UiT The Arctic University of Norway

Faculty of Health Sciences

Level of the Lipid Markers ApoA1 and ApoB in a General Youth Population. The Tromsø Study, Fit Futures 1

Gunnhild Hammervold Johnsen

Supervisors: Anne-Sofie Furberg (ISM) and Maria Averina (IBM)

Master's thesis in medicine MED-3950 Sept 2020

Foreword

Autumn 2018 I contacted Anne-Sofie Furberg because of my interest in using results from the Fit Futures study for my thesis. We decided on focusing the thesis around lipid-levels in the population, more accurately ApoA1 and ApoB levels in a youth population. This topic has to our knowledge not been investigated in any previous study in Norway.

Throughout my work with the thesis, Anne-Sofie Furberg was my main supervisor, and also Maria Averina was asked to join the project. She contributed as being co-supervisor.

I would like to thank my supervisors for help and guidance in the process of writing this thesis. The process of the writing of the thesis has been a challenging and educational supplement to my clinical practice in the study of medicine.

Table of contents

Summary

Background

Low serum Apolipoprotein A1 (ApoA1) and high Apolipoprotein B (ApoB) are risk factors for the development of cardiovascular disease. Studies have found tracking of lipid and lipoprotein levels form childhood and youth, into adult life. In order to identify an unfavorable lipid profile in young ages, knowledge about the distribution of these biomarkers in the general population is a prerequisite. Thus, the main aim of the thesis is to establish the distribution and reference intervals of serum ApoA1, ApoB, and ApoB/A1 ratio in a general youth population in Norway. To our knowledge, there are no previous Norwegian data on this topic. Furthermore, we will investigate if there are any associations between serum ApoA1 and ApoB and sex, BMI and waist-hip ratio, diet, and lifestyle factors such as tobacco use, alcohol use, and level of physical activity.

Material and methods

The analysis is based on data from 836 participants, aged 15-17 years in the Fit Futures 1 (FF1) study in 2010-2011 in the Tromsø region. The participants filled in an electronic questionnaire, and trained nurses collected blood samples, clinical measurements, and interview data. Reference intervals for ApoA1 and ApoB were made by establishing sex-specific 2.5 and 97.5 percentiles following the CLSI guidelines. Multivariable linear regression models were used to test whether sex, anthropometry, diet, and lifestyle were associated with serum concentrations of ApoA1 and ApoB.

Results

Our findings show reference intervals for serum ApoA1 (girls = 0.99-1.80 g/L, boys = 0.86-1.59 g/L), ApoB (girls 0.38-1.00 g/L, boys = 0.34-0.92 g/L), and ApoB/A1 ratio (girls = 0.27-0.82 g/L, boys = 0.27-0.86 g/L) for the FF1 population. Male sex (beta= -.149, p <0.001), BMI (beta= -.001, p=.001), snuff use (beta= -.034, p=.032), and intake of fat fish (beta= -.057, p=0.001) were negatively associated with ApoA1 concentrations, while a high intake of vegetables (beta= .054, p=0.17) and lean fish (beta =.029, p=0.83) were positively associated with ApoA1 concentration. Male sex (beta=-.047, p<0.001) and high intake of lean fish (beta = -.029, p=.014) were negatively associated with elevated ApoB concentrations, while BMI (beta = .012, p<.0001), intake of junk food (beta = .023, p=.023) and fat fish (beta = .028, p=0.017) were positively associated with elevated ApoB concentrations.

Conclusion

Reference intervals for ApoA1, ApoB, and ApoB/A1 were established for 15-17-year-olds in a general youth population in Norway. Our findings show that ApoA1 and ApoB concentrations are significantly associated with sex, diet, and BMI.

1 Background

1.1 ApoA1 and ApoB

Apolipoproteins are lipid-binding proteins that are responsible for the transport of lipids in the circulation, and play a role in lipid assembly, lipid transport and lipid metabolism by mediation interactions with receptors, enzymes and lipid transport proteins. The different apolipoproteins serve as cofactors for enzymes and ligands for receptors. All plasma lipids, except free fatty acids, are unsolvable in water and have to be transported in plasma as lipoproteins. The lipoproteins consist of a core of cholesterol and triglycerides, surrounded by a coat of phospholipids. The apolipoproteins are embedded in this outer shell (1-3).

Apolipoprotein B (ApoB) is the most essential protein in very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and low-density lipoprotein (LDL) cholesterol, also known as “bad cholesterol”. The concentration of ApoB is a good measurement for the number of these particles in plasma because there is only one ApoB-molecule per particle. Apolipoprotein A1 (ApoA1) is the main protein in high-density lipoprotein (HDL) cholesterol and can be used as a measurement for the concentration for HDL, also known as the “good cholesterol”. Although there can be more than one ApoA1-molecule in each HDL-particle, ApoA1 is therefore not an exact expression for the number of particles, as the case is with ApoB (1).

1.2 Cholesterol and Cardiovascular risk

Lipid analysis is commonly done as an assessment of cardiovascular risk. The strongest risk factors for adult cardiovascular disease (CVD) include high concentration of LDL, low concentration of HDL, elevated blood pressure, type 1 or 2 diabetes mellitus, cigarette smoking, and obesity (4). Hyperlipidemia wit high levels of “bad cholesterol” and low levels of “good cholesterol” promotes inflammatory responses in the arterial wall, leading to atherosclerosis. Atherosclerosis is the result of a complex interaction between blood elements, disturbed flow, vessel wall abnormality, involving several pathological processes (5-7). Atherosclerosis starts as small changes, fatty streaks, in the barrier between the blood and the blood vessel, the endothelia. When this barrier is weakened, lipids and inflammatory cells may enter the vessel. Most of the cells in the fatty streaks are macrophages, together with some T-cells. Fatty streaks can develop into atherosclerotic lesions (atheromas). Foam cells and extracellular lipid droplets form a core region in the center of an atheroma, which is surrounded by smooth muscle cells and a collagen-rich matrix, and infiltrated by T-cells,

macrophages, and mast cells. Atherosclerosis progresses over decades; however, the clinical manifestations usually appear from middle age. The pathological processes in atherosclerosis may lead to rupture, hemorrhage, and calcification of blood vessels, and thrombosis when mature plaques are present and flow-limiting.

The clinical manifestations include myocardial infarction, stable and unstable angina, stroke, transient ischemic attack, claudication, and critical limb ischemia (8). Atherosclerosis is highly prevalent worldwide, relatively few suffer a clinical event, but it is the most common cause of disability and premature death in the world (9).

A low serum concentration of ApoA1 and a high concentration of ApoB have been reported as risk factors for cardiovascular disease in case control-studies, studies of patients who have undergone angiography, and in prospective studies (10). It is encountered that a higher ApoB/ApoA1 ratio is associated with early subclinical atherosclerosis (11) and may be a more efficient indicator of atherosclerotic risk than other serum lipids (12-15). ApoB is closely related to the degree of progression of atherosclerosis and is widely accepted as the most important causal agent of atherosclerotic cardiovascular disease (16). Arterial injury causes endothelial dysfunction promoting modification of ApoB containing lipoproteins and infiltration of monocytes into the subendothelial space. Internalization of the ApoB containing lipoproteins by macrophages promotes foam cell formation, which is the hallmark of the fatty streak phase of atherosclerosis (11). Prevention of high cholesterol and associated cardiovascular risk includes the use of statins, that reduces the risk of stroke and mortality with patients with increased cholesterol levels (17), and lifestyle interventions (18, 19).

1.3 Prevention of Cardiovascular risk in a Youth Population

The clinical manifestations of cardiovascular disease typically appear during middle age, but the underlying atherosclerotic process has a long asymptomatic phase of development that often starts during early childhood, and it seems that this process occurs at an increasingly younger rate (20). Accelerated progression of atherosclerosis is linked to unhealthy lifestyle behaviors in preadolescent youth, and increased severity of asymptomatic coronary and aortic atherosclerosis along with an increased number of cardiovascular risk factors in autopsies performed among children, adolescents and young adults (21). In the Muscatine Study (22) and Young Finns Study (23, 24) elevated levels of cholesterol in childhood were associated with elevated levels in adult life. Thus, systemically identifying dyslipidemia in youths at an

early stage may allow for early targeted preventive measurements aiming to reduce the risk for atherosclerosis, and CVD morbidity and mortality later in life.

In the intergraded guidelines for cardiovascular health and risk reduction in children and adolescents provided by the National Heart, Lung and Blood Institute, the decision limits for high ApoB is ≤ 1.10 g/L (≤ 110 mg/dL) and for low is ApoA1 > 1.15 g/L (>115 mg/dL) in teenagers (8). The American Academy of Pediatrics (25) recommends children with obesity or family accumulation of hyperlipidemia to measure lipid concentration regularly.

