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COMPREHENSIVE METABOLIC PROFILE FROM CHILDHOOD TO ADULTHOOD

Associations with dietary intervention and lifestyle risk factors. The Special Turku Coronary Risk Factor Intervention Project

Miia Lehtovirta

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To My Boys

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MIIA LEHTOVIRTA: Comprehensive metabolic profile from childhood to adulthood – associations with dietary intervention and lifestyle risk factors
The Special Turku Coronary Risk Factor Intervention Project.
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ABSTRACT

BACKGROUND: Exposure to adverse lifestyle can lead to cardiovascular diseases in adulthood. Risk may be reduced with healthy diet, physical activity, and avoidance of tobacco smoke by inducing beneficial effects on metabolism, including serum lipoproteins and fatty acids. Still, little is known about these links at young age.
PARTICIPANTS: This thesis is part of the prospective, randomized Special Turku Coronary Risk Factor Intervention Project (STRIP) aiming to influence risk factors of cardiovascular diseases mainly via dietary counselling. The repeated dietary intervention, with a key target to decrease intake of saturated fat, continued from age 7 months until age 20 years. Data on diet, physical activity and smoking habits were gathered by food records and questionnaires. Serum cotinine (indicator of tobacco smoke exposure) was measured using gas chromatography and metabolic measures were quantified by nuclear magnetic resonance metabolomics in 338–554 participants between the ages of 9 and 19.
AIM: The aim of this thesis was to study the associations of dietary intervention, achieving the dietary targets of the intervention, physical activity, and exposure to tobacco smoke on a comprehensive metabolic profile from childhood to adulthood.
RESULTS: The intervention reduced intake of saturated fat and increased intake of polyunsaturated fat. The intervention and physical activity were directly associated with a less saturated serum fatty acid profile with inverse associations seen for monounsaturated fatty acids ratio while the associations for tobacco smoke exposure were opposite. The intervention and dietary targets were inversely linked with low-density lipoprotein lipid concentrations. Physical activity was inversely associated with very-low-density lipoprotein (VLDL) concentration and size, and directly with high-density lipoprotein (HDL) particle concentration, size, and cholesterol. Passive tobacco smoke exposure was directly associated with VLDL particle size, and inversely associated with HDL particle size. Non-lipid results were mostly weak.
CONCLUSIONS: Repeated dietary counselling, achieving its targets, or being physically active are beneficially associated with metabolic profile while exposure to tobacco smoke is detrimentally associated with circulating metabolic measures.

KEYWORDS: diet, intervention, lifestyle, risk factor, metabolic profile, longitudinal study, primordial prevention, cardiovascular disease

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kardiologia ja kardiovaskulaarilääketiede
MIIA LEHTOVIRTA: Kokonaisvaltainen metabolinen profiili lapsuudesta aikuisuuteen – yhteys ravitsemusinterventioon ja elämäntapatekijöihin Sepelvaltimotaudin riskitekijöiden interventioprojekti.
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Lokakuu 2023

TIIVISTELMÄ

TAUSTA: Haitalliset elintavat voivat johtaa kardiovaskulaarisairauksiin aikuisena. Riskiä voi pienentää ruokavalion, fyysisen aktiivisuuden, sekä vähäisen tupakansavualtistuksen tuomilla aineenvaihduntamuutoksilla, esim. lipoproteiineihin ja rasvahappoihin. Elintapojen yhteyksistä varhaisen iän aineenvaihduntaan tiedetään vähän.
AINEISTO: Tämä väitöskirjatyö on osa prospektiivista, satunnaistettua Sepelvaltimotaudin Riskitekijöiden Interventioprojekti (STRIP) -tutkimusta, joka pyrkii vaikuttamaan kardiovaskulaarisairauksien riskitekijöihin pääasiassa ravitsemusneuvonnan keinoin. Toistuva ravitsemusinterventio, päätavoitteena vähentää tyydyttyneen rasvan saantia, jatkui 7-kuukauden iästä 20-vuotiaaksi asti. Tietoa ravitsemuksesta, fyysisestä aktiivisuudesta sekä tupakoinnista kerättiin ruokapäiväkirjoilla sekä kyselylomakkeilla. Seerumin kotiniini (tupakansavualtistuksen indikaattori) määritettiin kaasukromatografilla ja aineenvaihduntatuotteet kvantifioitiin ydinmagneettisella resonanssispektroskopialla 338–554 osallistujalta 9–19 vuoden iässä.
TAVOITE: Tämän väitöskirjatutkimuksen tavoite oli tutkia ravitsemusintervention, intervention ravitsemustavoitteiden saavuttamisen, fyysisen aktiivisuuden sekä tupakansavualtistuksen yhteyttä metaboliseen profiiliin lapsuudesta aikuisuuteen.
TULOKSET: Interventio vähensi tyydyttyneen rasvan ja lisäsi monityydyttymättömän rasvan saantia. Interventio sekä fyysinen aktiivisuus olivat suoraan yhteydessä seerumin vähemmän tyydyttyneeseen rasvahappoprofiiliin sekä kääntäen yhteydessä kertatyydyttymättömien rasvahappojen osuuteen, tupakansavualtistuksen yhteyksien ollessa vastakkaiset. Interventio ja ravitsemustavoitteet olivat kääntäen yhteydessä LDL-partikkelien lipidipitoisuuteen. Fyysinen aktiivisuus oli kääntäen yhteydessä VLDL-partikkelien lukumäärään ja kokoon sekä suoraan yhteydessä HDL-partikkelien lukumäärään, kokoon sekä kolesterolipitoisuuteen. Passiivinen tupakointi oli suoraan yhteydessä VLDL-partikkelien kokoon ja kääntäen yhteydessä HDL-partikkelien kokoon. Pienimolekyylisten yhdisteiden tulokset olivat pääosin heikkoja.
JOHTOPÄÄTÖKSET: Toistuva ravitsemusneuvonta, sen tavoitteiden saavuttaminen ja fyysinen aktiivisuus ovat myönteisesti yhteydessä metaboliseen profiiliin kun taas tupakansavualtistus on haitallisesti yhteydessä verenkierron metaboliitteihin.

AVAINSANAT: ravitsemus, interventio, elämäntapa, riskitekijä, metabolinen profiili, pitkittäistutkimus, primordiaalipreventio, kardiovaskulaarisairaudet

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Abbreviations

ApoA-1	apolipoprotein A1
ApoB	apolipoprotein B
BMI	body mass index
C	cholesterol
CI	confidence interval
CRP	C-reactive protein
DHA	docosahexaenoic acid
E%	percentage of total energy intake
HDL	high-density lipoprotein
HDL-C	high-density lipoprotein cholesterol
HOMA-IR	homeostatic model assessment for insulin resistance
IDL	intermediate-density lipoprotein
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
LDL-P	low-density lipoprotein particle concentration
LA	linoleic acid
Lp(a)	lipoprotein (a)
LTPA	leisure-time physical activity
MET	metabolic equivalent
MUFA	monounsaturated fatty acids
NMR	nuclear magnetic resonance
PUFA	polyunsaturated fatty acids
SFA	saturated fatty acids
SD	standard deviation
STRIP	The Special Turku Coronary Risk Factor Intervention Project
VLDL	very-low-density lipoprotein

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals. Additional unpublished data are also presented.

I **Lehtovirta M**, Pahkala K, Niinikoski H, Kangas AJ, Soininen P, Lagström H, Viikari JSA, Rönnemaa T, Jula A, Ala-Korpela M, Würtz P, Raitakari OT. Effect of Dietary Counseling on a Comprehensive Metabolic Profile from Childhood to Adulthood. *Journal of Pediatrics*, 2018; 195: 190–198.

II **Lehtovirta M**, Matthews LA, Laitinen TT, Nuotio J, Niinikoski H, Rovio SP, Lagström H, Viikari JSA, Rönnemaa T, Jula A, Ala-Korpela M, Raitakari OT, Pahkala K. Achievement of the Targets of the 20-year Infancy Onset Dietary Intervention – Association with Metabolic Profile from Childhood to Adulthood. *Nutrients*, 2021; 13(2): 533.

III **Lehtovirta M**, Wu F, Rovio SP, Heinonen OJ, Laitinen TT, Niinikoski H, Lagström H, Viikari JSA, Rönnemaa T, Jula A, Ala-Korpela M, Raitakari OT, Pahkala K. Association of physical activity with metabolic profile from adolescence to adulthood. *Scandinavian Journal of Medicine & Science in Sports*, 2023; 33(3): 307–318.

IV **Lehtovirta M**, Pahkala K, Rovio SP, Magnussen CG, Laitinen TT, Niinikoski H, Lagström H, Viikari JSA, Rönnemaa T, Jula A, Ala-Korpela M, Raitakari OT. Association of Tobacco Smoke Exposure on Metabolic Profile from Childhood to Early Adulthood. The Special Turku Coronary Risk Factor Intervention Project (STRIP). *European Journal of Preventive Cardiology*, 2023; zwad285. Online ahead of print.

1 Introduction

Atherosclerosis is a lifelong process that starts in childhood and leads to cardiovascular complications later in life. Cardiovascular diseases are mostly the result of atherosclerosis, hardening and narrowing of the arteries, and they are the leading cause of death in Finland and worldwide. (Ference et al., 2017; Official Statistics of Finland (OSF), 2022) As atherosclerosis can develop asymptomatically for decades, efforts to prevent it should start at an early age and focus on tackling the establishment of factors attributing to its evolution. Central to this primordial prevention are lifestyle factors with diet, physical activity and tobacco smoke exposure being the most important. (Arnett et al., 2019) The aim of addressing these lifestyle risk factors is to prevent the evolution of adverse lipid levels, excess adiposity, and elevated blood pressure which act as intermediating risk factors in the development of atherosclerosis. (Mendis et al., 2011)

Lifestyle choices influence the way the human body functions. There are numerous small molecules in circulation representing the results of the interplay between genetics and external factors. (Ussher et al., 2016) These metabolites can be used to provide insights into disease pathophysiology, identify biomarkers, or evaluate disease risk. High-throughput nuclear magnetic resonance (NMR) metabolomics allows the quantification of numerous circulating metabolites, for example, lipoprotein subclasses, fatty acids, amino acids, and metabolites of glycolysis. (Soininen et al., 2015) This methodology has been used in large epidemiological studies revealing, for instance, novel markers for cardiovascular disease risk. (Deelen et al., 2019; Julkunen et al., 2023) Still, knowledge is limited on how lifestyle factors during childhood are reflected on this metabolic profile.

The Special Turku Coronary Risk Factor Intervention Project (STRIP), established in 1989, is a prospective, randomized study which focused on influencing the environmental risk factors of atherosclerosis from infancy to early adulthood. (Simell et al., 2009) The dietary counselling intervention given in the study specifically targeted the quality of dietary fat while additionally promoting the consumption of fruit, vegetables, and whole grains. The intervention phase of the study was started in infancy and lasted until the participants were 20 years of age. The study has an impressive and unique repository of data on lifestyle, biochemical,

and anthropometric measures assessed multiple times during the still ongoing study. The aim of this thesis was to investigate the effects of the dietary counselling intervention and the associations of quality of diet, physical activity, and exposure to tobacco smoke on a comprehensive serum metabolic profile measured repeatedly from childhood to adulthood.

