-parametric Mann Whitney U test was used to compare the concentration of lipoprotein subfractions between the high and low VO_{2max} groups. Spearman correlation analyses were performed within each group and for the whole cohort to compare VO_{2max} levels to the concentration of lipoprotein subfractions. A stringent significance level $p < 0.005$ was used to avoid random errors due to assessment of 99 non-independent lipoprotein subfractions.

2.8. 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.

3. Results

3.1. Clinical characteristics of study population

Clinical characteristics of the individuals in the two VO_{2max} groups are shown in Table 1. Significant differences were seen between the groups in body mass index (BMI), smoking status, and serum triglycerides. One outlier was identified in the high VO_{2max} group based on unsupervised principal component analysis (PCA) (Supplementary Fig. S1). This was due to a technical error in this sample and the participant was removed.

3.2. VO_{2max} and lipoprotein subfractions

Thirteen lipoprotein subfractions were significantly different between the groups of high and low VO_{2max} ($p < 0.005$) (Table 2), and all of them were higher in the low VO_{2max} group. The concentrations of Apo-B, free cholesterol, cholesterol, phospholipids, and triglycerides in the large VLDL particles (VLDL1-3) were significantly higher in the low VO_{2max} group compared to the high VO_{2max} group. The most pronounced differences were seen in lipid concentrations in the largest VLDL-subfraction that were found to be 41–50% higher in the low VO_{2max} group (Table 2). The associations between VO_{2max} groups and all quantified lipoprotein subfractions are shown in Supplementary Table S2.

In addition to comparing lipoprotein subfractions between the two

Table 1
Clinical characteristics of the final study population.

General	Low VO_{2max} group (n = 103)	High VO_{2max} group (n = 108)	*p*-value
VO_{2max} ($mL \cdot kg^{-1} \cdot min^{-1}$)	31.4 ± 4.9	47.1 ± 7.7*	0.001
Age, years	49.5 ± 5.9	49.5 ± 5.9	0.95
Gender (female/male)	61/42	62/46	0.95
Systolic blood pressure (mmHg)	129 ± 6	125 ± 14	0.08
Diastolic blood pressure (mmHg)	75 ± 10	72 ± 10	0.05
Body mass index	27.5 ± 4,0	24.8 ± 2.6*	0.01
Daily smoker (n = yes)	20	11*	0.02
Physical activity index score	3.7 ± 1.9	3.7 ± 1.9	0.95
Fasting status (hours)	2.0 ± 1.0	2.0 ± 1.0	0.99
Non-fasting glucose (mmol/L)	5.5 ± 0.9	5.2 ± 0.8	0.09
Total cholesterol (mmol/L)	5.6 ± 0.9	5.5 ± 0.9	0.36
LDL-cholesterol (mmol/L)	3.4 ± 0.8	3.4 ± 0.8	0.99
HDL-cholesterol (mmol/L)	1.4 ± 0.4	1.4 ± 0.3	0.10
Triglycerides (mmol/L)	1.7 ± 0.9	1.3 ± 0.7*	0.01
Triglycerides/HDL-ratio	1.4 ± 1.2	1.0 ± 0.7*	0.03

Data are mean ± SD. VO_{2max}: maximal oxygen uptake, LDL: low-density lipoprotein, HDL: high-density lipoprotein. *Variables significantly different between the low VO_{2max} group and high VO_{2max} group at $p < 0.05$.

Table 2
Overview of lipoprotein subfractions that differed in concentration between the high VO_{2max} and the low VO_{2max} group ($p < 0.005$).