General preventive measure in relation to CVD include improvement of diet and exercise patterns to treat or prevent obesity, and efforts to prevent smoking, and development towards hyperglycemia and hypertension (11, 26). More specifically, in the dietary guidelines for children and adolescents provided by The American Heart Association, the recommendations are a balanced caloric intake with sufficient physical activity to achieve an appropriate weight and consume more fruits, vegetables, fish, whole grains, and low-fat dairy products. It is also recommended to limit the intake of fruit juice, sugar-sweetened beverages and foods, and salt (11).

To our knowledge, there are no published data on the distribution of ApoA1 and ApoB in a general youth population in Norway, and related anthropometry and lifestyle factors.

2 Objective

The main aim of this study is to establish the distribution and reference intervals of ApoA1, ApoB, and ApoB/A1 ratio in a general youth population in Norway. Secondary aim is to investigate if there are any associations between ApoA1 and ApoB and sex, clinical measurements as BMI and waist-hip ratio (WHR), lifestyle factors such as tobacco use, alcohol use, level of physical activity, and diet.

3 Material and method

3.1 Study population

The Fit Futures 1 study invited all first-year upper secondary school students in the municipality of Tromsø and Balsfjord to a comprehensive health survey in 2010-2011 (27). The invited cohort comprised 1117 adolescents, and 1038 (508 girls and 530 boys) attended the survey (attendance rate 93%). The participants met at the Clinical research unit, the

University Hospital of North Norway. The survey included self-administered electronic questionnaire, clinical measurements, blood samples and interview by trained nurses.

In the present study, 77 participants age 18 or above were excluded. As the study aims to establish reference values for ApoA1, ApoB, and ApoB/A1 in a youth population and test whether these are associated with anthropometry and lifestyle factors in this age group, 112 participants with missing blood sample values were excluded. Three participants diabetes type 1 and were also excluded from further analysis. None of the participants reported use of lipid-reducing drugs.

Chauvenet's criterion (28) was used to define SD for ApoA1, ApoB, and ApoB/A1 for the remaining 846 participants. The method is based on creating an acceptable band of data around the mean and eliminating any data outside the band (i.e. data considered to be outliers). Based on a population size of 850, participants with ApoA1, ApoB, or ApoB/A1 values outside 3.42 SD (N=10) were rejected from further analysis.

Thus, we included 836 adolescents, 398 girls, and 438 boys aged 15-17 years in the present analysis. The majority were 16 years of age (N = 672), 117 participants were 17 years, while a small group of 47 were 15 years.

Figure 1 shows the selection of the study population based on the participants in FF1.

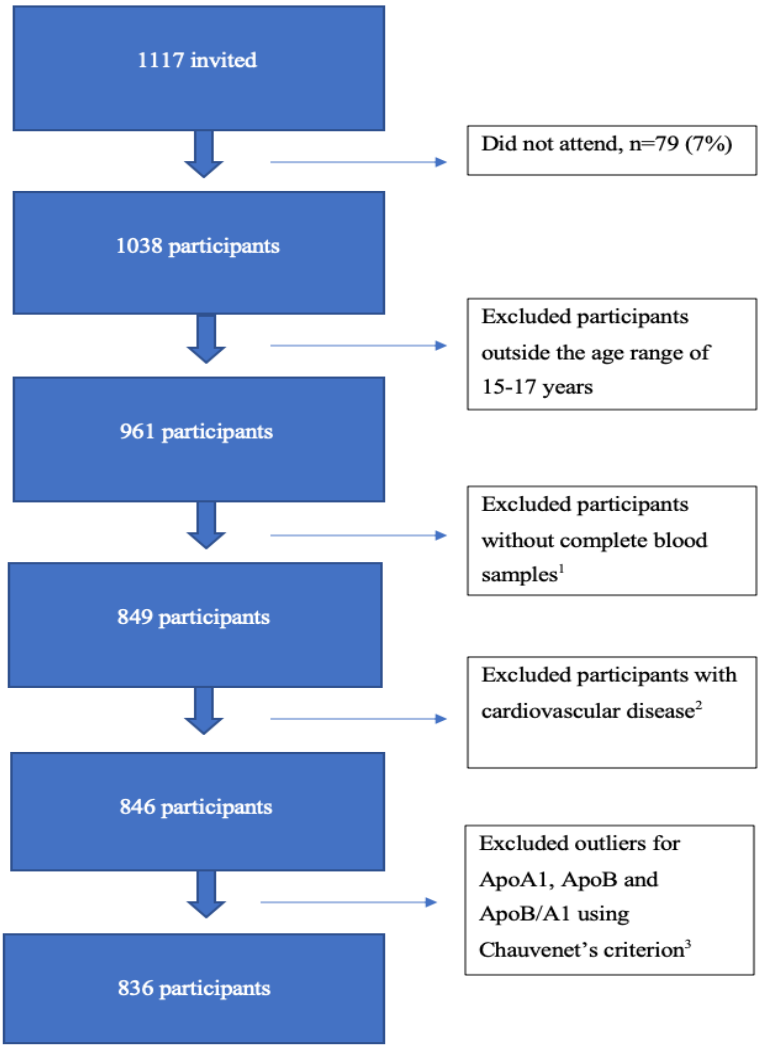

Figure 1. Flow chart for the FF1 study.

[1] 112 participants did not have complete blood samples.

[2] Three participants had self-reported diabetes type I.

[3] Data outside 3.42 SD were rejected due to a population of 850.

3.2 Measurements

Body height was measured in cm and weight in kg (kilograms). Kilograms were measured to the nearest 0.1 unit with participants wearing light clothing and no shoes on an automatic electronic scale, the Jenix DS 102 Stadiometer (Dong Sahn Jenix, Seoul, Korea). BMI was calculated as weight divided by height squared (kg/m^2). Hip and waist circumference were measured twice to the nearest cm without outerwear by using measuring tape. Hip circumference was measured around the widest part of the hip while waist circumference was measured at the umbilical line. Waist-to-hip ratio (WHR), the ratio of the circumference of

the waist to that of the hip, was calculated. WHR was defined as abdominal obesity in the analysis. The cut-off values for abdominal obesity were 0.90 waist/hip ratio for men and 0.85 for women (29).

3.3 Electronic Questionnaire

An electronic questionnaire (Questback) was used to collect data about ethnicity, diet, physical activity, alcohol intake and use of tobacco.

3.3.1 Diet

The participants were asked about their dietary habits. They were asked how often they ate cheese (all kinds), fat fish (salmon, trout, mackerel, herring), lean fish (cod, saithe, haddock), junk food (pizza, hamburger, hot dogs), chocolate sweets (chocolate, candy), and snacks (chips, biscuits, cakes, buns), or drank full-fat dairy drinks (whole milk, kefir, and yogurt) and fat-reduced dairy drinks (semi-skimmed milk, cultura, fat reduced yogurt). The participants could choose between the answers “Rarely/never”, “1-3 times per month”, “1-3 times per week”, “4-6 times per week”, “Every day”. For the question about intake of fruit and vegetable the answers were “Rarely/never”, “1-3 times per month”, “1-3 times per week”, “1-2 times per day”, “3-4 times per day”, “5 times or more”.

Some of the categories were merged and recoded in the analysis due to too few participants in each category.

3.3.2 Physical activity

Questions about level of physical activity was included in Questback. The participants were asked to assign their usual level of physical activity in leisure time, the past 12 months, on a four-level scale. The question was validated among men and women aged 40-42 in the sixth Tromsø Study 07-08; the participants were able to rate their leisure time activity level in correspondence with their objectively measured physical activity (30).

3.3.3 Use of Tobacco

Current use of tobacco was assessed by the questions “Do you smoke?” and “Do you use snuff?”, the answers were “No, never”, “Sometimes” and “Daily”. Due to few participants answering that they were smoking daily, “Sometimes” and “Daily” were recoded into “Sometimes or daily” for the question “Do you smoke?”.

3.3.4 Frequency of alcohol intake

Frequency of alcohol was assessed by the question: “How often do you drink alcohol?” (“Never”, “Once per month or less”, “2-4 times per month”, “2-3 times per week”, “4 times or more per week”). The three categories with the highest frequency of alcohol intake were merged and recoded to “≥ 2times per month” in the analysis.

3.3.5 Ethnicity

The participants were given the following questions: “Do you consider yourself as: Norwegian? (Yes/No). “Do you consider yourself as: Sami?” (Yes/No). “Do you consider yourself as: Kven?” (Yes/No). “Do you consider yourself as: Other?” (Yes/No). The participants could answer “Yes” to multiple questions. In the analysis, “Norwegian” was recoded to those who only considered themselves as Norwegian. “Other” was recoded to include also those who answered “Yes” to Sami or Kven.