2 Review of the Literature

2.1 Atherosclerosis

2.1.1 Development of atherosclerosis

Atherosclerotic cardiovascular diseases are the principal causes of morbidity and mortality. (Ference et al., 2017) Their clinical manifestations, such as coronary heart disease and ischemic stroke, typically occur from middle age as the end stages of atherosclerosis, a slow and dynamic process beginning early in life and initiated by the retention and consecutive aggregation of apolipoprotein B (ApoB) containing lipoproteins from the bloodstream into the arterial intima at vascular sites predisposed for plaque formation. (Skålén et al., 2002; Tabas et al., 2007) The likelihood of ApoB-containing lipoprotein particles, of which low-density lipoprotein (LDL) is most abundant, to enter and leave the arterial intima is dependent on, for example, their sustained plasma concentration, and lipid and protein composition. (Borén & Williams, 2016; Williams & Tabas, 1995) Higher plasma concentrations increase the probability of these particles to become retained in the artery wall as does their composition modulated affinity for arterial-wall proteoglycans. The risk associated with increased lipoprotein concentrations in the plasma are commonly assessed by measuring the circulating LDL cholesterol concentration (LDL-C). Native hunter-gatherer populations and primates, all of whom do not show signs of atherosclerosis, retain their LDL-C concentrations at less than 1.8 mmol/L. (O'Keefe et al., 2004) Additionally, individuals with certain genetic variants present LDL-C concentrations of less than 2 mmol/L throughout their life and are virtually free from developing atherosclerosis. (Cohen et al., 2006) This implies that the concentration of ApoB-containing lipoprotein particles needs to increase above certain levels for atherosclerosis to develop, after which the probability of atherosclerotic plaque development increases in a dose-response manner. (Ference et al., 2017; Goldstein & Brown, 2015)

Lipoproteins normally flow into and out of the arterial wall, though the molecular mechanisms behind this movement are not completely understood. ApoB-containing lipoproteins that due to their size are capable of efficiently cross an intact endothelium are chylomicron remnants, smaller very-low-density lipoproteins

(VLDL), intermediate-density lipoproteins (IDL), previously mentioned LDL particles, and lipoprotein (a) [Lp(a)]. (Borén & Williams, 2016) Even among these, the smaller ones pass the endothelium more effortlessly. Only a small percentage of the ApoB-containing lipoproteins entering the artery wall start to aggregate as they are bound by proteoglycans in the extracellular matrix. The inclination of LDL particles to aggregate varies considerably between individuals, predicts death from atherosclerotic cardiovascular disease, and can be modified through diet. (Ruuth et al., 2018) The retained and aggregated ApoB-containing lipoproteins provoke several local responses, are taken up by macrophages and vascular smooth muscle cells leading to foam-cell formation and to the growth of atherosclerotic plaques. (Tabas et al., 2007) Subsequently, more maladaptive processes occur after plaque initiation, such as the acceleration of ApoB-containing lipoprotein retention, and activation of inflammatory cells and prothrombotic pathways. (Abela et al., 2009; Duewell et al., 2010) As the plaques grow and start to obstruct the blood flow in the arterial lumen, the clinical manifestations of atherosclerotic cardiovascular diseases commonly emerge (Figure 1).

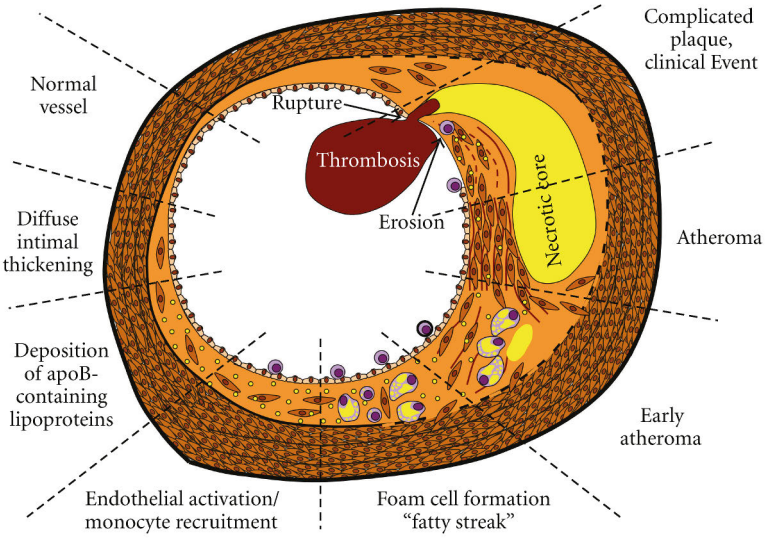

Figure 1. Atherosclerotic plaque formation. Reprinted from International Journal of Vascular Medicine vol. 2012 by (Funk et al., 2012).

2.1.2 Risk factors

Myriad of factors have a role in the development of atherosclerosis, commonly called risk factors. The Framingham Heart Study, one of the earliest longitudinal observational studies on coronary heart disease, described it to be associated with,

for example, high serum cholesterol, physical inactivity, and tobacco smoking. It also identified the need for a preventive approach partly due to the high rate of sudden death as the only clinical manifestation of this disease. (Dawber & Kannel, 1966) More recently, the World Health Organization listed unhealthy diet, physical inactivity, tobacco use and harmful use of alcohol as the main behavioural risk factors for atherosclerosis and elevated concentrations of blood lipids as one of its metabolic risk factors stating that a large percentage of atherosclerotic cardiovascular diseases are preventable through the reduction of behavioural risk factors. (Mendis et al., 2011)

Risk factors can be classified as nonmodifiable (for example age and genetics) or modifiable. The latter are strategic in the attempts to prevent atherosclerosis to begin with by maintaining optimal risk factor levels (primordial prevention) or by decreasing non-optimal levels (primary prevention) through lifestyle changes or medication. A recent statement from the American Society for Preventive Cardiology recognises lifestyle modifications, such as healthy diet and physical activity, as the foundation for cardiovascular disease prevention and state that they should be implemented early in life since poor health behaviour is difficult to reverse once it becomes customary. (German et al., 2022) Additionally, as atherosclerosis is a dynamic process, it could be more reversible the earlier the preventive measures are initiated. (Björkegren et al., 2014) Accordingly, among the major modifiable childhood risk factors linked with preclinical atherosclerotic phenotypes in adults are dyslipidemia (a disorder of lipoprotein metabolism), unhealthy diet, exposure to tobacco smoke, and physical inactivity. (Morton et al., 2022; Pool et al., 2021).

2.1.2.1 Dyslipidemia

LDL particle concentration and LDL cholesterol concentration are highly correlated under most conditions, and LDL particles account for the majority of ApoB-containing lipoproteins (~90%). (Ference et al., 2017) Thus, LDL cholesterol is commonly used to assess cardiovascular disease risk as a good approximation for LDL particle and ApoB-containing lipoprotein particle concentrations which are not generally measured in the current situation in the clinical practice. (Dyslipidemiat. Käypä hoito -suositus., 2022; Ference et al., 2017; Holmes & Ala-Korpela, 2019) Screening for dyslipidemia has traditionally included in addition to LDL-C the quantification of serum concentrations of total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triglycerides.

In adult populations blood total cholesterol concentration, and especially LDL-C concentration, have been directly linked with atherosclerotic cardiovascular diseases, and LDL particles are acknowledged as having a causal role in the development of atherosclerosis. (Ference et al., 2017; Keys et al., 1984; MacMahon

et al., 2007) Mendelian randomization studies involving over 300 000 participants demonstrated that long-term exposure to lower plasma concentrations of LDL-C beginning early in life associated with considerably lower risk for coronary artery disease than reducing the concentration of LDL-C later in life. (Ference et al., 2012) Maintaining serum LDL cholesterol concentration of < 2 mmol/L throughout the life course seems to protect from atherosclerotic diseases even when multiple non-lipid risk factors are present. (Cohen et al., 2006) These findings suggest that the causal effect of LDL on atherosclerotic cardiovascular disease is determined by the concentration and cumulative duration of exposure to LDL-C.

Large observational studies have discovered a strong inverse association between HDL-C concentration and atherosclerotic cardiovascular diseases, though no reduction in cardiovascular morbidity or mortality was found by therapeutically induced increase in the concentration of HDL-C. (Di Angelantonio et al., 2009; MacMahon et al., 2007) Neither has there been clear evidence from Mendelian randomization studies for HDL-C concentration to be causally associated with the risk of atherosclerotic cardiovascular disease. (Brandts & Ray, 2023) Low plasma HDL-C concentration is commonly found in conjunction with cardiometabolic conditions, such as type 2 diabetes, and it is associated with increased concentration of triglycerides. (Taskinen & Borén, 2015) Thus, contrary to HDL-C, triglyceride concentrations have been shown to be directly associated with cardiovascular disease risk. (Borén et al., 2014) Triglycerides are the major constituents of chylomicrons and VLDLs, both ApoB-containing lipoproteins, whose contribution to atherosclerotic cardiovascular disease and its risk assessment might be better captured through indices beyond traditional dyslipidemia screening. (Borén et al., 2014; Budoff, 2016; Gaggini et al., 2022) For example, direct measurement of ApoB concentration (as each ApoB-containing lipoprotein contains a single ApoB molecule) or non-HDL cholesterol concentration have been shown to be better markers of atherogenic dyslipidemia than LDL-C concentration. (Carr et al., 2019; Raitakari et al., 2013)

Clinical manifestations of atherosclerotic cardiovascular diseases during childhood are rare. However, autopsy studies on children and young adults (with non-cardiovascular cause of death) have shown early atherosclerotic changes already at a young age and that their severity is correlated with risk factors such as LDL cholesterol concentration. (Berenson et al., 1998; McGill et al., 1997) Using non-invasive imaging techniques, children with hypercholesterolemia have been shown to demonstrate more pronounced vascular changes indicative of subclinical atherosclerosis compared to healthy children, while even in healthy children LDL-C concentration has been inversely associated with measures of arterial distensibility. (Järvisalo et al., 2001; Mikola et al., 2017) Large cohort studies launched, and cross-sectional studies conducted in the 1970s and 1980s demonstrated that adverse levels

of childhood risk factors, especially if observed at or after 9 years of age are predictive of subclinical atherosclerosis in adulthood. (Juonala et al., 2010) More specifically, there is strong evidence that childhood dyslipidemia is associated with vascular changes indicative of subclinical atherosclerosis in adulthood. (Koskinen et al., 2020; Pool et al., 2021) Additionally, some studies indicate that if childhood dyslipidemia is resolved by adulthood, it greatly reduces the risk of developing these preclinical vascular features. (Juonala et al., 2020; Magnussen et al., 2009)