Lipoprotein subfractions	Abbreviations	Low VO_{2max} group (mean ± SD)	High VO_{2max} group (mean ± SD)	Percentage higher in low VO_{2max}group (%)	*p*-value
HDL-subfractions (mg/dL)					
Triglycerides in HDL3	H3TG	2.0 ± 0.8	1.7 ± 0.7	18%	0.002
LDL-subfractions (mg/dL)					
Triglycerides in LDL5	L5TG	3.5 ± 1.4	3.2 ± 1.6	10%	0.004
VLDL-subfractions (mg/dL)					
Apo-B in VLDL	VLAB	7.9 ± 3.0	6.9 ± 3.1	15%	0.001
Free cholesterol in VLDL	VLFC	12.6 ± 5.7	10.6 ± 5.4	19%	0.004
Phospholipids in VLDL	VLPL	25.1 ± 11.7	20.6 ± 11.2	22%	0.002
Triglycerides in VLDL	VLTG	110.8 ± 58.0	88.9 ± 51.6	25%	0.001
Cholesterol in VLDL1	V1CH	9.1 ± 8.2	6.3 ± 6.6	44%	0.004
Free cholesterol in VLDL1	V1FC	3.0 ± 2.7	2.0 ± 2.4	50%	0.001
Phospholipids in VLDL1	V1PL	7.3 ± 6.1	5.0 ± 5.4	46%	0.001
Triglycerides in VLDL1	V1TG	52 ± 42	37 ± 35	41%	0.001
Cholesterol in VLDL3	V3CH	4.9 ± 2.7	4.1 ± 3.0	20%	0.004
Free cholesterol in VLDL3	V3FC	2.3 ± 1.4	1.8 ± 1.3	28%	0.003
Phospholipids in VLDL3	V3PL	5.2 ± 2.3	4.4 ± 2.4	18%	0.003

VO_{2max}: maximal oxygen uptake, SD: standard deviation, HDL: high-density lipoprotein, LDL: low-density lipoprotein, VLDL: very-low density lipoprotein.

Table 3
Lipoprotein subfractions correlation with levels of VO_{2max}.

Lipoprotein subfraction	Abbreviations	Low VO_{2max} group		High VO_{2max} group		All	
		r	*p*-value	r	*p* -value	r	*p* -value
Cholesterol in HDL3	H3CH	−0.33	<0.0005	−0.27	0.004	−0.19	0.004
Phospholipids in HDL3	H3PL	−0.35	<0.0005	−0.28	0.003	−0.24	<0.0005

VO_{2max}: maximal oxygen uptake, r: correlation coefficient, HDL: high-density lipoprotein.

groups with high and low VO_{2max}, correlation analyses were performed between lipoprotein subfractions and VO_{2max}. Lipoprotein subfractions that correlated significantly with VO_{2max} both within each group separately and in all samples together are shown in Table 3.

4. Discussion

We performed lipidomic analyses using NMR spectroscopy to quantify the differences in circulating lipoprotein subfractions between individuals with high and low cardiorespiratory fitness level. To our knowledge, no previous study has performed association analysis between gold standard quantified VO_{2max} and a large number of lipoprotein subfractions. We observed group differences, and inverse correlations between VO_{2max} levels and lipoprotein subfractions. Standard clinical analyses showed similar levels of total cholesterol and HDL-cholesterol between the groups, and slightly higher levels of triglycerides in participants with low VO_{2max}. Anthropometric and physiological variables were relatively similar between the groups, and within normal ranges. A significant, but small difference in BMI was found between the groups. There were also more daily smokers in the low VO_{2max} group.

Thirteen lipoprotein subfractions were higher in the low VO_{2max} group compared to the high VO_{2max} group ($p < 0.005$). The largest differences were seen among subfractions of VLDLs. In addition, the triglyceride concentration in small-sized HDL and LDL subfractions were significantly higher in individuals with a low VO_{2max}. Using correlation analyses, the concentration of phospholipids and cholesterol in small-sized HDL subfractions were found to be inversly associated with VO_{2max}. Interestingly, the lipoprotein profile of healthy individuals with low VO_{2max} seemed to match the lipoprotein profile seen in insulin resistant individuals with increases in large VLDL particles, as well as higher levels of small HDL and LDL subfractions [19]. Several studies have previously shown that VO_{2max} is associated with insulin resistance in different populations [20,21]. In our previous biomarker study of the

same cohort, a pro-atherogenic profile was associated with the low VO_{2max} group, indicated by higher levels of both choline and unsaturated fatty acids.