3.4 Clinical analysis

3.4.1 Blood samples

Non-fasting blood samples were collected from an antecubital vein and analyzed consecutively at the Department of Laboratory Medicine, UNN, Tromsø. Serum lipids were analyzed by enzymatic colorimetric methods. The analysis was performed on Cobas 8000 instrument with reagents from Roche Diagnostics Norway AS. CVa (analytic coefficient of variation) was <2% for total cholesterol, HDL cholesterol, and LDL cholesterol, and <5% for ApoA1 and ApoB. Serum assays were obtained from the first blood specimen taken from each participant. ApoB/A1 ratio was calculated and coded into a new variable based on the levels of ApoA1 and ApoB.

3.5 Statistical analysis

ApoA1 and ApoB were tested for normality (Figure 2 and Figure 3). We made a presentation of reference values for ApoA1 and ApoB with sex-specific 2.5 and 97.5 percentiles derived form a relatively healthy general population sample by following the Clinical & Laboratory Standards Institute (CLSI) guidelines for defining, establishing, and verifying reference intervals (31). Reference intervals describes the normal variation expected to find in a group of well-defined individuals, unlike decision limits that indicates levels significantly correlated with higher risk of adverse outcomes or are diagnostic for the presence of a specific disease (32).

Multivariable linear regression is a method that establishes the relationship between a dependent variable and multiple explanatory variables. The method was used in this thesis to examine if sex, anthropometry and lifestyle factors could explain the variations in levels of ApoA1 and ApoB. The independent variables were selected based on what could affect lipid levels. Categorical variables were recoded to dummy variables in the regression analysis. The variables (Age, Sex, BMI, Abdominal Obesity, Frequency of Alcohol Intake, Smoking, Snuff use, Level of Physical Activity in Leisure Time, Intake of Lean Fish, Fat Fish, Cheese, Semi-Skimmed Milk, Junk Food, Chocolate Sweets, Snacks, Fruits, and Vegetables) were put in the model for ApoA1 and ApoB, respectively. The same analyses were also run with whole milk instead of semi-skimmed milk. We used backwards elimination and evaluated the result of the p-value for each potential explanatory variable; non-significant variables were eliminated from the model in a stepwise manner to find the model that could best explain the data. The models were run with all variables (age, sex, ethnicity, physical activity, BMI, WHR, smoking, snuff use, alcohol intake, vegetable intake, fruit intake, junk food intake, cheese, snacks, chocolate/sweets intake, milk intake, fat fish and lean fish intake). R^2 was used as a goodness-of-fit measure for the regression models. Interactions were tested by creating interaction variables for the variables desired to test for interaction, respectively. The interaction was tested for interaction for sex and the following variables: vegetables, junk food, fat fish, lean fish. Interaction between vegetables and alcohol frequency, junk food and alcohol frequency, and interaction between lean and fat fish were also tested. No statistically significant interactions were found. All statistical analyses were done in SPSS version 26, and the level of statistical significance was set to $P<0.05$.

3.6 Ethics

Informed consent was obtained from all participants in the study. For individuals below 16 years, parental consent was also obtained. Participants were paid 200 Norwegian kroner on completion of the study. All procedures were approved by the Regional Committees for Medical and Health Research Ethics North Norway (REK) and the National Data Protection Authority. This study was approved by REK (2019/1135 Fettstoffer i blodet hos ungdom, Personvernombudet University Hospital North Norway, and The Tromsø Study – Data and publication committee (DPU).

4 Results

4.1 Characteristics of the study population of the FF1 study

The baseline characteristics of the study population are presented in Tables 1 and 2. The study population included 398 girls and 438 boys who had measured ApoA1 and ApoB. The mean age was 16.12 for boys and 16.05 for girls (see Table 1). Table 2 shows the dietary characteristics of the study population. The decimals were rounded up to the nearest tenth/hundredth. The boys had lower in HDL concentrations compared to the girls (mean HDL boys = 1.24 mmol/L vs. mean HDL girls 1.46 mmol/L). There was no difference in BMI (mean BMI girls = 22.25 kg/m^2, mean BMI boys = 22.24 kg/m^2) between the sexes. 12.4% of the girls and 11.7% of the boys had abdominal obesity based on WHR above 0.85 for females and WHR above 0.9 for males.

Table 1. Characteristics of participants in the FF1 study. Numbers are means and standard deviations (SD) for continuous variables and percent for categorical variables.

	Boys	*Girls*
	N = 438[1]	*N = 398*[1]
Age, years	16.1 (.5)	16.1 (.4)
Weight, kg	69.9 (13.6)	60.6 (11.5)
Height, cm	177.1 (6.7)	165.0 (6.4)
BMI, kg/m^2	22.2 (3.9)	22.3 (4.0)
Hip circumference, cm	97.4 (8.4)	97.5 (7.9)
Waist circumference, cm	81.7 (10.8)	76.9 (10.0)
Waist-hip ratio	.84 (.06)	.79 (.06)
Total cholesterol, mmol/L	3.87 (.69)	4.22 (.71)

[1] Numbers may vary due to missing value.

HDL cholesterol, mmol/L	1.24 (.28)	1.46 (.33)
LDL cholesterol, mmol/L	2.26 (.61)	2.44 (.64)
Triglycerides, mmol/L	1.12 (.52)	1.03 (.47)
Frequency of alcohol intake, %		
Never	31.4	25.5
Once per month or less	38.6	44.7
≥2-4 times per month	30.0	29.8
Smoking, %		
Never	76.6	81.2
Sometimes or daily	23.4	18.8
Snuff use, %		
Never	60.6	68.6
Sometimes	12.8	13.9
Daily	26.7	17.5
Ethnicity, %		
Norwegian	87.7	91.4
Other[2]	12.3	8.6
Physical activity in leisure time, %		
Sedentary	28.5	12.6
Moderate	25.0	40.2
High	46.5	47.2

[2] Defined as Sami, Kven or other ethnicities than Norwegian.

Table 2 shows the dietary characteristics of the study population. The decimals were rounded up to the nearest tenth/hundredth. The participants had a relatively low intake of fish in their diet. Only 1/3 of the participants reported eating fat or/and lean fish one time a week or more. Chocolate/sweets and snacks intake was similar for boys and girls, while boys ate more often junk food and girls ate more often fruits and vegetables.

Table 2. Dietary characteristics of participants of the FF1 study. Numbers are percent.

	Boys	*Girls*
	N = 438[1]	N = 398[1]
Cheese		
≤1-3 times per month	17.8	20.8
1-3 times per week	39.3	42.8
4-6 times per week	28.7	26.3
Every day	14.3	10.1
Junk food[2]		
Rarely/never	5.8	9.6
1-3 times per month	37.4	50.8
1-3 times per week	47.4	35.4
≥4-6 times per week	9.3	4.3
Chocolate sweets[3]		
≤1-3 times per month	30.8	29.8
1-3 times per week	56.1	56.6

[1] Numbers may vary due to missing value.

[2] Hamburger, pizza or hot dogs.

[3] Chocolate, candy.

≥4-6 times per week	13.1	13.6
Snacks[4]		
≤1-3 times per month	32.3	34.4
1-3 times per week	53.6	52.4
≥4-6 times per week	14.2	13.2
Fruit		
≤1-3 times per month	18.4	9.8
1-3 times per week	37.0	25.0
4-6 times per week	20.5	24.2
1-2 times per day	14.9	24.5
≥3-4 times per day	9.3	16.4
Vegetables		
≤1-3 times per month	13.8	9.8
1-3 times per week	28.3	21.3
4-6 times per week	32.1	35.0
≥1-2 times per day	25.7	34.0
Fat fish		
Rarely/never	22.1	18.8
1-3 times per month	44.7	50.4
≥1-3 times per week	33.3	30.8
Lean fish		

[4] Chips, biscuits, cakes, buns.

Rarely/never	18.5	19.5
1-3 times per month	47.4	48.7
≥1-3 times per week	34.1	31.7
Whole milk		
Rarely/never	54.1	52.3
1-3 times per month	32.3	41.4
≥1-3 times per week	13.7	6.4
Semi-skimmed milk		
Rarely/never	33.1	30.9
1-3 times per month	26.6	37.8
1-3 times per week	16.4	15.3
≥4-6 times per week	23.8	16.1

4.2 Reference intervals and distribution for ApoA1, ApoB, and ApoB/ApoA1

The 2.5, 50, 95, and 97.5 percentiles, SD, and mean for serum ApoA1, ApoB, and ApoB/ApoA1 for boys, girls, and the total study population are presented in Table 3. ApoA1 (skewness = .277, kurtosis = .009) (Figure 2) and ApoB (skewness = .515, kurtosis = .199) (Figure 3) were close to normally distributed. ApoA concentrations were statistically significantly lower in males compared to females (P=0.001), as well as ApoB concentrations (P=0.047). 22.5% of the participants were below the clinical decision limit for teenagers established by the National Heart, Lung and Blood Institute (8) for ApoA1 (>1.15 g/L), and 0.36% of the participants were above the limit for ApoB (≤ 1.10 g/L). 32.5% of the boys in this study were under the decision limit for ApoA1, compared to 12.6% of the girls. 0.36% of the participants were over the limit for ApoB.