Studies linking childhood dyslipidemia with clinical manifestations of atherosclerotic cardiovascular diseases in adults are limited. International Childhood Cardiovascular Cohort Consortium, a prospective study with seven cohorts in three different countries and over 38 000 participants, demonstrated that five atherosclerotic cardiovascular disease risk factors (including total cholesterol and triglyceride concentration) were associated with cardiovascular events, both individually and combined. (Jacobs et al., 2022) Importantly, independent of LDL cholesterol concentration during middle age, the cumulative exposure to elevated LDL cholesterol concentration during earlier life might be directly associated with cardiovascular event risk underlining the importance of maintaining optimal LDL cholesterol concentration from an early age onwards. (Domanski et al., 2020; Zhang et al., 2021)

2.1.2.2 Unhealthy diet

According to the Global Burden of Disease Study 2017, poor quality of diet was among the top three risk factors for cardiovascular disease along with high systolic blood pressure and high LDL-C concentration. (Dai et al., 2022) As one of the main behavioural risk factors identified for cardiovascular diseases by World Health Organization, diet profoundly affects the cardiometabolic risk factors such as diabetes, obesity, hypertension, and dyslipidemia. (Mendis et al., 2011) Justifiably, diet is an integral part of all cardiovascular disease prevention guidelines. (Arnett et al., 2019; Belardo et al., 2022; Mach et al., 2019; Visseren et al., 2022) Guidelines currently focus more on dietary patterns, which represent the combination of foods that are usually consumed, instead of single nutrients or foods. (Blomhoff et al., 2023; Mozaffarian, 2016; Sievenpiper & Dworatzek, 2013) The recommended beneficial dietary patterns share many common features favouring vegetables, legumes, fruits, whole grains, and lean protein sources while avoiding for example trans-fats, sugar sweetened beverages and processed foods high in saturated fatty acids (SFAs), sugar, and salt. (American Diabetes Association, 2021; The Diabetes and Nutrition Study Group (DNSG) of the European Association for the Study of Diabetes (EASD), 2023) Identifying a healthy diet in Mendelian randomization studies has proved difficult as comprehensive dietary reporting is challenging and

these studies seem more suitable for assessing effects of dietary components with measurable biomarkers. (Schooling & Zhao, 2023) Thus, knowledge on the role of, for example, dietary fat on cardiometabolic disease risk is derived mainly from interventional and observational studies. (Sellem et al., 2023) Dietary patterns with the most evidence for prevention of cardiovascular disease are the Mediterranean diet, the Dietary Approaches to Stop Hypertension (DASH) diet, and the healthy plant-based diet. (Blomhoff et al., 2023; Diab et al., 2023)

In large observational studies the Mediterranean diet has been associated with reductions in cardiovascular disease events and all-cause mortality. (Stewart et al., 2016; Trichopoulou et al., 2003) The Lyon Diet Heart Study, a randomized controlled trial, was initiated in 1988 and followed 605 participants after their first myocardial infarction for four years. It found Mediterranean style diet to reduce mortality by 56% and morbidity from recurrent non-fatal myocardial infarction by 72%. (Belardo et al., 2022) The Prevención con Dieta Mediterránea (PREDIMED) was launched in 2003 and assessed the Mediterranean diet for the primary prevention of cardiovascular disease by randomizing 7 447 Spanish patients at elevated cardiovascular disease risk to three dietary groups (Mediterranean diet with either extra virgin olive oil or nuts, or a control diet). After nearly five years, approximately 30% reduction in cardiovascular events was found with groups consuming the Mediterranean diet. (Martínez-González et al., 2015) Additionally, a recent meta-analysis of 13 prospective cohort studies found an inverse association between olive oil consumption and the risk of cardiovascular disease and all-cause mortality and hypothesized that anti-inflammatory and antioxidant effects along with substituting dietary SFAs with unsaturated fats played a role. (Hooper et al., 2020; Xia et al., 2022) In general, there is evidence that replacing dietary SFAs with unsaturated fatty acids is more beneficial with regards to cardiovascular disease risk than replacing dietary SFA with other nutrients. (Public Health England, 2019; Schulze et al., 2020)

The DASH diet was designed in the late 1990s in the United States by the National Heart, Lung, and Blood Institute to prevent and manage hypertension by taking into account the known dietary factors affecting blood pressure, and as such included a sodium restriction compared to the other diets. (Appel et al., 1997) A review of the DASH dietary pattern consisting of prospective cohort studies (n = 942 140) with cardiometabolic disease outcomes and effect on cardiometabolic risk factors in controlled trials (n = 4 414) found DASH dietary pattern to be associated with, for example, decreased incident cardiovascular disease and coronary artery disease (about 20% reduction) in the prospective cohort studies, and decreased LDL-C concentration (-0.10 mmol/L; 95% CI: -0.20, -0.01) in controlled trials. (Chiavaroli et al., 2019) Plant-based diets, which are diets consisting mainly of food of plant origin, can be diverse. A healthy plant-based diet has low SFA and high fibre content and has been in observational cohort studies shown to associate

with lower cardiovascular disease risk, with reductions in cardiometabolic risk factors such as LDL-C concentration, blood pressure, and overweight. (Choi et al., 2021; Diab et al., 2023) A meta-analysis of 11 randomized controlled trials with a total of 832 participants and an average duration of 24 weeks showed vegetarian diets to significantly lower risk factors such as total cholesterol (-0.36 mmol/L; 95% CI: -0.55, -0.17), and LDL-C concentration (-0.34 mmol/L; 95% CI: -057, -0.11). (Wang et al., 2015)

The Cardiovascular Risk in Young Finns Study is a prospective longitudinal study launched in Finland in 1980 (n = 3596, age 3–18 years) to investigate childhood risk factors for cardiometabolic outcomes in adulthood. It has shown, for example, that childhood lifestyle (diet, physical activity) is predictive of diet in adulthood and that dietary patterns are adopted in childhood, carry over into adulthood, and are associated with coronary heart disease risk factors. (Juonala et al., 2013) For example, continuously elevated consumption of fruits and vegetables from childhood to adulthood was positively associated with a marker of subclinical atherosclerosis in adulthood whereas dietary pattern in which their intake was reduced showed opposite results, especially in men (Mikkilä et al., 2005) Accordingly, American Heart Association recommends adopting a heart-healthy diet early in life and maintaining it. (Lichtenstein et al., 2021) Some randomized dietary intervention trials in children have been conducted. The Dietary Intervention Study in Children initiated in 1987 was a randomized trial consisting of U.S. preadolescents (n = 663, 8–10 years old) with elevated LDL-C concentrations. It aimed to lower total (target 28% of energy from fat), SFA (<8% of energy) as well as cholesterol (<75mg/1000 kcal) dietary intake with a mean follow-up time of 7.4 years. The dietary behavioural intervention resulted in reduced intakes of total and SFAs as well as cholesterol throughout the intervention period with a mean reduction of LDL-C concentration by 0.09 mmol/L. (Obarzanek et al., 2001)

The STRIP study is a prospective, randomized intervention trial targeting to influence the biological and behavioural risk factors of atherosclerosis. (Simell et al., 2009) The dietary intervention given in the study was based on Nordic Nutrition Recommendations and aimed primarily to replace dietary SFAs with unsaturated fat and reduce the intake of cholesterol, and additionally promoted increased intake of fruits, vegetables, and whole grains, and reduced intake of sodium, and sucrose. Families with 1062 7-month-old infants were randomized to an intervention or a control group beginning in 1990 in the city of Turku, Finland. The intervention phase lasted until the children were 20 years of age. Participants in the intervention group had lower LDL-C concentrations throughout the study compared to the control participants (mean difference in females of -0.10 mmol/L with 95% CI: -0.19, -0.01, and in males -0.18 mmol/L with 95% CI: -0.26, -0.10). (Niinikoski et al., 2012) Additionally, the dietary counselling given in the STRIP study has been shown to

result in higher fibre intake, lower SFA intake, and higher unsaturated fatty acids to SFA ratio as well as improved insulin sensitivity in adolescents between 15 to 20 years. (Oranta et al., 2013) The study has also shown, for example, that achieving the main dietary targets of the intervention was favourably associated with arterial health. (Laitinen et al., 2020)

2.1.2.3 Physical inactivity

World Health Organization recognizes physical inactivity as one of the behavioural risk factors for atherosclerotic diseases, and physical activity recommendations are present in guidelines for cardiovascular disease prevention and management. (Arnett et al., 2019; Ferraro et al., 2020) Physical Activity Guidelines for Americans recommend 150 minutes of moderate intensity or 75 minutes of vigorous intensity physical activity per week for adults while being less inactive in general is recommended, and at least an hour per day of moderate to vigorous intensity physical activity for children and adolescents aged 6 to 17. (U.S. Department of Health and Human Services, 2018) In Finnish adults, 39% of men and 34% of women reached these goals in 2017. (Koponen et al., 2018) In a study comprising of 1.6 million adolescents aged 11 to 16 years from 146 countries examined during 2001 and 2016, the World Health Organization observed that 81% did not reach the goal of at least an hour of moderate to vigorous physical activity daily, with no improvement during the study period. (Guthold et al., 2020) In Finland, this goal was reached by about 36% of 7- to 15-year-old children during 2022 with younger children being more active than older children. (Kokko & Martin, 2023)

In adults, physical activity has been associated with reduced risk for cardiovascular disease and mortality in cohort studies. (Lear et al., 2017; Lee et al., 2021; Sattelmair et al., 2011) This may primarily be through influence on other risk factors, such as improved weight, reduced risk for dyslipidemia, type 2 diabetes, or hypertension. (Cheng et al., 2018; German et al., 2022) For example, endurance type of exercise has been shown to influence lipid and lipoprotein metabolism as it increases lipoprotein lipase concentration and activity, accelerate lipid transfer, decomposition and excretion leading to reduced concentration of triglycerides and increased concentration of HDL-C. (Franczyk et al., 2023) This type of activity has also been shown to reduce total cholesterol and LDL-C concentrations. (Sulague et al., 2022) Additionally, physical activity has been associated with reduced progression of measures of subclinical atherosclerosis. (Kozákova et al., 2010; Sato et al., 2008)

Randomized controlled trials evaluating the effect of physical activity on cardiovascular endpoints are scarce. In a randomized intervention trial of 1 635 sedentary participants aged 70 to 89 years, a moderate intensity physical activity

program was followed by the participants in the intervention group for 2.6 years on average, and no reduction in cardiovascular events was observed. (Newman et al., 2016) The Finnish Diabetes Prevention Study (DPS), a controlled and randomized study conducted in the 1990s on 522 middle-aged participants with impaired glucose tolerance, showed several beneficial and long-term changes in, for example, physical activity, and blood glucose concentrations. (Lindström et al., 2003, 2013) The main physical activity goal in the study was moderate intensity physical activity for at least 30 minutes daily and the intervention participants were guided to increase their overall level of physical activity through endurance exercise and supervised, individually tailored circuit-type moderate intensity resistance training sessions offered free of charge. The DPS showed that type 2 diabetes is preventable with lifestyle intervention. Still, Mendelian randomization studies have failed to find a causal role for physical activity on cardiovascular disease risk or longevity. (Bahls et al., 2021; van Oort et al., 2021) Further, physical activity levels might be influenced by the same genetic factors that impact the risk of death, offering a possible explanation for the association between higher physical activity and lower mortality. (Karvinen et al., 2015; Kujala, 2018) Thus, further study is needed to unravel the ways through which physical activity influences health. Despite this uncertainty, observational studies have associated childhood physical activity with improved measures of subclinical atherosclerosis and lower prevalence of dyslipidemia in adulthood. (da Silva et al., 2023; Fernandes et al., 2011; Pool et al., 2021)