4.1. VLDL-subfractions

Despite similar fasting status and relatively small differences in cardiometabolic risk factors between the groups with high and low VO_{2max}, the participants with low VO_{2max} had about 50% more lipid-content in their large VLDL particles. Previous studies have shown that triglyceride levels in the large VLDL-1 particles are closely correlated with insulin resistance and VO_{2max} level [19,22,23]. In the absence of direct measures of insulin resistance in our cohort (e.g., insulin levels, oral glucose test or HbA1C), the triglyceride/HDL-ratio was calculated to estimate insulin sensitivity [24,25]. The low VO_{2max} group had an average triglyceride/HDL-ratio of 1.4 and the high VO_{2max} group had a ratio of 1.0. Although no standard reference range is established for the triglyceride/HDL-ratio, there are indications that triglyceride/HDL has an incremental association with insulin resistance [26–28]. Hence, it is likely that the individuals in the low VO_{2max} group are more insulin resistant than those in the high VO_{2max} group.

It has been recognized that elevation of the large-sized VLDL1 particles initiates a sequence of events that lead to dyslipidemia, including the formation of small dense LDL and HDL subfractions. This is supported by enrichment of small LDL5 and HDL3 subfractions among individuals with low VO_{2max}. In addition, low VO_{2max} was associated with increased levels of Apo-B in VLDL particles. As each VLDL particle contains one molecule of Apo-B, this means that individuals of the low VO_{2max} group have 15% more circulating VLDL particles than the high VO_{2max} group. Increased levels of VLDL particles have previously been associated with visceral adiposity and type-2 diabetes [29].

4.2. HDL-subfractions

HDL is highly heterogeneous in its size and composition. This heterogeneity arises partly during reverse cholesterol transport, as there is a constant remodeling of lipoproteins [30]. Standard clinical measurement of HDL-C fails to capture this heterogeneity in composition, size, and biological function. Recently, it has been suggested that the protective role of HDL against atherogenesis may be related to the composition of the particles rather than the concentration [31]. In the current study, clinically measured total HDL-C was slightly higher in participants in the high VO_{2max} group (non-significant). However, when separating HDL into subfractions according to size, we found the opposite; that the levels of cholesterol in HDL3 (H3CH) particles were inversely associated with VO_{2max}. This indicates a shift towards the small-sized HDL particles with decreasing VO_{2max}. These results are in line with a previous study comparing twins with long-term differences in physical activity level that reported less VLDLs, and a shift towards large-sized HDL particles in long-term physically active participants [32]. Additionally, a recent study by Jones et al. reported increased concentration of cholesterol in the large VLDL subclasses and increased triglycerides in the small-sized HDL subfractions in children with low cardiorespiratory fitness [33].

Previous studies have shown that larger HDL particles seem to protect against atherogenesis, while the smaller subclasses are positively correlated with the risk of CVD [19,34–44]. When measuring HDL-C by traditional methods, the positive and negative associations of small and large HDL particles may counteract each other. Hence, important information may be lost, and interpretation of HDL-C alone may not reflect the true clinical status. Our results are in line with previous statements suggesting that HDL size affects the function and could be clinically valuable for cardiovascular risk prediction [30,41]. However, conflicting data still exists on HDL-subfractions and CVD-risk [45,46].

It is proposed that high levels of large HDL-C are more protective than high levels of small and dense HDL-C [34,47]. An NMR study by Holmes et al. identified an inverse relationship between cholesterol concentration in medium and large HDL particles with risk of myocardial infarction [46]. These data are somewhat in line with our study, as H3CH was found to be inversely associated with VO_{2max}. In 2018, Sacks et al. proposed the hypothesis that mainly medium and large-sized HDL are responsible for reverse cholesterol transport as cholesterol enters and exits without changing the HDL size [39]. If their hypothesis is correct, the small and dense HDL particles are potentially not as atheroprotective as the larger HDL particles. Our results support previous statements that measurement of cholesterol in HDL subfractions may be more valuable than the traditional HDL-C [34,44,47].