Table 3. Distribution of ApoA1 (g/L), ApoB (g/L) and ApoB/A1 in the FF1 study.

	Percentiles					
	2.5	***50***	***95***	***97.5***	***Mean***	***SD***
Total (N = 836)						
ApoA1	.92	1.28	1.64	1.74	1.22	.20
ApoB	.36	.61	.91	.95	.62	.16
ApoB/A1	.27	.45	.77	.84	.49	.15
Girls (N = 398)						
ApoA1	.99	1.36	1.73	1.80	1.37	.20
ApoB	.38	.63	.93	1.00	.65	.16
ApoB/A1	.27	.47	.77	.82	.49	.15
Boys (N = 438)						
ApoA1	.86	1.21	1.54	1.59	1.22	.18
ApoB	.34	.60	.86	.92	.60	.15
ApoB/A1	.27	.49	.77	.86	.50	.14

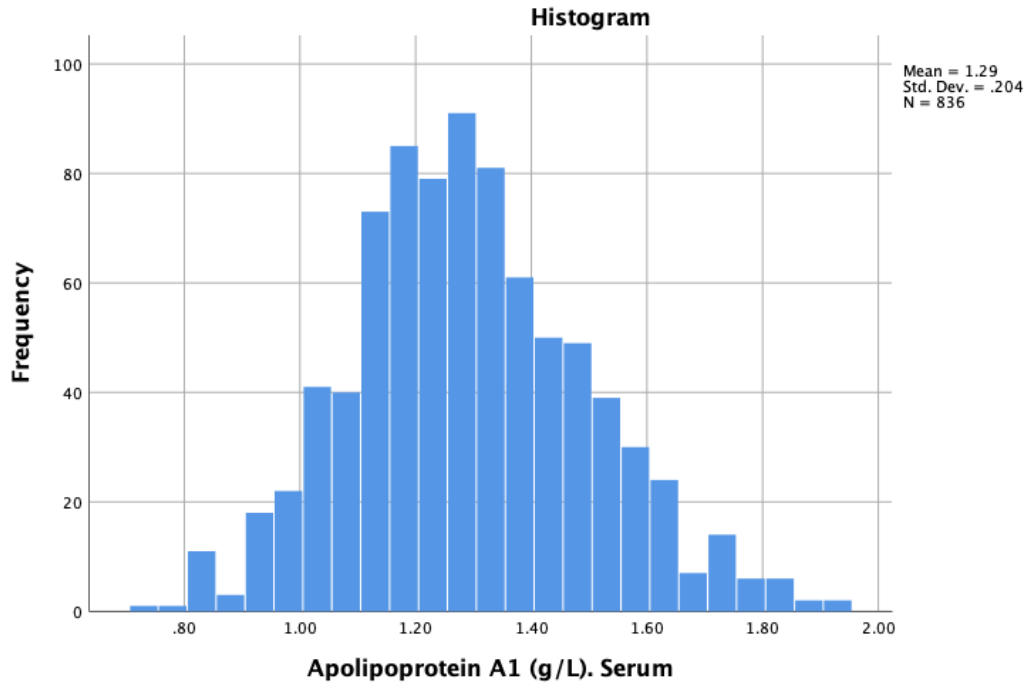

Figure 2. The Distribution of ApoA1 for the FF1 population.

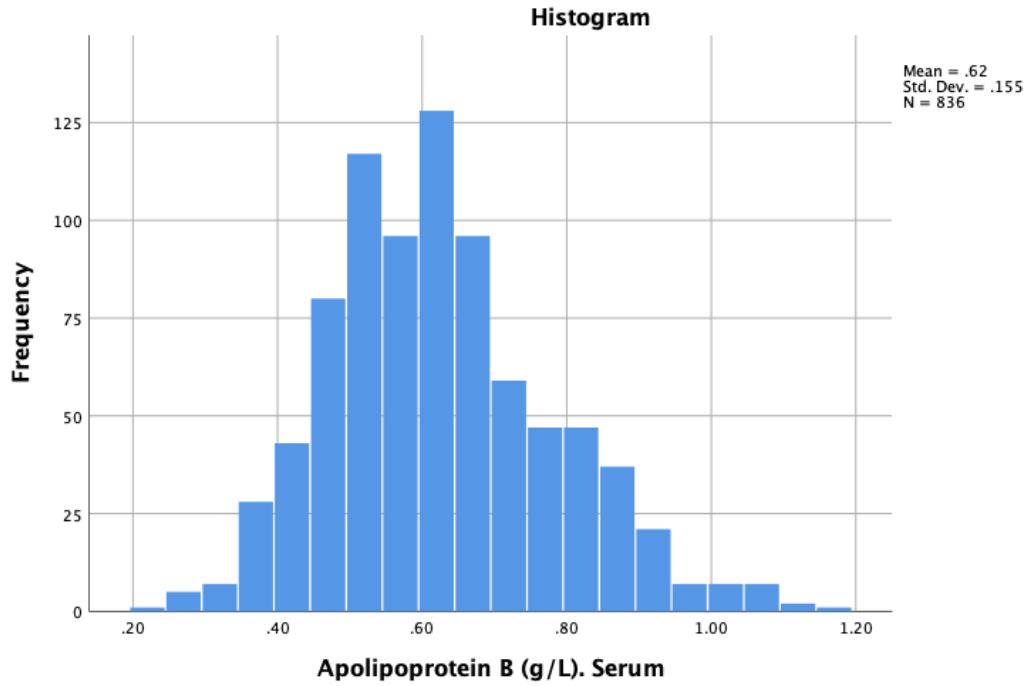

Figure 3. The Distribution of ApoB for the FF1 population.

4.3 Multivariable linear regression

Multivariable linear regression analysis was used to determine whether there was an association between ApoA1 and ApoB values and the chosen variables (Sex, Age, BMI, Abdominal Obesity, Smoking, Snuff Use, Frequency of Alcohol Intake, intake of Fat Fish,

Lean fish, Semi-skimmed Milk and Whole Milk, Vegetables, Fruits, Snacks, Chocolate Sweets, Junk Food, and Cheese). The models were run with all variables, and later separately tested for interaction, as described in the Material and method section. None of the interaction terms were statistically significant and thus were not included in the final regression model. The regression model for ApoA1(R^2 = .170) is presented in Table 4, and the regression model for ApoB (R^2 = .135) is presented in Table 5.

Alcohol, cigarette smoking, physical activity, milk and cheese intake, fruits intake and chocolate/sweets and snacks intake and abdominal obesity were not associated with either ApoA1 or ApoB values in the FF1 study population.

Table 4. Multivariable linear regression analysis ApoA1 (g/L) in the FF1 study.

	Beta	*P-value.*
Boys vs girls	-.149	.000
Age, years	.001	.938
BMI, kg/m²	-.006	.001
Daily snuff use vs others	-.034	.032
Vegetables vs ≤1-3 times per month		
1-3 per week	.037	.095
4-6 times per week	.049	.023
≥1 time per day	.054	.017
Fat fish vs rarely/never intake		
1-3 times per month	-.057	.001

≥1-3 times per week	-.60	.005
Lean fish vs rarely/never intake		
≥1-3 times per week	.029	.083

The final model of the multiple linear regression analysis shows a significant positive association between ApoA1 serum concentrations and frequent intake of vegetables (more than 4 times per week vs. <3 times per month) and a tendency to a positive association with intake of lean fish more than once per week (vs. never/rarely). ApoA1 serum concentration were significantly negatively associated with male sex, BMI, daily use of snuff and fat fish intake once per month or more often vs. never/rarely intake.

Table 5. Multivariable linear regression analysis of ApoB (g/L) in the FF1 study.

	Beta	*P-value.*
Boys vs girls	-.047	.000
Age, years	.010	.382
BMI, kg/m²	.012	.000
Junk food vs ≤		
1-3 per month	.023	.023
Fat fish vs rarely/never		
≥ 1-3 times per month	.028	.017
Lean fish vs rarely/never		
≥1-3 times per month	-.028	.014

The final model of the multiple linear regression analysis shows a significant positive association between ApoB serum concentrations and BMI, frequent intake of fat fish once per month or more often (vs. never/rarely) and intake of junk food (1-3 times per month vs. ≤ 1-3 times per month). ApoB serum concentration were significantly negatively associated with male sex, and lean fish intake once per month or more often vs. never/rarely intake.