Physical activity in children and adolescents, especially vigorous activity, has been favourably associated with, for example, concentrations of triglycerides, total cholesterol, and glucose. (Poitras et al., 2016) Additionally, there is evidence that being physically active might attenuate the development of subclinical atherosclerosis in healthy adolescents. (Pahkala et al., 2011). A 2-year school-based physical intervention study on 256 nine-year-old Norwegian children beneficially altered the children's clustered cardiovascular disease risk profile. (Resaland et al., 2018) The Child and Adolescent Trial for Cardiovascular Health (CATCH) launched in 1991 was a randomized controlled trial involving 24 elementary public schools with a total of 5 106 students at four locations in the U.S. It aimed to promote healthy eating, physical activity and non-smoking with dietary and physical activity targets set at both the school and individual level. The 2.5-year intervention managed to reduce the fat content of school lunches and to increase the intensity of physical activity in physical education with similar improvements seen in the health behaviours of the intervention school children while no differences in cholesterol measures, or blood pressure were seen between treatment groups. (Luepker et al., 1996)

The ongoing Physical Activity and Nutrition in Children (PANIC) intervention study investigates the effects of a combined physical activity and dietary counselling intervention on cardiometabolic risk factors on 504 children from the city of Kuopio, Finland. (Eloranta et al., 2014) The physical activity intervention was based on the Finnish Recommendations for Physical Activity of School-aged Children advocating for example at least two hours of brisk physical activity daily, and the dietary intervention was based on the Finnish Nutrition Recommendations. (Ministry of Social Affairs and Health, 2005; Wahlqvist, 2005) The study included 6- to 9-year-old first-graders from 16 schools who began their school from 2007 to 2009. The children were divided into an intervention group (n = 306) and a control group (n = 198) according to the school that they attended. The intervention aimed to increase total physical activity, decrease sedentary behaviour and the intakes of SFAs, salt, and sugar, while increasing the intakes of fibre and polyunsaturated fatty acids (PUFAs). The intervention consisted of six physical activity and dietary counselling sessions until the study's 2-year follow-up. These counselling sessions were individualized, attended by both the children and their parents who received verbal and written guidance as well as some material support. The intervention led to increased self-reported physical activity and consumption of vegetables, high-fat vegetable-oil-based margarin, as well as low-fat milk. These were reflected as higher dietary intake of fibre and 0.8% lower dietary intake of SFAs with a 0.05 mmol/L average decrease in the serum LDL cholesterol concentration. (Eloranta et al., 2021)

2.1.2.4 Tobacco smoke exposure

Active smoking, second-hand smoke, and the use of chewing tobacco accounted for nearly nine million deaths worldwide in 2019, and this number has been increasing since 1990, with roughly 37% of these deaths due to cardiovascular disease. (Roth et al., 2020) In Finland, the number of active smokers is on decline, but still about one in four men and one in five women smoked in 2017. (Koponen et al., 2018) A large Mendelian randomization study concluded a causal association between smoking and a broad range of cardiovascular diseases, particularly coronary artery disease. (Larsson et al., 2020) Potential mechanisms include lipid oxidation, endothelial dysfunction, and augmented coagulability, and nicotine might provoke acute cardiovascular events through increased myocardial contractility and vasoconstriction. (Rigotti & Clair, 2013) Lipoproteins have been suggested to play a mediating role as well, possibly through increased lipolysis in adipose tissue due to nicotine, as active smoking has been shown to affect serum lipoprotein concentrations with reduced HDL-C and increased triglyceride concentrations being commonly reported. (Jain & Ducatman, 2018; Song et al., 2020) Additionally, active smoking during childhood and adolescence has been associated with impaired

markers of subclinical atherosclerosis and cardiovascular events in adulthood. (Jacobs et al., 2022; Raitakari et al., 2003)

Even brief exposure to second-hand smoke increases the risk of acute myocardial infarction by around 30%, and evidence from epidemiological studies suggests that the effects of second-hand smoke exposure on cardiovascular system could be nearly as large as active smoking with multiple and interacting mechanisms including increased platelet activity, and atherosclerosis. (Barnoya & Glantz, 2005; Glantz & Parmley, 1991) A cross-sectional study found second-hand smoke exposure to be associated with dyslipidemia, and though evidence is scarce, speculated nicotine-induced release of catecholamines or similar mechanisms as with active smoking to be involved. (Okekunle et al., 2022) Additionally, evidence exists that exposure to second-hand smoke or parental smoking during childhood is associated with markers of subclinical atherosclerosis in adulthood. (Chen et al., 2015; Gall et al., 2014; West et al., 2015)

The proportion of adolescents who have experimented with smoking has been declining in Finland from the turn of the century. For example, in 2001 about 30% of 12-year-old males reported to have experimented with smoking while in 2019 only 3% did the same. Daily use of tobacco products among 14- to 18-year-olds during the same period was 25% and 10%, respectively. (Kinnunen et al., 2019) A meta-analysis of 12 mostly cross-sectional studies (n = 5 279) found both active and passive smoking in childhood to be directly associated with measures of subclinical atherosclerosis. (Georgiopoulos et al., 2021) Additionally, a study (n = 90 278) comparing current and former smokers found that those who started smoking before the age of 17 were at a higher risk for developing cardiovascular disease compared to those who started smoking at a later age. (Choi & Stommel, 2017) Still, the few cross-sectional studies that have evaluated the associations between childhood exposure to tobacco smoke and measures of dyslipidemia have yielded mixed results. (Ebrahimi et al., 2019; Merianos et al., 2018; Zakhar et al., 2016)

A review of U.S. based intervention studies (n = 44 521) to discourage the use of tobacco products in children and adolescents found educational and behavioural interventions to significantly reduce the rate of smoking initiation in 7- to 17-year-old children. (Selph et al., 2020) As part of the STRIP intervention, the prevention of smoking was introduced at the age of 8 years. It aimed to increase the understanding of the health risks associated with both active and passive smoking, and supported the self-image of those children who did not smoke while discussing topics such as attitudes towards smoking, how to avoid passive smoking, or how to refuse an offered tobacco. The prevalence of smoking has been similar between the study groups, except at the age of 20 years when fewer intervention group participants smoked regularly compared with control group participants (10%, n = 162 vs. 17%, n = 213). (Pahkala et al., 2020)

2.2 Metabolomics

The measures that are commonly used to evaluate dyslipidemia and hence the risk of atherosclerotic cardiovascular disease are not always sensitive or specific enough to identify persons at risk. In certain conditions, such as metabolic syndrome or diabetes, LDL-C concentration might not accurately reflect the LDL particle concentration as there is a predominance of small, dense, and cholesterol-poor LDL particles. (Ference et al., 2017) Beyond mere triglyceride concentration, the lipoprotein particles that are its main carriers (triglyceride-rich lipoproteins) have been shown to be causally associated with cardiovascular disease and all-cause mortality. (Thomsen et al., 2014) Additionally, because of the discrepancies observed in studies assessing the role of HDL-C in atherosclerosis and cardiovascular disease it has been proposed that indexes of HDL particle structure and function might be better in mediating the effects of HDL. (Pappa et al., 2020)

Metabolomics is the systematic study of the metabolome, the product of the interaction between external exposures (such as diet, physical activity, or smoking) and the genome-transcriptome-proteome. (Ussher et al., 2016) Thus, it also serves as a reflection of gene and protein functional activity, and whereas genetics are largely static, metabolic measures are relatively dynamic. (Cheng et al., 2017) In a statement from 2017, the American Heart Association recognized these aspects of metabolomics to be critical for understanding complicated diseases such as cardiovascular disease, and the potential of metabolomics in providing novel insights into the disease pathophysiology, individual risk assessment, and disease prevention. (Cheng et al., 2017)

The metabolome consists of metabolites, an immense amount of endogenous small molecules, including fatty acids, lipids, amino acids, nucleic acids, as well as exogenous chemicals, with largely varying chemical characteristics and concentrations. Thus, measurement of all metabolites with a single analytical technique is unfeasible, and combined analytical approaches are used to compass the diversity of the metabolome. (Shah et al., 2012) The primary methods to assess metabolome are NMR spectroscopy, and mass spectrometry which is often coupled to a prior chromatographic technique. They can be done either in a targeted or untargeted manner. Targeted metabolomics allows the measurement of at most several hundred known metabolites at a time, and it assesses a distinct, well-characterized set of metabolites. Absolute quantification of metabolites can be obtained through isotopic labelling of endogenous metabolites across a range of concentrations. In comparison, untargeted approach aims to analyse all small molecules within a sample while usually not being able to be quantitative. (Ussher et al., 2016)

In mass spectrometry metabolites are identified based on their mass to charge ratio. The sample's metabolic components are often initially separated by a chromatography in which a sample is dissolved into a solvent and this mobile phase

is passed through a stationary phase which allows the metabolites in the solvent to pass through at varying speeds or remain. After possible separation, metabolites are ionized, and mass dependent ion separation can be performed by different techniques. (Roberts & Gerszten, 2013) NMR spectroscopy takes advantage of the magnetic properties of select atomic nuclei to determine the number and type of metabolites in a sample. (Lewis et al., 2008) In a strong magnetic field, NMR-active nuclei in the molecule absorb electromagnetic radiation at a particular magnetic resonance frequency. This resonance signal is then recorded by a spectrometer as a function of time and usually transformed into an NMR spectrum (plot of frequency against absorption). Neighbouring atoms of the nuclei influence this spectrum in an identifiable way, and quantitative information can be induced from signal intensity. (Roberts & Gerszten, 2013) The benefits of NMR spectroscopy include the need for little sample preparation, non-destructiveness (same sample can be analysed multiple times), and that it is capable of absolute quantification without isotope labelling. However, compared with MS, it has relatively poor sensitivity.