Additionally, there was a negative correlation between VO_{2max} and the phospholipid-load in HDL3 particles. To our knowledge, the concentration of phospholipid HDL-subfractions has not been investigated in association with cardiorespiratory fitness and CVD risk. Phospholipids are believed to influence the anti-oxidative properties of HDL, but the mechanism is not known [48].

4.3. LDL-subfractions

It is widely accepted that LDL has a role in initiation and progression of atherosclerosis, and increasing evidence suggests that small LDL particles are of special importance [49,50]. Increased levels of small LDL particles have previously been associated with the atherosclerotic risk independently of traditional risk factors such as LDL-C [11,51,52]. The small LDL particles can more easily penetrate the arterial wall, are more prone to atherogenic modifications and binding to the arterial wall [53]. In the present study, there was no association between total LDL-C and VO_{2max}. However, triglyceride levels within the small and dense LDL5 particles were increased in participants with low VO_{2max} compared to high VO_{2max}. A possible explanation for the higher concentration of triglycerides in LDL5 is the slightly higher BMI of the participants in the low VO_{2max} group. Enrichment of triglycerides in LDL has previously been observed in visceral obesity [54]. Increased triglyceride levels in LDL5 may impair lipoprotein-lipase activity, thus reducing the efficiency of VLDL conversion to LDL via hydrolysis of triglycerides. Such events are observed with insulin resistance and type 2 diabetes [55,56].

4.4. Limitations of the study

Information on statin therapy was not available from the HUNT database. The main effect of statin therapy is lowering LDL-cholesterol and possibly also slightly increasing the amount of HDL cholesterol [57,58]. Participants in our study may have altered lipoprotein subfractions as a result of their statin use, but it is quite unlikely due to their age and strict inclusion criteria (being healthy with no previous heart disease) [59].

As diet is likely to contribute to the levels of the quantified lipoprotein subfractions, more information on long-term diet and a comparable diet at the day of blood samplings would be preferable. However, knowledge of the participants diet is spares from the HUNT study, so we were only able to control for the number of hours of fasting before blood sampling.

Attention should also be given to the need of a standardized method to analyze lipoprotein subfractions. Different methods utilize different techniques for separation of subfractions, which makes it challenging to directly compare results [33,60,61]. Promising studies illustrating the relevance of lipoprotein subfractions for risk prediction of CVD make it even more important. If subfractions are to be included in the clinic, more investigation should be performed to find the most suitable method.

4.5. Conclusion

Low VO_{2max} was associated with an atherogenic lipidomics profile in healthy individuals. Individuals with low VO_{2max} had more VLDL

particles, as well as increased lipid content in large-sized VLDL, and small-sized HDL and LDL particles. The lipoprotein profile of individuals with low VO_{2max} resembles the profile seen in patients with insulin resistance. The atherogenic lipoprotein profile in these otherwise healthy individuals may represent one of the links between low VO_{2max} and CVD risk. Additional studies are needed to test whether some of these lipoprotein subfractions may represent early risk factors for CVD.

Financial support

The study was supported by grants from the Norwegian Health Association and the Liaison Committee between the St. Olavs Hospital and the Faculty of Medicine at the Norwegian University of Science and Technology (NTNU). There are no disclosures to report.

CRediT authorship contribution statement

Markus Nodeland: Data analysis, literature search, writing, publication. **Marie Klevjer:** Data analysis, statistics, writing, publication. **Julie Sæther:** Data analysis, literature search, writing, publication. **Guro Giskeødegård:** Data analysis, statistics, writing, publication. **Tone Frost Bathen:** Design, sample analysis, Data analysis, statistics, writing, publication. **Ulrik Wisløff:** Design, cohort development, writing, publication. **Anja Bye:** Design, cohort development, data analysis, literature search, writing, publication.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Trøndelag Health Study (The HUNT Study) is a collaboration between HUNT Research Centre (The Faculty of Medicine, Norwegian University of Science and Technology NTNU), Helse Midt-Norge, Trøndelag County Council and The Norwegian Institute of Public Health.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.atherosclerosis.2022.01.001.

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