5 Discussion

In this FF1 study, the higher value of the reference interval for ApoB was set to 0.95 g/L. The decision limit from the National Heart, Lung and Blood Institute for high level of ApoB was ≤ 1.10 g/L, and only 0.36% of the participants of this study were above the high concentration value. The lower value of the reference interval ApoA1 in this study was 0.92 g/L, while the decision limit of low ApoA1 is >1.15 g/L. 22.5% of the participants of the study were below the decision limit of ApoA1. Thus, our data show that only a few adolescents have increased ApoB concentrations defined by the decision limit, but a relatively large part of the population have a reduced ApoA1 concentration defined by the decision limit. Especially boys had a high prevalence of low ApoA2 concentration (32.5% boys under the decision limit vs. 12.6% girls). The boys were also lower in HDL compared to the girls (mean HDL boys = 1.24 mmol/L vs. mean HDL girls 1.46 mmol/L).

University Hospital North Norway´s donor study (33) collected data from 120 healthy adult blood donors (60 males and 60 females). The population is comparable to our study population because both studies are from a general healthy population from the same geographical region and the laboratory test were performed at the same laboratory by the same analytical methods. The lower reference values for adults of ApoA1(34) were 1.04 g/L for males and 1.08 g/L for females, that are slightly higher compared to .86 g/L for boys and .99 g/L for girls of our study. The higher reference value of ApoB (35) in adults were 1.33 g/L for males and 1.17 g/L in females, that are considerably higher compared to the higher reference value of the boys (.95 g/L) and the girls (1.00 g/L). Our result show that teenagers from the Fit Futures study had more favorable ApoB, but not ApoA1 profile than the healthy adult population from the same geographic area.

Even though most of the studies of apolipoproteins and cardiovascular disease are seemingly based on adults, it is of interest to discuss findings in a youth population due to the possibility of early intervention and possible prevention of cardiovascular disease and premature death.

The findings of this study may contribute to the knowledge basis for early risk assessment connected to risk factors for cardiovascular disease and early preventive measures to avoid premature death. The distribution of ApoA1, ApoB, and ApoB/ApoA1 ratio in a Norwegian youth population will hopefully be a tool for clinicians in the evaluation of risk assessment of cardiovascular disease, by identifying youth with elevated ApoB and ApoB/A1 ratio and intervening at an early stage to with preventive measurements. Diet and exercise intervention, closer follow-ups with a focus on avoiding other risk factors for CVD as hypertension, smoking, and hyperglycemia. Perhaps this may lead to better preventive measurements to avoid cardiovascular disease and premature death, by identifying youths with an elevated risk of cardiovascular disease.

This study shows the distribution of ApoA1 and ApoB in a general youth population in Norway and identifies associations between anthropometry, lifestyle factors, and ApoA1 and ApoB levels. There have been several surveys of serum lipid levels among youths (36, 37). Only a few of these have included ApoA1 and B.

Previous studies have identified lifestyle risk factors associated with elevated lipoprotein levels and atherosclerosis. Most of these studies are conducted on an adult population. The findings from this FF1 youth population are similar in the study for adults (10), except the association between fat fish intake and lower ApoA1 and higher ApoB, and the lack of association with alcohol intake.

The findings of association with vegetables, lean fish, and junk food and ApoA1 and ApoB levels were similar to findings in other studies. High vegetable intake in the diet, as in the DASH (Dietary Approaches to Stop Hypertension) diet, has been shown to reduce blood pressure as well as triglycerides and LDL cholesterol in a randomized controlled trial (38). The analysis in this FF1 study show association between the intake of vegetables and high ApoA1 levels, compared to those participants who never or rarely eat vegetables. Fish contains a variety of nutrients such as protein, fat, vitamin D and vitamin B12, selenium, and iodine (39). Lean fish consumption is associated with a decreased risk of having metabolic syndrome, decreased serum triglyceride, and increased HDL-cholesterol (40). In our FF1 study intake of lean fish was associated with higher ApoA1 levels and lower ApoB levels compared to individuals who never or rarely eat lean fish. Junk food consumption is associated with premature heart disease as junk food is known to have a high level of salt and saturated fat, which is associated with elevated blood pressure and an unfavorable lipid profile

with a high level of LDL and low level of HDL. Elevated ApoB levels were associated with increased junk food intake in our study.

A possible explanation for the unexpected result for fish intake and lipoproteins ApoA1 and ApoB in this FF1 study are environmental pollutants. Fat fish obviously contains more fat than lean fish, and farmed fat fish allegedly contains more fat than wild fatty fish. The total fat content in farmed salmon was significantly higher than in wild salmon due to a higher content of saturated and monounsaturated fatty acids, as well as a higher content of omega-6 fatty acids (41). Fat fish also contains pollutants, accumulated in the food chain and stored in fatty tissues, that influence lipid levels. There have not been used any data of pollutants in this study, but fat fish is the strongest predictor of organic pollutants PFAS concentration in serum in the FF1 study population (42). PFAS concentrations in serum were associated with higher total cholesterol, higher LDL-cholesterol, and higher ApoB in the same FF1 population (43).

There have been observations of associations between high ApoA1 and excessive alcohol consumption in studies of adults (10, 44). An explanation for the lack of association in this FF1 population to alcohol can be related to underreporting or relatively low consumption over time in this age group.

The strengths of our study are a high attendance rate (93%), which may contribute to reducing selection bias. Due to the large data set we had access to in this study, there was a wide range of information about each of the participants, and this made it possible to adjust for known risk factors. The study is population-based and therefore to a large degree representative for the general population. It is however conducted on a limited age-group, and the results cannot be transferred to other age groups without further research.

A weakness of this study is that the dietary factors, tobacco, and alcohol use was self-reported, which may have led to under-reporting. As the present study is a cross-sectional study, it only gives a “here and now” representation of the population instead of following the development over time – therefore can these results only represent association, but not causality. The analysis was limited to the participants with complete blood samples, which lead to exclusion of 112 participants. However, there is no reason to believe that those without blood samples had different lipoprotein levels than those included in the study.

6 Conclusion

Our findings show reference intervals for ApoA1(girls = 0.99-1.80, boys = 0.86-1.59), ApoB (girls 0.38-1.00, boys = 0.34-0.92), and ApoB/A1 ratio (girls = 0.27-0.82, boys = 0.27-0.86) in a general youth population in Norway. The reference interval for ApoB corresponds well with clinical decision limits established by the National Heart, Lung and Blood Institute (8). However, about 1/5th of the study population (22,5%) has ApoA1 concentrations below the clinical decision limit, therefore the lower value of the reference interval for ApoA1 is below this limit. ApoA1 and ApoB concentration are influenced by sex, diet (fish intake, vegetable intake and junk food intake), snuff use, and BMI. Identifying risk factors connected to ApoA1 and ApoB levels may contribute to awareness and preventive measures to further reduce cardiovascular risk and premature death. Reducing BMI and intake of junk food, increasing intake of lean fish and vegetables could be potential preventive measures to improve lipoprotein profile in the general adolescent population. Future observational studies and intervention trials are necessary to determine the effectiveness of preventive measurements against the risk of development of cardiovascular disease in youths.