2.2.1 Novel biomarkers for cardiovascular disease

Triglyceride-rich lipoproteins have gained increasing attention in atherosclerotic cardiovascular disease risk assessment partly due to the increased prevalence of hypertriglyceridemia. (Ganda, 2023) Chylomicrons are secreted into the circulation from the intestine while VLDL particles are synthesized in hepatocytes. Triglycerides in these particles are hydrolysed by lipoprotein lipase, resulting in VLDL and chylomicron remnants as well as IDL particles some of which can be taken up by the liver and cleared. Those remaining in the circulation are further modified, resulting in LDL particles. (Budoff, 2016; Ma et al., 2021) Composition and physical-chemical properties of major lipoproteins are shown in Figure 2. A study on 27 673 initially healthy U.S. women who were followed for over an 11-year period found that the concentration of VLDL particles, its subclasses, and IDL particles all independently predicted cardiovascular events. (Mora et al., 2009) Similarly, in a Chinese cohort of 4 662 participants all VLDL subclass concentrations associated with myocardial infarction and ischemic stroke while IDL associated only with myocardial infarction. (Holmes et al., 2018) Additionally, remnant cholesterol (cholesterol in triglyceride-rich lipoproteins), which is mainly VLDL cholesterol (VLDL-C), and the triglyceride concentration in VLDL particles are currently of growing interest with regards to cardiovascular disease risk. (Balling et al., 2020; Lawler et al., 2017) Smaller remnant cholesterol particles contain 5 – 20 times more cholesterol than LDL particles, and there is evidence suggesting that they are retained more easily in the subendothelium, taken up by macrophages directly,

and that they can exacerbate the process of atherosclerosis. (Basu & Bornfeldt, 2020; Rosenson et al., 2014; Shin et al., 2004)

As LDL-C and LDL particle (LDL-P) concentrations may be discordant, the Multi-Ethnic Study of Atherosclerosis assessed in a cross-sectional setting 4 499 participants with these discordant measures (LDL-C calculated, LDL-P measured by NMR) and found subjects with higher LDL-P concentration but normal LDL-C to have the strongest association with a marker of subclinical atherosclerosis. (Otvos et al., 2011) Reflecting this, small LDL particles are considered more atherogenic than large LDL particles, though conflicting evidence exists. (Mora et al., 2007; Williams et al., 2014) Still, all LDL subclasses have been directly associated with myocardial infarction and stroke. (Holmes et al., 2018)

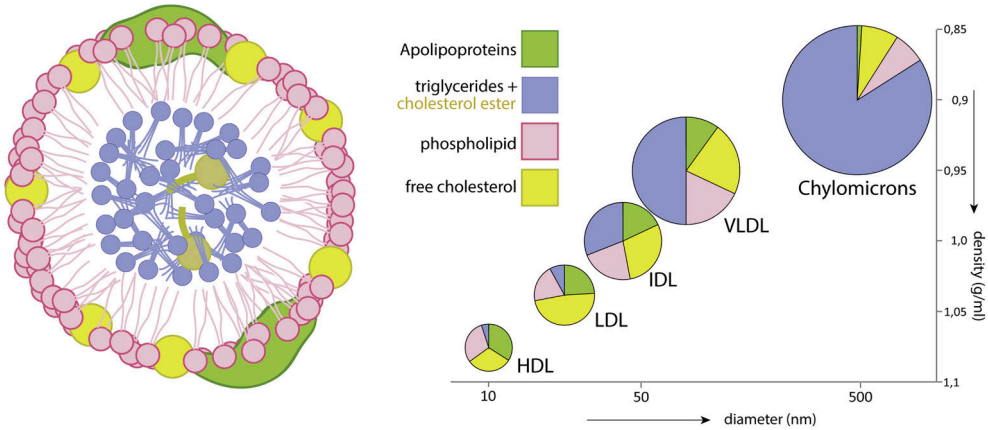

Figure 2. Composition and main physical-chemical properties of major lipoprotein classes. Reprinted from Progress in Retinal and Eye Research (van Leeuwen et al., 2018) with permission from Elsevier.

LDL particles can be removed from the circulation by hepatic LDL receptors, or they can be transported into cells via endocytosis. Nascent HDL particles are formed from apolipoprotein A1 (ApoA-1) secreted mainly by the liver and its interaction with plasma phospholipids. Small HDL particles remove cholesterol from arterial wall, primarily from lipid-overloaded macrophages, expanding into larger particles. All forms of HDL particles are capable of taking up cholesterol out of the cells. HDL particles can transfer their cholesterol to hepatocytes (selectively taking the cholesterol or by endocytosis of the HDL particle leading to its possible degradation) or to ApoB-containing lipoproteins (in exchange for triglycerides), converting them back into smaller HDL particles that repeat the process. (Ma et al., 2021; Röhrl & Stangl, 2013; Trajkovska & Topuzovska, 2017) A study of 5 598 U.S. adults showed HDL particle concentration, unlike HDL-C, to be inversely associated with a

measure of subclinical atherosclerosis and incident coronary heart disease, even after adjusting for LDL-P. (MacKey et al., 2012) Similar association with incident coronary heart disease was found in The Dallas Heart Study consisting of 2 924 adults who were followed for an average of 9.4 years. (Rohatgi et al., 2014) With regards to HDL subclasses, some studies have found larger HDL subclasses to be more beneficial than small HDL subclass. (Holmes et al., 2018; Mutharasan et al., 2017) Contrary to these findings, a cross-sectional study of more than 6 000 participants found small- and medium-sized HDL particles to be inversely associated with measures of subclinical atherosclerosis. (Kim et al., 2016) As results on HDL subclasses are conflicting, HDL quality has become of interest with investigations on e.g., the role of ApoA-1, the major protein of HDL particles that ensures structural stability and cholesterol efflux from cells to HDL. (El Harchaoui et al., 2009; Mora et al., 2009; van der Steeg et al., 2008)

Metabolomics has revealed several other metabolites associated with cardiovascular disease. A targeted NMR-study aimed to identify biomarkers for incident cardiovascular disease on three large cohorts from Finland and Britain with over 13 000 participants during long-term follow-up. (Wurtz et al., 2015) In meta-analyses two metabolites were directly associated with future cardiovascular events, namely serum phenylalanine concentration and the ratio of monounsaturated fatty acids (MUFAs) to total fatty acids, while omega-6 and docosahexaenoic (DHA) fatty acid concentrations showed inverse association. The associations were further corroborated with mass spectrometry in nearly 3 000 participants, and the results remained even after adjusting for risk factors. (Wurtz et al., 2015) A non-targeted metabolomics study with three Swedish cohorts and about 3 600 participants applied mass spectrometry to evaluate associations with incident cardiovascular disease. It found inverse associations for two lysophosphatidylcholines, and a sphingomyelin, whereas a direct association with a monoglyceride was found, independent of main cardiovascular risk factors. (Ganna et al., 2014) Lysophosphatidylcholines and sphingomyelins belong to a diverse group of membrane lipids, amphiphilic molecules that are the major components of cell and lipoprotein membranes, with possible involvement in the pathogenesis of cardiovascular diseases. (Di Pietro et al., 2023) A targeted quantitative mass spectrometry study included 3 865 participants from the Malmö Diet and Cancer – Cardiovascular Cohort with an average follow-up time of over 20 years and examined the associations of a set of lipid measures with incident coronary artery disease. It found, for example, the concentration of a certain sphingomyelin to be associated with an increased risk of incident coronary artery disease. (Ottosson et al., 2021) As inflammation is an integral part of atherosclerosis, a measure of glycoprotein acetyls, reflecting the serum concentrations of several acute-phase reactants, has been directly associated

with markers of subclinical atherosclerosis, cardiovascular events, and all-cause mortality. (Duprez et al., 2016; Holmes et al., 2018; Tibuakuu et al., 2019)

A recent cross-sectional, targeted NMR metabolomics study utilising the UK Biobank data from nearly 120 000 participants studied the associations of a panel of plasma metabolic measures with the prevalence, incidence, and mortality of over 700 diseases. (Julkunen et al., 2023) The results show, for example, that the ratio of MUFAs to total fatty acids is associated with nearly 200 different disease endpoints. Among the most prominent associations for glycoprotein acetyls were type 2 diabetes, and myocardial infarction. Branched-chain amino acids showed direct associations with metabolic disease risk. Generally, major cardiovascular events were directly associated with plasma metabolic measures including alanine, phenylalanine, tyrosine, isoleucine, and the ratios of MUFAs and SFAs to total fatty acids. Inverse associations were found for the concentrations of glycine, histidine, ApoA-1, HDL-C, and the ratios of omega-3 and omega-6 fatty acids to total fatty acids, among others. The writers speculate that the many disease associations observed for plasma fatty acid ratios may make them better markers of systemic inflammation than of diet. (Julkunen et al., 2023) Findings from previous metabolomics studies show similar results as leucine and isoleucine have been associated with a measure of preclinical atherosclerosis, and branched chain amino acids with insulin resistance and type 2 diabetes. (Cheng et al., 2017; Jiang et al., 2020; Wurtz et al., 2013) A nested case-control targeted plasma NMR metabolomics study with roughly 4 500 participants from China Kadoorie Biobank investigated the associations of metabolic measures with three cardiovascular disease events and found concentrations of glycoprotein acetyls, glucose, β-hydroxybutyrate, creatinine, and acetoacetate to be directly associated with most of the cardiovascular events, with an inverse association found for the ratio of DHA to total fatty acids. The plasma ratio of MUFAs to total fatty acids was directly associated with only one disease event, myocardial infarction. (Holmes et al., 2018)

Studies examining the associations between metabolic profile and subclinical measures of atherosclerosis in children and adolescents are scarce. One study used targeted NMR metabolomics to evaluate the cross-sectional associations (n = 1 178, age 11–12) and longitudinal (n = 4 249, metabolites at 7–8 years of age, vascular measure at 10–12 years of age) associations between 69 metabolic measures and markers of subclinical atherosclerosis. No associations were found, and the authors hypothesized that possibly a longer exposure period would be needed. Additionally, the association observed between metabolic measures and cardiovascular events requires an existing atherosclerotic plaque along with factors contributing to its instability and rupture. (Juonala et al., 2019) Consequently, in a study on 1 595 young adults aged 24–39 from the Cardiovascular Risk in Young Finns Study a marker of subclinical atherosclerosis was measured within a 6-year interval and

NMR spectroscopy assessed serum metabolites were evaluated at baseline to predict incidence of subclinical atherosclerosis or atherosclerotic plaque. The study found higher serum DHA concentrations to be associated with reduced risk whereas tyrosine and glutamine serum concentrations were directly associated with a 6-year incident of subclinical atherosclerosis. (Würtz et al., 2012)

To recapitulate, ApoB-containing lipoprotein subclass concentrations have been associated with increased risk for cardiovascular disease events with some studies seeing small LDL particles as more atherogenic, though conflicting evidence exists. (Lawler et al., 2017; Ma et al., 2021) Specifically, triglyceride-rich lipoprotein subclasses and their lipid composition seem to promote endothelial dysfunction, but the mechanisms are still unclear. (Ma et al., 2021) HDL particle concentration has been inversely associated with cardiovascular disease events with some studies suggesting larger HDL particles to be more beneficial. (Holmes et al., 2018; MacKey et al., 2012) Though HDL is the only protective lipoprotein in circulation with respect to atherosclerotic cardiovascular disease, it is unclear which of its subclasses exerts the most benefits and by what mechanism. (Ma et al., 2021)