Works cited

1. Hagve T-A, Berg JP. Klinisk biokjemi og fysiologi. Berg JP, editor. Oslo: Gyldendal; 2015.
2. Nelson DL, Cox M. Lehninger - Principles of Biochemistry. New York: Freeman; 2013.
3. Ramasamy I. Recent advances in physiological lipoprotein metabolism. Clinical Chemistry and Laboratory Medicine (CCLM). 2014;52(12):1695.
4. Buttar H, Li T, Ravi N. Prevention of cardiovascular diseases: Role of exercise, dietary interventions, obesity and smoking cessation. Clinical Cardiology. 2005;10(4):229-49.
5. Lusis AJ. Atherosclerosis. Nature. 2000;407(6801):233-41.
6. Falk E, Shah PK, Fuster V. Coronary Plaque Disruption. Circulation. 1995;92(3):657-71.
7. Rafieian-Kopaei M, Setorki M, Doudi M, Baradaran A, Nasri H. Atherosclerosis: process, indicators, risk factors and new hopes. Int J Prev Med. 2014;5(8):927-46.
8. Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents: Summary Report. PEDIATRICS. 2011;128(5):213-56.
9. Herrington W, Lacey B, Sherliker P, Armitage J, Lewington S. Epidemiology of Atherosclerosis and the Potential to Reduce the Global Burden of Atherothrombotic Disease. Circulation Research. 2016;118(4):535-46.
10. Averina M, Nilssen O, Brenn T, Brox J, Arkhipovsky VL, Kalinin AG. Factors behind the Increase in Cardiovascular Mortality in Russia: Apolipoprotein AI and B Distribution in the Arkhangelsk Study 2000. Clinical Chemistry. 2004;50(2):346-54.
11. Panayiotou A, Griffin M, Georgiou N, Bond D, Tyllis T, Tziakouri-Shiakalli C, et al. ApoB/ApoA1 ratio and subclinical atherosclerosis. International Angiology. 2008;27(1):74-80.
12. Walldius G, Jungner I. The apoB/apoA-I ratio: a strong, new risk factor forcardiovascular disease and a target for lipid-lowering therapy– a review of the evidence. Journal of Internal Medicine. 2006;259(5):493-519.
13. Tamang HK, Timilsina U, Sing KP, Shrestha S, Raman RK, Panta P, et al. Apo B/Apo A-I Ratio is Statistically A Better Predictor of Cardiovascular Disease (CVD) than Conventional Lipid Profile: A Study from Kathmandu Valley, Nepal. Journal of Clinical and Diagnostic Research. 2014;8(2):34-6.
14. Sedlis SP, Schechtman KB, Ludbrook PA, Sobel BE, Schonefeld G. Plasma apoproteins and the severity of coronary artery disease. Circulation. 1986;73:978-86.
15. Tognon G, Berg C, Mehlig K, Thelle D, Strandhagen E, Gustavsson J, et al. Comparison of Apolipoprotein (apoB/apoA-I) and Lipoprotein (Total Cholesterol/HDL) Ratio Determinants. Focus on Obesity, Diet and Alcohol Intake. Plos ONE. 2012;7(7).
16. Shapiro MD, Fazio S. Apolipoprotein B-containing lipoproteins and atherosclerotic cardiovascular disease. F1000Research. 2017;6:134-.
17. Chan K, Gaziano J, Hebert P. Cholesterol lowering with statin drugs, risk of stroke, and total mortality – an overview of randomized trials. JAMA. 1997;278(4):313-21.
18. Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Saturated Fat, Carbohydrate, and Cardiovascular disease. American journal of clinical nutrition. 2010;91(3):502-9.
19. Kopin L, Lowenstein CJ. Dyslipidemia. Annals of Internal Medicine. 2017;167(11):ITC81-ITC96.
20. Stoner L, Weatherall M, Skidmore P, Castro N, Lark S, Faulkner J, et al. Cardiometabolic Risk Variables in Preadolescent Children: A Factor Analysis. J Am Heart Assoc. 2017;6(10):e007071.

21. Bernenson GS, Srinivasan SR, Bao W, Newman WP, Tracy RE, Wattigney WA. Association between Multiple Cardiovascular Risk Factors and Atherosclerosis in Children and Young Adults. The New England Journal of Medicine. 1998;338(23):1650-6.
22. Lauer RM, Lee J, Clarke W. Factors Affecting the Relationship Between Childhood and Adult Cholesterol Levels: The Muscantine Study. PEDIATRICS. 1988;82(3):309-17.
23. Juonala M, Viikari JSA, Kähönen M, Solakivi T, Helenius H, Jula A, et al. Childhood Levels of Serum Apolipoproteins B and A-I Predict Carotid Intima-Media Thickness and Brachial Endothelial Function in Adulthood: The Cardiovascular Risk in Young Finns Study. Journal of the American College of Cardiology. 2008;52(4):293-9.
24. Juhola J, Magnussen CG, Viikari JSA, Kähönen M, Hutri-Kähönen N, Jula A, et al. Tracking Serum Lipid Levels, Blood Pressure, and Body Mass Index from Childhood to Adulthood: The Cardiovascular Risk in Young Finns Study. The Journal of Pediatrics. 2011;159:584-90.
25. Daniels SR, Greer FR, the Committee on Nutrition. Lipid Screening and Cardiovascular Health in Childhood. The American Acadamy of Pediatrics. 2008;122(1):198-208.
26. Daniels SR, Pratt CA, Hayman LL. Reduction of risk for cardiovascular disease in children and adolescents. Circulation. 2011;124(15):1673-86.
27. Nilsen OA, Ahmed LA, Winther A, Christoffersen T, Furberg A-S, Grimnes G, et al. Changes and tracking of bone mineral density in late adolescence: the Tromsø Study, Fit Futures. Archives of Osteoporosis. 2017;12(1):37.
28. Barnett V, Lewis T. Outliers in Statistical Data. Chichester: J.Wiley and Sons; 1994.
29. Waist circumference and waist-hip ratio report of a WHO expert consultation. Geneva: World Health Organisation; 2011.
30. Emaus A, Degerstrøm J, Wilsgaard T, Hansen BH, Dieli-Conwright C, Furberg A-S, et al. Does a variation in self-reported physical activity reflect variation in objectively measured physical activity, resting heart rate, and physical fitness? Results from the Tromsø study. Scandinavian Journal of Public Health. 2010;38(5):105-18.
31. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guidelines; 28(3).
32. Solberg HE. Tidsskriftet. 2004;124.
33. Labhåndbok UNN [Available from: https://labhandbok.unn.no/.
34. APOLIPOPROTEIN A1 Laboratoriehåndbok: Universitetssykehuset Nord-Norge HF; 2017 [updated 02.07.2020. Available from: https://labhandbok.unn.no/medisinsk-biokjemi/apolipoprotein-a1-article2008-816.html.
35. APOLIPOPROTEIN B Laboratoriehåndbok: Universitetssykehuset Nord-Norge HF; 2017 [updated 07.02.2020. Available from: https://labhandbok.unn.no/medisinsk-biokjemi/apolipoprotein-b-article2009-816.html.
36. Nielsen TRH, Lausen-Thomsen U, Fonvig CE, Bøjsøe C, Pedersen L, Bratholm PS, et al. Dyslipidemia and Reference Values for Fasting Plasma Lipid Concentrations in Danish/North-European White Children and Adolescents. BMC Pediatrics. 2017;17(1):116-.
37. Ata N, Maguire B, Hamilton DC, Kuhle S. Percentile curves for cardiometabolic disease markers in Canadian children and youth: a cross-sectional study. BMC Pediatrics. 2018;18(1):314.
38. Chiu S, Bergerom N, Williams P, Bray G, Sutherland B, Krauss RM. Comparison of the DASH (Dietary Approaches to Stop Hypertension) diet and a higher-fat DASH diet on blood pressure and lipids and lipoproteins: a randomized controlled trial. American Society for Nutrition. 2016;103:341-7.
39. Alexander J, Frøyland L, Hemre G-I, Jacobsen BK, Lund E, Meltzer HM, et al. A comprehensive assessment of fish and other seafood in the Norwegian diet. Oslo; 2007.

40. Tørris C, Molin M, Cvancarova MS. Lean fish consumption is associated with lower risk of metabolic syndrome: a Norwegian cross sectional study. BMC Public Health. 2016;19(16):347.
41. Lundebye A-K, Lock E-J, Rasinger JD, Nøstbakken OJ, Hanninsdal R, Karlsbakk E, et al. Lower levels of Persistent Organic Pollutants, metals and the marine omega 3-fatty acid DHA in farmed compared to wild Atlantic salmon (Salmo salar). Enviromental Research. 2017;155:49-59.
42. Averina M, Brox J, Huber S, Furberg A-S. Perfluoroalkyl Substances in Adolescents in Northern Norway: Lifestyle and Dietary Predictors. The Tromsø Study, Fit Futures 1. Enviroment International. 2018;114:123-30.
43. Averina M, et. a. A possible link between exposure to perfluoroalkyl substances (PFAS) and dyslipidemia, hypertension and obesity in adolescents. The Tromsø study Fit Futures. Submitted to Environmental Research 2020.
44. Hao G, Wang Z, Zhang L, Chen Z, Wang X. Relationship Between Alcohol Consumption and Serum Lipid Profiles Among Middle-Aged Population in China: A Multiple-Center Cardiovascular Epidemiological Study. 2015;66(8):753-8.