Direct association between the serum ratio of MUFAs to total fatty acids and cardiovascular events has been reported by many studies with the serum ratio of SFAs to total fatty acids showing similar associations. (Akbaraly et al., 2018; Julkunen et al., 2023; Wurtz et al., 2015) Conversely, the ratios of PUFA-related measures to total fatty acids have shown inverse associations with cardiovascular events. These observations in fatty acid ratios to total fatty acids might reflect inflammation better than the composition of diet. (Julkunen et al., 2023) Glycoprotein acetylation is considered a biomarker of systemic inflammation and has been shown to be associated with subclinical atherosclerosis, cardiovascular events, and type 2 diabetes. (Duprez et al., 2016; Holmes et al., 2018; Julkunen et al., 2023) However, it is unclear whether glycoprotein acetylation is a determinant or a mere indicator of atherosclerotic progression. (Ma et al., 2021) Several studies have associated branched-chain amino acids (especially isoleucine and leucine) with type 2 diabetes, subclinical atherosclerosis, and the risk for cardiovascular events. (Cheng et al., 2017; Jiang et al., 2020; Wurtz et al., 2013) Mechanisms for the association between branched-chain amino acids and cardiovascular disease are unclear, but include mitochondrial dysfunction, and platelet activation. (McGarrah & White, 2023) Other amino acids, such as phenylalanine, tyrosine, alanine, and glutamine have been associated with increased risk for subclinical atherosclerosis or cardiovascular events. (Julkunen et al., 2023; Würtz et al., 2012) Conflicting evidence exists also for these measures and the mechanisms are largely unclear. (Tzoulaki et al., 2019) Membrane lipids, such as sphingomyelins, have shown conflicting results with regards to cardiovascular disease risk and are a growing interest of study. (Ganna et al., 2014; Ottosson et al., 2021)

2.3 Lifestyle risk factors and metabolic profile

The latest guideline of the American College of Cardiology/American Heart Association on the primary prevention of cardiovascular diseases acknowledges healthy lifestyle throughout life as the most important way of prevention. It stresses that strong focus should be on lifestyle optimization including improving diet, being physically active, and avoiding tobacco use and exposure to passive smoking. (Arnett et al., 2019) Other guidelines also acknowledge the importance of these lifestyle factors. (Blomhoff et al., 2023; Dyslipidemiat. Käypä hoito -suositus., 2022; Visseren et al., 2022) Several studies have examined how these lifestyle risk factors are reflected in the metabolic profile to, for example, provide mechanistic insight, or improve risk prediction.

2.3.1 Diet

Metabolic profiling reflects not only dietary intake but genetic variations and the interplay between diet and gut microbiota as well. A study combining data from the PREDIMED trial (n = 1 859), and three U.S. based prospective cohorts (n = 6 868), aimed to identify a metabolic profile that would reflect adherence to the Mediterranean diet and additionally study whether this profile was associated with cardiovascular disease risk. (Li et al., 2020) Plasma metabolome was obtained by targeted mass spectrometry qualifying 302 known metabolites. The study found that 67 metabolites associated with adherence to the Mediterranean diet. Some metabolites that were directly associated with adherence to the Mediterranean diet (such as certain unsaturated phosphatidylcholines) were inversely associated with cardiovascular disease events after adjusting for known risk factors. Additionally, by using Mendelian randomization the study showed that the genetic component of the metabolic profile was inversely associated with the risk of cardiac heart disease. Another cross-sectional study of 10 806 adult participants free of diabetes from the U.K. used targeted mass spectroscopy metabolomics to evaluate the associations between adherence to Mediterranean diet and 175 plasma metabolites. It found 66 metabolites to be associated with adherence to the Mediterranean diet, and that a set of these metabolites (carnitines, amines, and phospholipid species) moderately explained the association of the diet with dyslipidemia and insulin resistance. (Tong et al., 2020)

A study using targeted serum NMR metabolomics assessed the adherence of 4 824 participants to the Alternative Healthy Eating Index, which reflects dietary guidelines adapted to the UK framework, with 80 metabolites and whether these metabolites related to reduced risk of cardiovascular disease. (Akbaraly et al., 2018) A total of 41 metabolites showed association with healthy diet and as a key finding of the study the authors highlight the fatty acid results which show that higher serum

ratios of PUFAs, and lower ratios of SFAs and MUFAs, to total fatty acids, associated with healthy diet. Additionally, adherence to healthy dietary recommendations was inversely associated with, for example, concentrations of certain amino acids (isoleucine, leucine, phenylalanine), VLDL particle size, lipids in VLDL, IDL, and LDL subclasses, as well as glycoprotein acetyls. (Akbaraly et al., 2018) The results remained after further adjustments with ethnicity, physical activity, smoking habits, and cardiovascular risk factors. Of these 41 metabolites those showing direct associations with future cardiovascular disease risk were, among others, amino acids, glycoprotein acetyls, the size of VLDL particles, and total lipid concentrations in lipoprotein subclasses (except for IDL). In fatty acid measures, the degree of fatty acid unsaturation was inversely associated with cardiovascular disease risk, along with the serum ratios of PUFAs, proportion of omega-3 fatty acids, and DHA, to total fatty acids. The absolute concentrations of SFAs and MUFAs, and the ratio of MUFAs to total fatty acids, were directly associated with cardiovascular disease risk. (Akbaraly et al., 2018) A study based on the China Kadoorie Biobank and consisting of 4 778 participants assessed the effects of red meat consumption on 225 plasma metabolic markers quantified by NMR spectroscopy. (Pan et al., 2022) Of the metabolites, 46 were associated with red meat consumption with direct associations seen, for example, for IDL, small HDL, and the size of LDL. Of these metabolites, 29 associated with cardiovascular disease risk, and, in general, the associations of metabolic measures between red meat consumption and cardiovascular disease risk were in the same direction.

A secondary analysis consisting of participants from the Dietary Intervention and VAScular function (DIVAS) randomized controlled trial (n = 113) and from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort study (n = 3 557) aimed to assess the effects of replacing dietary SFAs with unsaturated fatty acids on plasma lipids in relation to cardiometabolic disease. Mass spectrometry was used to measure the absolute concentrations of 987 molecular species. (Sellem et al., 2023) DIVAS study consisted of three 16-week high-fat dietary interventions: a SFA-rich diet, a MUFA-rich diet, and a MUFA/PUFA-rich diet. The study identified fatty acids sensitive to replacement of dietary SFAs with unsaturated fatty acids in the DIVAS study and their association with cardiometabolic risk in the EPIC-Potsdam study. High unsaturated fatty acid interventions in the DIVAS study reduced especially the plasma concentrations of SFA-containing glycerolipids and sphingolipids which in turn were directly associated with a higher cardiovascular disease risk in the EPIC-Potsdam study. Additionally, the MUFA-rich diet in the DIVAS trial resulted in reduced concentrations of certain MUFAs in plasma sphingolipids, which might be explained by findings suggesting that SFAs are used as precursors in the endogenous synthesis of MUFA-containing sphingolipids, and that dietary SFA and MUFA are strongly

correlated in the Western diet. (Chalfant & Del Poeta, 2010; Sellem et al., 2023; Sundström et al., 2001)

These previous studies were conducted on adult populations, and studies examining the associations between diet and metabolic profile are scarce in the young. A study on 663 young adults (aged 21–25 years) from the FinnTwin12 study analysed the associations between habitual diet (identifying 5 dietary patterns), and NMR spectroscopy measured lipoprotein subclasses. (Bogl et al., 2013) After adjustments for e.g., physical activity and smoking status, a "junk food" pattern, which correlated positively with the energy from fat and sucrose and negatively with total carbohydrates and dietary fibre, was directly associated with serum triglycerides. VLDL subclass distribution was shifted toward larger particles and LDLs toward smaller particles, and higher scores of the "junk food" pattern directly associated with concentrations of small HDL particles. Additionally, habitual fish consumption was inversely associated with VLDL particle size and directly associated with HDL particle size. (Bogl et al., 2013) In adults, fatty fish consumption has also been linked with beneficial effects on HDL particles, such as increased HDL particle size and cholesterol concentration. (Erkkilä et al., 2014; Manninen et al., 2018) A cross-sectional and a prospective study analysed how higher consumption of ultra-processed foods is associated with NMR-measured plasma metabolic profile in the British Avon Longitudinal Study of Parents And Children (ALSPAC). (Handakas et al., 2022) The cross-sectional study included 4 528 participants who were 7 years of age, whereas in the prospective analysis the consumption of ultra-processed foods was assessed at the age of 13-years, and the metabolic profile was obtained at the age of 17 years (n = 3 086). At 7 years, greater intake of ultra-processed foods was inversely associated with, for example, plasma concentrations of aromatic amino acids, branched-chain amino acids, phosphoglycerides, total cholesterol, HDL particle size, and the ratios of omega-3 fatty acids and PUFAs to total fatty acids. A direct association was seen for the ratio of MUFAs to total fatty acids and VLDL particle size. In the longitudinal analysis inverse associations between plasma concentrations of omega-3 fatty acids and the ratio of DHA to total fatty acids were observed. The study indicated a role for e.g., glutamine, citrate, isoleucine, leucine, tyrosine, and MUFA as a ratio of total fatty acids measured at 7 years of age in the association between the consumption of ultra-processed foods and successive fat mass accumulation. In the study, ultra-processed foods comprised of foods that are typically created by industrial technologies and processes, such as soft drinks, cookies, and pizza dishes, while it is worth noting that a general definition is hard to give. (The Diabetes and Nutrition Study Group (DNSG) of the European Association for the Study of Diabetes (EASD), 2023)

To summarise, unhealthy dietary patterns seem to be directly associated with various ApoB-containing lipoproteins and their subclasses while adhering to a

healthy dietary pattern showed inverse associations on these measures. (Akbaraly et al., 2018; Bogl et al., 2013; Handakas et al., 2022; Pan et al., 2022) Additionally, some unhealthy dietary patterns have shown direct associations with small HDL particles or inverse association with HDL particle size. (Bogl et al., 2013; Handakas et al., 2022; Pan et al., 2022) One healthy dietary pattern has been directly associated with the serum ratio of PUFAs to total fatty acids while observing inverse associations with the ratios of MUFAs and SFAs to total fatty acids. (Akbaraly et al., 2018) This healthy dietary pattern showed inverse associations with some amino acids, such as isoleucine and leucine, while conflictingly, an unhealthy dietary pattern was also inversely associated with these branched-chain amino acids and partly contributed this to reduced intake of protein containing foods rather than to physiological mechanisms. (Akbaraly et al., 2018; Handakas et al., 2022) Dietary patterns have been associated with various membrane lipids as well, but further research is needed to solidify all these associations. (Martínez-González et al., 2015; Sellem et al., 2023)

2.3.2 Physical activity

A large body of literature has evaluated the acute metabolic effects of physical activity or exercise whereas many health outcomes, such as atherosclerotic cardiovascular diseases, manifest over decades. Therefore, studies aiming to understand the longer-term associations between physical activity and metabolic profile are warranted to unveil a possible chronic adaptation in the metabolome in response to persistent physical activity. (Kelly et al., 2020)