Reference: Tognon G, Berg C, Mehlig K, Thelle D, Strandhagen E, Gustavsson J, et al. Comparison of Apolipoprotein (apoB/apoA-I) and Lipoprotein (Total Cholesterol/HDL) Ratio Determinants. Focus on Obesity, Diet and Alcohol Intake. Plos ONE. 2012;7(7).	**Study design**: Cross-sectional study	
	Evidence level:	VII
	GRADE	Medium

Purpose	**Material and methods**	**Results**	**Discussion and comments**
To investigate the cross-sectional association of obesity, diet and lifestyle factors with ApoB/ApoA1 and total cholesterol/HDL, respectively, in a Swedish population. **Conclusion** The study showed that apolipoproteins and lipoproteins share common determinants. A positive association of alcoholic beverages on the lipid profile has also been confirmed for both ratios and was stronger in women than in men. With the exception of ethanol, diet played no, or only a minor part, in explaining the association of obesity with either ratio. **Country** **Sweden** **Year of data sampling** **2001 to 2004**	The study population consisted of randomly selected women and men aged 25-74 years living in the Västra Götaland Region at the time of the sampling. 3614 (42%) out of 8626 eligible individuals participated in the study. Body height, weight, waist and hip circumference were measured. Usual dietary intake was assessed with a validated self-administered food frequency questionnaire. The questionnaire also included information about health, physical activity, socioeconomic, and lifestyle factors. Blood samples were taken at different times of the day on subjets fasting for at least four hours. Due to implausible energy intake, 155 subjects were excluded. 7 subjects were excluded due to pregnancy, 189 subjects were excluded because they were taking statin at enrolment. 166 subjects were excluded from the analysis because of missing ApoB/ApoA1 ratio and 2 because of outlier values for this ratio.	Analyses at food level for one food item at a time, adjusted for age, smoking status, physical activity, marital status and menopausal status plus estrogen use in women, showed that two food groups were consistently associated with both ratios in men and women: sweetened products and alcoholic drinks. The apolipoprotein and lipoprotein ratios were highly correlated, particularly in women, and obesity was strongly associated with both. Additionally, age, cigarette smoking and alcohol intake were important determinants of these ratios. Alcohol was the only dietary factor that appreciably attenuated the association between obesity and each of the ratios, with a stronger attenuation in women. Other dietary intake and lifestyle-related factors such as smoking status and physical activity had a lower effect on this association.	**Checklist:** • **Is the purpose clearly defined? Yes.** • **Is the selection representative for the population? Participants were more likely to be women, of higher age and with a higher education level than non-participants.** • **Is the inclusion criteria clearly defined? Yes.** • **Did responders deviate from non-responders?** • **Does the study use methods that are reliable for what is being measured? Yes.** • **Is data collection standardised? Yes.** • **Are important confunding factors taken into account in design/execution/analyzing? Yes.** • **Do you trust the results? Yes. Obesity and dietary habits.** • **Can results be transferred into practice? Yes.** **Strenghts** It is a population-based cohort including both men and women where weight status, cardiovascular risk factors and dietary intakes have been assessed The availability of data concerning various potential confounders, the possibility to capture different aspects of diet and particularly to discriminate ethanol intake from different beverages, the availability of both apolipoprotein and lipoprotein measures from samples collected on the same occasion and finally a sufficient study population size to allow analyses on men and women separately. **Weaknesses** The cross- sectional design that does not allow inference on possible causative relationships but only on associations. The diet data was based on food frequency questionnaires which were semi- quantitative with regard to quantities consumed and thus likely to contain obesity-related underreporting. Both smoking status and obesity were both strongly associated with dyslipidemia so it cannot exclude some residual confounding by these factors.

Reference: Juhola J, Magnussen CG, Viikari JSA, Kähönen M, Hutri-Kähönen N, Jula A, et al. Tracking Serum Lipid Levels, Blood Pressure, and Body Mass Index from Childhood to Adulthood: The Cardiovascular Risk in Young Finns Study. The Journal of Pediatrics. 2011;159:584-90.	Study design: Cross-sectional study
	Evidence level: VII
	GRADE: Medium

Purpose	Material and methods	Results	Discussion and comments
To examine tracking and predictiveness of childhood lipid levels, blood pressure, and body mass index for risk profile in adulthood and the best age to measure childhood risk factor levels. **Conclusion** Childhood blood pressure, serum lipid levels, and body mass index correlate strongly with values measured in middle age. **Country** **Finland** **Year of data sampling** **1980 to 2007**	In 1980 4320 Finnish children of the ages 3, 6, 9, 12, 15, and 18 years were invited, and 3596 (83.2%) participated in the cross-sectional survey conducted at 5 university cities in Finland. Subjects were randomly chosen from a national register. The follow-up studies for the whole study group were performed in 1983, 1986, 2001, and 2007 when 2991 (83.2%), 2799 (78.3%), 2283 (63.5%), and 2204 (61.3%) subjects participated, respectively. Blood samples were collected after a 12 hour fast, measuring total cholesterol, triglycerides, HDL, and LDL. Measurements of height and weight were obtained, and BMI was calculated. Blood pressure was measured. *Statistical method* *A 27-year tracking of cardiovascular risk factors was estimated by calcutaing Spearman´s rank-order correlation co-efficients stratified by sex and age. For blood pressure analysis, subjects based 3 years at baseline were excluded due to different measuring methods. The ability of the high-risk childhood levels in predicting abnormal adult levels was assessed by using sensitivity, specificity, positive predictive value, and negative predictive value.*	In 2007, male subjects had higher BMI, SBP, DBP, total cholesterol, LDL-C, and TG levels compared with female subjects, and HDL-C levels were lower in male subjects. Significant 27-year tracking was observed both in male and female subjects. In general, the correlation co-efficients were statistically significant; in all age groups, however, tracking was statistically non-significant for DBP in 12-year-old male and female subjects and TG in 9-year-old male and female subjects and 3-year-old female subjects. The strongest correlations were observed in 12- to 18-year old subjects. With the exception of high TG levels in younger girls, subjects with high childhood risk factor levels were at significantly increased odds of the development of abnormal risk factor levels 27 years later. Older girls were at higher odds of the development of adult obesity or dyslipidemia because of the presence of the childhood risk factor. Older boys who were overweight or obese were at substantially increased odds of the development in adulthood compared with younger boys who were obese/overweight. The specificity rate of obesity was highest in female subjects in the 6- and 9-year age groups, and PPV was highest in 15-year old girls. In boys, the sensitivity rate was the highest in the 12-year old group and PPV was highest in the 15-year old group. For hypertention, there did not seem to be an age difference. For total cholesterol and LDL-C levels, sensitivity and specificity rates and PPV were highest in older age groups (12, 15, and 18 years), especially in female subjects. For HDL-C level, there was not much distinction in age groups in female subjects. In male subjects, childhood HDL-C levels were most predictive in the 6-, 9-, and 12-year age groups.	**Checklist:** • **Is the purpose clearly defined? Yes.** • **Is the selection representative for the population? Yes, subjects were randomly chosen from a national register.** • **Is the inclusion criteria clearly defined? Yes.** • **Did responders deviate from non-responders? Non-participation in the follow-up study, if differential, may have led to bias. But the authors refer to other studies that has previously shown that subjects lost to follow-up differ by sex and age, but not in blood lipid levels, blood-pressure or BMI.** • **Does the study use methods that are reliable for what is being measured? Yes.** • **Is data collection standardised? Yes, but may differ due to age of the subjects and year of the data sampling. This is taken into account in the analysis.** • **Are important confunding factors taken into account in design/execution/analyzing? Yes.** • **Do you trust the results? Yes** • **Can results be transferred into practice? Yes.** **Strengths** Randomly selected cohort of young adults followed 27 years since childhood. They were able to provide detailed information about the persistence of childhood risk factor levels to adulthood in 6 different age groups and to determine the best age for measurements of risk factors. **Weaknesses** Non-participation in the follow-up study, may have led to bias. Possible errors and biases with blood pressure method include terminal digit preference and variability between observers. The results are limited to white European subjects.

Reference: Bernenson GS, Srinivasan SR, Bao W, Newman WP, Tracy RE, Wattigney WA. Association between Multiple Cardiovascular Risk Factors and Atherosclerosis in Children and Young Adults. The New England Journal of Medicine. 1998;338(23):1650-6.	**Study design**: Cross-sectional study
	Evidence level: VII
	GRADE Medium

Purpose	Material and methods	Results	Discussion and comments
To gather information on the relation of multiple risk factors to the extent of asymptomatic atherosclerosis in young people. **Conclusion** As the number of cardiovascular risk factors increases, so does the severity of asymptomatic coronary and atherosclerosis in young people. **Country** **USA** **Year of data sampling** **1973 to 1996**	Autopsies were performed on 204 young people 2 to 39 years of age, who had died from various causes, principally trauma. Data on antemortem risk factors were available for 93 of these persons, who were the focus of this study. Risk factors were correlated with the extent of atherosclerosis in the aorta and coronary arteries. Spearman correlation analysis was used to examine the association between the extent of fatty-streak and fibrous plaque lesions in the aorta and coronary arteries and age at death and the z scores of individual risk-factor variables. A multivariate technique was then used to examine the association between the two sets of variables.	The extent of fatty streaks and fibrous plaques in the aorta and coronary arteries increased with age. The association between fatty streaks and fibrous plaques was much stronger in the coronary arteries ($r=0.60$, $P<0.001$) than in aorta ($r=0.23$, $P=0.03$). Among the cardiovascular risk factors, body-mass index, systolic and diastolic blood pressure, and serum concentrations of total cholesterol, triglycerides, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol, as a group, were strongly associated with the extent of lesions in the aorta and coronary arteries. In addition, cigarette smoking increased the percentage of the intimal surface involved with fibrous plaques in the aorta (1.22 percent in smokers vs. 0.12 percent in nonsmokers, $P=0.02$) and fatty streaks in the in the coronary vessels (8.27 percent vs. 2.89 percent, $P=0.04$). The effect of multiple risk factors on the extent of atherosclerosis was quite evident. subjects with zero 1, 2, and 3 or 4 risk factors had, respectively, 19.1 percent, 30.3, 37.9 and 35.0 percent of the intimal surface covered with fatty streaks in the aorta. The comparable figures for the coronary arteries were 1.2 percent, 2.5 percent, 7.9 percent and 11.0 percent, respectively, for fatty streaks and 0.6 percent, 0.7 percent, 2.4 percent and 7.2 percent for the collagenous fibrous plaques.	**Checklist:** • Is the purpose clearly defined? **Yes** • Is the selection representative for the population? **Yes.** • Is the inclusion criteria clearly defined? **Yes.** • Did responders deviate from non-responders? **Most deaths were due to accidents or homicide, about 10 percent were due to renal, neoplastic, or infectional diseases or suicide.** • Does the study use methods that are reliable for what is being measured? **Yes,the extent of the intimal surface covered in fatty streaks and raised fibrous plaques in the vessls was graded visually according to procedures developed in the International Atherosclerosis Project.** • Is data collection standardised**? Yes.** • Are important confunding factors taken into account in design/execution/analyzing? **Plasma insulin and glucose concentrations were not measured, but it is reasonable to suggest that these variables may be a part of the cluster of risk factors in the study population.** • Do you trust the results? **Yes.** • Can results be transferred into practice? **Yes.** **Strenghts** The extent of development of fatty streaks and fibrous plaque was evaluated by three different pathologists independently. **Weaknesses** Could not examine the effect of multiple risk factors on the extent of atherosclerosis separately according to race and sex because of the small numbers of persons in each group within the sample of the study. Only 93 participants had data on antemortem risk factors.

Reference: Averina M, Nilssen O, Brenn T, Brox J, Arkhipovsky VL, Kalinin AG. Factors behind the Increase in Cardiovascular Mortality in Russia: Apolipoprotein AI and B Distribution in the Arkhangelsk Study 2000. Clinical Chemistry. 2004;50(2):346-54.	**Study design**: Cross-sectional study	
	Evidence level:	VII
	GRADE	Medium

Purpose	Material and methods	Results	Discussion and comments
Investigate indicators of atherosclerotic risk factors in a Russian population hence to the markedly higher cardiovascular mortality in Russia compared to Western Europe and the US. Apparently first study of apolipoprotein A1 and B in Russia.	ApoA1 and ApoB were measured by immunoturbidimetric assay in 3694 men and women from Arkhangelsk, Russia, in 1999-2000 along with questionnaire, physical examination and other laboratory analyses.	The age-related distribution of ApoB was similar to that in other countries, whereas the ApoA1 was different. For men over or 20 years of age, ApoA1 was considerably higher than in studies from other countries. Women had also relatively high ApoA1, although the difference was not as pronounced as in men. The ApoA1 concentration was positively associated with age and lifestyle variables such as alcohol consumption and physical activity, and negatively associated with body mass index and self-reported myocardial infection. γ-Glutamyltransferase was positively associated with ApoA1 in both sexes.	**Checklist:** • Is the purpose clearly defined? **Yes.** • Is the selection representative for the population? **Yes, most likely.** • Is the inclusion criteria clearly defined? **Yes.** • Did responders deviate from non-responders? **No.** • Does the study use methods that are reliable for what is being measured? **Yes.** • Is data collection standardised? **Yes.** • Are important confunding factors taken into account in design/execution/analyzing? **Yes.** • Do you trust the results? **Yes.** • Can results be transferred into practice? **Yes.** **Strenghts** The first report av ApoA1 and ApoB distributions in Russia based on the WHO-IFCC Reference Materials. The study was carried out in a region with similar mortality rates to those in Russia as a whole in 2000. **Weaknesses** Participants were recruited through the primary healthcare system. The possibility of different distribution of CVD risk factors in some geographic districts of Russia cannot be excluded. Recruitment of the participants during the obligatory medical examination might have led to underreporting of alcohol consumption.
Conclusion The apparently favorable apolipoprotein profiles contrast with official death statistics indicating high cardiovascular mortality in Russia. High ApoA1 might indicate excessive alcohol consumption.			
Country **Russia**			
Year of data sampling 1999-2000			

Reference: Lauer RM, Lee J, Clarke W. Factors Affecting the Relationship Between Childhood and Adult Cholesterol Levels: The Muscantine Study. PEDIATRICS. 1988;82(3):309-17.	**Study design**: Longitidual cohort
	Evidence level: IV
	GRADE: Medium

Purpose	Material and methods	Results	Discussion and comments
Investigate associations between cardiovascular risk factors and cholesterol level in a study of population of children observed into adult life. **Conclusion** Elevated levels of cholesterol during childhood were associated with elevation in adult life. Obesity acquired in adolescence and the young adult years, oral contraceptive use, and cigarette smoking had deleterious effects on adult cholesterol levels and lipoprotein fraction. **Country** **USA** **Year of data sampling** **1971-1985**	A group of 2246 subjects, schoolchildren of Muscatine, Iowa, initially examined at 8-18 years of age were reexamined as young adults of 20 to 25 or 26 to 30 years of age. In the initially examination, the subjects were survey for their levels of height, weight, triceps skinfold thickness, BP, plasma cholesterol, and triglyceride. At the time of the recall these examinations were repeated, in addition to a questionnaire including disease, lifestyle factors and medication. Cholesterol percentiles were calculated for each adult age-gender group. These percentiles were dichotomized into those less than 90th percentile for age and gender and those equal to or greater than the 90th percentile. Logistic regression was used to estimate the probability that adult levels would reach or exceed the 90th percentile. Analysis of variance were used to examine the effects of tobacco and alcohol use on adult lipid levels. Student`s t-test were used to determine the effects of oral contraceptive use on lipid levels. Stepwise linear regression was used to quantify the percentage of explained variability in adult cholesterol level attributable to childhood cholesterol, change in BMI, and oral contraceptive use.	After 15 years of age, BMI increased slightly in girls while boys had a greater increase. Triceps skinfold thickness decreased slightly in pubertal years for boys, suggesting that boys were becoming more muscular. Among girls, triceps skinfold measurement increased among with BMI, suggesting a greater acquisition of body fat than among boys. Pearson's correlation of the childhood age- and gender specific levels of total cholesterol, LDL cholesterol, HDS-cholesterol and LDL/HDL cholesterol ratios, and BMI indicate a high degree of tracking if the population levels of cholesterol and BMI from childhood to adult life. For adult aged 20 to 25 years of age, there was little risk for adult levels of plasma total cholesterol in excess of the 90th percentile if childhood levels were less than the 50th percentile. For both genders, the risk was progressively higher for elevated adult levels when childhood levels were greater than the 50th percentile of total cholesterol. Cholesterol measurements obtained in childhood are predictive of adult levels of total and LDL cholesterol, with 25% to 50% of adult cholesterol variability explained by childhood levels. Of children with cholesterol levels initially found to be greater than the 90th percentile in a single measurement, 43% were found to have levels greater than the 90th percentile at 20 to 30 years of age, with 62% greater than the 75th percentile and 81% greater than the 50th percentile at adult ages. LDL cholesterol and HDL cholesterol levels and their ratio were affected by a number of acquired lifestyles, including development of obesity, cigarette smoking, alcohol consumption and oral contraceptive use. In addition, a family history of ischemic heart disease was an important correlate of elevated cholesterol levels in both children and adults, which suggest that heritable factors play an important role in control of cholesterol levels.	Checklist: • Is the purpose clearly defined? **Yes.** • Is the selection representative for the population? **Yes.** • Is the inclusion criteria clearly defined? **Yes.** • Does the study use methods that are reliable for what is being measured? **Yes.** • Is data collection standardised? **Yes.** • Are important confunding factors taken into account in design/execution/analyzing? **Yes.** • Do you trust the results? **Yes.** • Can results be transferred into practice? **Yes.** Strenghts - Large study population of 2246 participants initially examined at 8-18 years of age and reexamined as young adults of 20-30 years of age. - Standardized clinical and laboratory measurements. Weaknesses - The results are limited to white American subjects.