In one such cross-sectional study the associations between self-reported daily physical activity and 115 plasma metabolites obtained with targeted mass spectrometry in over 1 000 Japanese men were examined. The men filled a questionnaire evaluating the average time spent in various physical activities during the last 12 months. The study found higher physical activity and reduced sitting time to be inversely associated, for example, with isoleucine and triglycerides, and directly with HDL cholesterol. (Fukai et al., 2016) Another study examined the associations between self-reported habitual physical activity and 337 metabolites obtained by targeted mass spectrometry in 5 197 U.S. participants from 4 large cohort studies. Physical activity was defined as the average amount of physical activity calculated from two questionnaires applied closest in time before and after blood sample collection (time between questionnaires 2–4 years). The study found that physical activity was associated with 20 metabolites after adjustments, including citrulline, glycine, phosphocholines and lysophosphatidylcholines. (Ding et al., 2019) As long-term randomized controlled exercise trials are scarce, one study aimed to control for childhood environments and genetic factors by comparing 16

baseline-healthy twin pairs with over 30 years of discordance for physical activity. (Kujala et al., 2013) Additionally, 1 037 age- and sex-matched persistently active and inactive pairs from three population-based cohorts were studied as well. Metabolic profile was obtained by a targeted NMR spectroscopy. Being persistently physically active associated with a metabolic profile with lower serum fasting concentrations of isoleucine, glycoprotein acetyls, glucose, VLDLs, and a less saturated fatty acid profile, whereas the concentrations of large and very large HDL particles were increased. Generally, studies on the links between physical activity (or exercise) and metabolomics have found a "healthier metabolic profile" defined as, for example, lower concentrations of isoleucine, VLDL cholesterol, triglycerides, glucose, and a less saturated fatty acid profile. (Kelly et al., 2020)

Few studies have examined the association between physical activity and metabolic profile in children and adolescents with a paucity in longitudinal studies. In the ALSPAC study, 1 826 adolescents' total activity and sedentary time were measured using an accelerometer at the age of 12, 14, and 15 years, and 230 metabolites were assessed at the age of 15 years by a targeted NMR spectroscopy. (Bell et al., 2018) Higher total activity was associated with, for example, higher concentration of HDL-C, and lower cholesterol and triglyceride concentrations in VLDL particles as well as lower concentration of glycoprotein acetyls. Another cross-sectional study included 880 Norwegian children with a mean age of about 10 years with physical activity intensity and sedentary time measured by accelerometer which was worn for seven consecutive days, and 30 measures of lipoproteins obtained by NMR spectroscopy. (Jones et al., 2019) It found, for example, that the time spent in moderate-to-vigorous intensity physical activity was inversely associated with most ApoB-containing lipoprotein subclasses and triglyceride measures, and directly associated with HDL cholesterol concentration, particle size, and larger HDL subclasses, independent of the time spent sedentary.

A supervised play-based intervention of 22 healthy, overweight U.S. preadolescent (8–12 years of age) children assessed their metabolic profile from urine samples by untargeted mass spectrometry after either a 4-week or an 8-week intervention, or an unsupervised summer break (control group). (Meucci et al., 2017) The 8-week intervention induced the greatest shift in the metabolites (including an increase in lysine and creatinine concentrations) whereas the four-week intervention did not affect the metabolic profile compared to the control group. Cardiorespiratory fitness refers to the capacity of the circulatory and respiratory systems to supply oxygen to skeletal muscle mitochondria during physical activity, and it is a marker of physical health. (Raghuveer et al., 2020) A cross-sectional study of 450 children aged 6–8 years from the PANIC study investigated the associations of cardiorespiratory fitness with serum NMR assessed metabolic measures. (Haapala et al., 2022) The study found that cardiorespiratory fitness was directly associated with

HDL-C, medium-sized HDL particles, ApoA-1, glutamine, and phenylalanine after adjusting with body fat percentage.

To conclude, physical activity seems to present a "healthier metabolic profile" described as e.g., less saturated fatty acid profile with inverse associations seen for concentrations of isoleucine, triglyceride, glucose, and VLDL-C. (Kelly et al., 2020; Kujala et al., 2013) Additionally, many studies show direct associations between physical activity and HDL-C as well as with various HDL measures. (Fukai et al., 2016; Haapala et al., 2022; Kujala et al., 2013) Physical activity has been directly associated with various amino acids, such as glutamine, glycine, lysine, creatinine, and inversely with glycoprotein acetylation. (Bell et al., 2018; Ding et al., 2019; Kujala et al., 2013) It is hypothesized that the inverse association observed for branched-chain fatty acids, isoleucine included, results from their increased break-down during physical activity which in turn results in more efficient lipid metabolism possibly leading to improved insulin sensitivity, while the beneficial associations seen in the fatty acid profile due to long-term physical activity seem to stem from multiple phenomena related to oxidation and desaturation. (Kelly et al., 2020; Kujala et al., 2013)

2.3.3 Tobacco smoke exposure

Exposure to tobacco smoke can be detrimental to health and there are over 4 000 identified chemicals in tobacco which can affect health through different biological pathways. (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2004) Metabolomics studies of smoking are scarce even though they could give insight into how exposure to tobacco smoke is reflected on host's metabolic adaptations and smoking-related diseases. (Gu et al., 2016)

A study using untargeted mass spectrometry examined the associations between cigarette smoking and metabolites in 892 middle-aged participants from four cohorts in Italy, U.S., China, and Finland. (Gu et al., 2016) The study applied two smoking phenotypes, current smoking status and cigarettes per day. A total of 24 metabolites associated with the smoking behaviours: 15 xenobiotic, 3 amino acids, 2 lipids, 4 vitamins or cofactors, and one carbohydrate. The findings were consistent in direction across the four cohorts. Bilirubin was one of the endogenous metabolites with lower concentrations observed for current smokers than for former or never smokers. Circulating bilirubin is considered an antioxidant, and it has been inversely associated with, for example, cardiovascular disease. (Lin et al., 2006; Stocker et al., 1987) Another study used different untargeted mass spectrometry and NMR techniques to compare active smokers (n = 55, smoking 10–30 cigarettes daily for at least 5 years) to never-smokers (n = 57) in Germany with BMI in the healthy range. The participants were enrolled in the study for a period of 183 and 164 days,

respectively, and they kept a diary of e.g., cigarette consumption, diet, and exercise. They were in confinement for 48 hours with controlled diet before sample collection. The pathways most impacted by smoking belonged to, for example, sphingolipid and glycerophospholipid metabolism, and amino acid metabolism. Additionally, an increase in the concentrations of small LDL particles and ApoB was observed and a decrease in the concentration of bilirubin. (Kaluarachchi et al., 2016) In a 24-hour diet-controlled study comparing 25 male smokers and 25 non-smokers with untargeted mass spectrometry, elevated ratios of MUFAs to total fatty acids were observed along with alterations in SFAs and PUFAs. As the main fraction of fatty acids in plasma is esterified to phospholipids, further analyses of two phospholipid subspecies were made and it revealed them to be significantly elevated. (Muller et al., 2014) Similar changes were observed in a cross-sectional, case-controlled study comparing lipidomes of current smokers, smokers with mild chronic obstructive pulmonary disease, and never-smokers (n = 40 per group) obtained by untargeted and targeted mass spectrometry. The study found an increase in glycerophospholipids and MUFAs whereas a decrease in omega-3 fatty acids was observed. (Titz et al., 2016) A more recent study on 113 male participants diagnosed with coronary artery disease (46 current smokers, 34 former smokers, and 33 never-smokers) using untargeted mass spectrometry found smoking to be associated with concentration variations in sphingolipids, glycerophospholipids, and amino acids. (Hu et al., 2021)

Only a few studies utilizing metabolomics to examine the effects of passive tobacco smoke exposure in children have been conducted. One study of 52 children (19 with cystic fibrosis and passive tobacco smoke exposure, 23 with cystic fibrosis but without tobacco smoke exposure, and 10 with neither) used hair nicotine as a measure of passive tobacco smoke exposure and mass spectrometry to evaluate differences in metabolites. (Wisniewski et al., 2020) Alterations with passive tobacco smoke exposure were identified in fatty acid metabolism, steroid biosynthesis, cysteine metabolism and oxidative stress. Another cross-sectional study using untargeted mass spectrometry included Chinese children with low, medium, and high passive tobacco smoke exposure (n = 17 in each group, aged 2–5 years) determined by urine cotinine concentrations to assess urinary metabolites. (Zhu et al., 2021) Additionally, a before – after study was carried out before and two months after intervention for the smokers in the family to discontinue smoking. Forty-three metabolites were associated with passive tobacco smoke exposure in the cross-sectional analysis, and only few of these were confirmed to be directly associated in the longitudinal analysis, such as kynurenine which has been associated with the risk of lung cancer. (Huang et al., 2020)

To summarize, studies exploring the associations of tobacco smoke exposure with metabolic profile are scarce, mostly cross-sectional and with small sample sizes

with differing methods used for metabolite assessment further hindering comparison. Still, active smoking has been directly associated with small LDL particle and ApoB concentrations as well as with the ratio of MUFAs to total fatty acids. (Kaluarachchi et al., 2016; Muller et al., 2014) Inverse associations have been observed for concentrations of bilirubin and long-chain omega-3 fatty acids. (Gu et al., 2016; Lin et al., 2006; Titz et al., 2016) Additionally, active smoking has been associated with pathways relating to e.g., sphingolipid and amino acid metabolism. (Hu et al., 2021; Kaluarachchi et al., 2016) Passive tobacco smoke exposure in children has shown alterations in, for example, fatty acid metabolism, and steroid biosynthesis. One possible mechanism for the observed increases in the ratio of MUFAs to total fatty acids in the total fatty acid profiles and phospholipid profiles is due to an altered endogenous fatty acid desaturation (Hodson & Fielding, 2013; Muller et al., 2014) As PUFAs (but not MUFAs) are sensitive to oxidative damage, the observed alterations in PUFAs could reflect increase in the oxidative stress of smokers whereas the alterations in the concentrations of glycerophospholipids might be due to diet, or be a more direct effect of smoking, such as inhibition of lipoprotein lipase by nicotine possibly resulting in reduced clearance of lipids. (Titz et al., 2016)

3 Aims

This thesis is based on findings from the Special Turku Coronary Risk Factor Intervention Project. The main purpose was to study the effects of the randomized dietary counselling intervention, as well as the associations of achieving the dietary targets of the intervention, physical activity, and tobacco smoke exposure with comprehensive serum metabolic profile.

The specific aims were:

1. to study the effects of the randomized STRIP dietary counselling intervention trial on metabolic profile from childhood to adulthood (Study I)
2. to study how achieving the dietary targets of the STRIP study are associated with metabolic profile from childhood to adulthood (Study II)
3. to examine the associations of physical activity with metabolic profile from adolescence to adulthood (Study III)
4. to assess the association of tobacco smoke exposure with metabolic profile from childhood to adulthood (Study IV)

4 Subjects and Methods

This study included subjects who participated in the STRIP study between the ages of 9 and 19 years and who provided data on metabolic measures.

4.1 The Special Turku Coronary Risk Factor Intervention Project (STRIP)

The STRIP study is a prospective, randomized, infancy-onset intervention trial targeting atherosclerosis risk factors. (Niinikoski et al., 2012; Simell et al., 2009) The families of healthy 5-month-old infants, born between July 1989 and December 1991, were recruited to the study by nurses during routine visits from well-baby clinics in Turku, Finland (n = 1 880). When the infants were 6-months old, their families received detailed information about the study and a total of 1 062 infants (56.5% of the eligible age cohort) then embarked upon the study. At the age of seven months, they were randomly allocated either to a dietary intervention group (n = 540, 256 females) or a control group (n= 522, 256 females). Both groups attended study visits led by a nutritionist and a pediatrician or a nurse. A group of 45 children, born from March to July 1989, was analysed as well, recruited, and randomized (intervention n = 22, control n = 23) to first test the study protocols and thus serve as a 'pilot' group.

The intervention group received individualized dietary counselling at 1- to 3-month intervals until the child was two years of age and biannually thereafter until 20 years of age. The intervention was primarily targeted at the parents until the child was seven years of age, after which separate sessions were increasingly organized for the child. The parents were carefully informed about the contents of the child's counselling session, and they were encouraged to further discuss the same topics with the child at home. The control group had biannual visits until the child was seven years of age, after which the families were seen annually until the child was 20 years old. The children in the control group received only basic health education routinely given at Finnish well-baby clinics and by school health care. Similar measurements were performed for both study groups, and they met the same study personnel. The STRIP study is still ongoing.

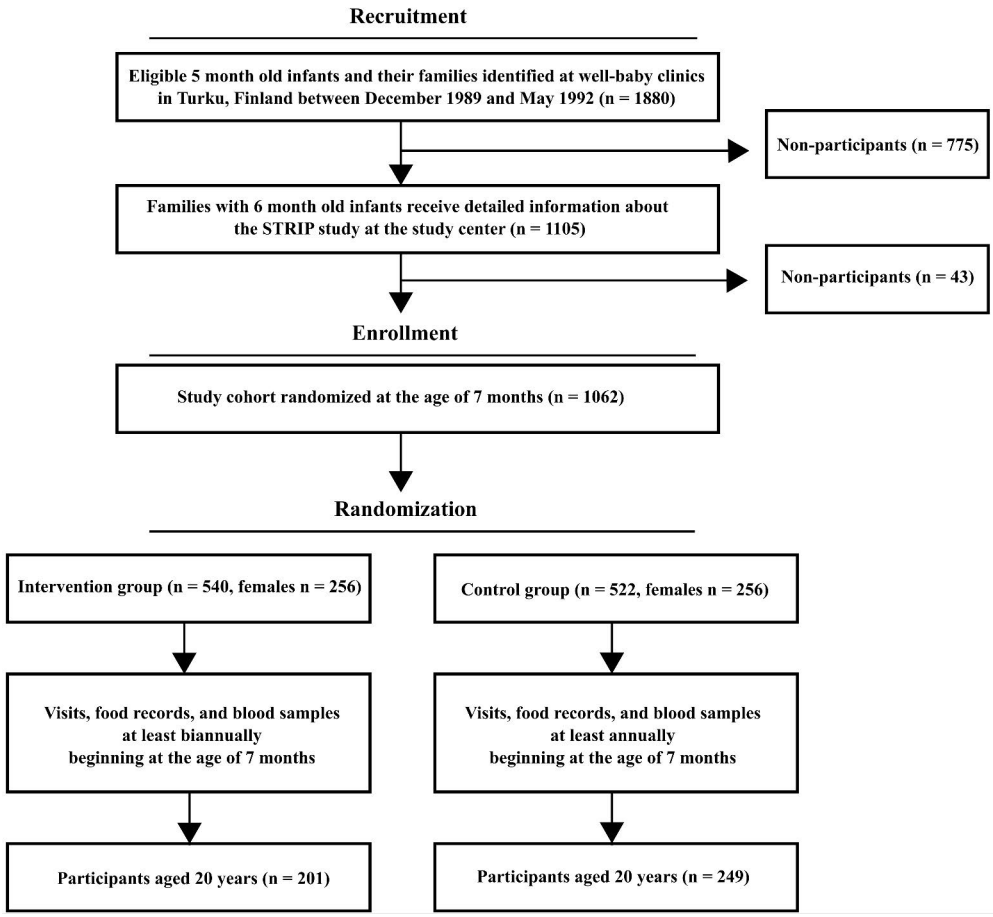

Figure 3. Flow chart of the STRIP cohort. The intervention period continued until the age of 20 years. Most common reasons for discontinuing in the study were insufficient time, birth of a younger sibling, child's recurrent infections, moving away from Turku area, and reluctance to blood sampling.

4.2 Subjects

The subjects included in the studies of this thesis consisted of children and adolescents who participated in the STRIP study between the ages of 9 and 19 years (1998 and 2010) and provided data on metabolic measures quantified by high-throughput serum NMR.

Study I examined the effects of the randomized STRIP dietary intervention on a comprehensive serum metabolic profile measured repeatedly from childhood to early adulthood. The study comprised of participants for whom data on metabolic measures were available from serum samples drawn at the age of 9 (n = 554), 11 (n = 553), 13 (n = 508), 15 (n = 517), 17 (n = 457), and 19 (n = 417) years. This

represents 92–99% of the total number of study participants. Intervention effect on dietary intake of different fatty acid types was also evaluated.

Study II assessed how achieving the targets of the STRIP dietary intervention, regardless of study group allocation, was associated with serum metabolic profile measured repeatedly from childhood to adulthood. This study cohort included also the 'pilot' group children and comprised of participants who reported dietary data and had metabolic measures quantified at the same time point at the age of 9 (n = 549), 11 (n = 518), 13 (n = 485), 15 (n = 472), 17 (n = 410), and 19 (n = 338) years.

Table 1. Number of participants (F=female, M=male) in the four studies at different ages. Number of participants in the active tobacco smoke exposure cohort in parenthesis.

Study / Age	I		II		III		IV	
	F	M	F	M	F	M	F	M
9	268	286	272	277	NA	NA	263	276
11	264	289	250	268	NA	NA	257	279
13	245	263	240	245	241	262	256	268
15	248	269	222	250	232	240	224 (181)	249 (192)
17	225	232	211	199	236	230	216 (151)	203 (124)
19	211	206	181	157	188	173	192 (124)	168 (86)

Study III examined the association of physical activity during adolescence with serum metabolic profile. The study cohort comprised of 13- to 19-year-old adolescents, including 'pilot' group participants, who provided physical activity data and had metabolic measures quantified at the same time point at the age of 13 (n = 503), 15 (n = 472), 17 (n = 466), and 19 (n = 361) years.

Study IV investigated the association of tobacco smoke exposure, both passive and active, with serum metabolic profile measured repeatedly during childhood and adolescence. The study cohorts included the 'pilot' group children. Passive tobacco smoke exposure study cohort excluded participants who reported tobacco smoking at least once a week and comprised of participants who provided serum metabolic measures and serum cotinine concentrations at the same time point at the age of 9 (n = 539), 11 (n = 536), 13 (n = 524), 15 (n = 473), 17 (n = 419), and 19 (n = 360) years. Active tobacco smoke exposure study cohort comprised of 15- to 19-year-old participants who reported at least daily smoking or reported no tobacco use and who

provided serum metabolic measures and serum cotinine concentrations at the same time point at the age of 15 (n = 373, 13 daily smokers), 17 (n = 275, 36), and 19 (n = 210, 49) years of age. Additionally, the associations between passive tobacco smoke exposure and serum metabolic profile were analysed from the perspective of parents' smoking status and child's cotinine concentration.

4.3 Metabolite quantification

A high-throughput nuclear magnetic resonance metabolomics platform was used to quantify 75 serum lipid and metabolite measures from fasting samples collected at 6 time points when the study participants were aged 9–19 years. This platform provides quantification of e.g., clinical lipoprotein measures, and total lipid, cholesterol, esterified cholesterol, free cholesterol, triglyceride, phospholipid, and particle concentrations of 14 lipoprotein subclasses and their major subfractions. Further, it offers quantification of numerous fatty acids, amino acids, ketone bodies, and gluconeogenesis related metabolites.

In the proton NMR spectroscopy method applied in this study, each molecule with hydrogen atoms gives a characteristic signal with a distinctive shape and the area of which is proportional to the concentration of the molecule. (Mihaleva et al., 2014) It provides metabolite concentrations in physiological units, and thus the data can be analyzed as any other biomarker data. The full process and methods of sample preparation and quantification are extensively described elsewhere. (Soininen et al., 2009, 2015) Briefly, serum (or plasma) samples are handled in plates containing 2 quality control samples. Before the NMR measurements, 260 μL of serum and 260 μL of a sodium phosphate buffer are mixed and moved to the NMR tubes with a PerkinElmer JANUS Automated Workstation. NMR metabolomics combines Bruker AVANCE III 500 MHz and Bruker AVANCE III HD 600 MHz spectrometers with SampleJet robotic sample changer. For native serum samples the lipoprotein and low-molecular-weight metabolites data can be automatically collected with either spectrometer. After these measurements, the samples go through a standardized lipid extraction procedure done manually with Integra Biosciences VIAFLO 96 channel electronic pipette. These extracts are then moved into NMR tubes and lipid data are collected in full automation with the 600 MHz instrument using a standard parameter set. Initial data processing is then done using the computers that control the spectrometers. The 14 lipoprotein subclass sizes are defined as follows: extremely large VLDL with particle diameters from 75 nm upwards and a possible contribution of chylomicrons, five VLDL subclasses (average particle diameters of 64.0 nm, 53.6 nm, 44.5 nm, 36.8 nm, and 31.3 nm), IDL (28.6 nm), three LDL subclasses (25.5 nm, 23.0 nm, and 18.7 nm), and four HDL subclasses (14.3 nm, 12.1 nm, 10.9 nm, and 8.7 nm). The mean size for VLDL,

LDL and HDL particles was calculated by weighing the corresponding subclass diameters with their particle concentrations.

4.4 Dietary and lifestyle intervention

The individualized dietary counselling given in the STRIP study was designed to meet the Nordic Dietary Recommendations available at the time. (Lapinleimu et al., 1995; Sandström et al., 1996) The main dietary goal was to replace SFAs with unsaturated fat in the diet without reducing total fat intake. Additionally, low intake of dietary cholesterol, sodium and sucrose were targeted, coupled with enhancing consumption of whole-grain products, fruit, and vegetables. Optimal diet comprised of energy without restrictions with 10–15% of all energy (E%) from protein, 50–60 E% from carbohydrates, and up to 30 E% from fat, except between the ages of 1 and 2 years, when 30–35 E% from fat was recommended. Intervention aimed at unsaturated to saturated fatty acid ratio of 2:1, and cholesterol intake of < 200 mg/day. A fixed diet was never demanded, rather, counselling was individualized, and dietary changes were suggested based on child's food records (for example replacement of dairy fat-blend spreads with vegetable oil-based spreads).

In the beginning of the intervention trial, breast feeding, or formula was advised until one year of age, and after that, 0.5–0.6 litres of skimmed milk was recommended for the intervention children. The intervention families were also advised to add 2 to 3 teaspoonfuls of soft margarine or vegetable oil to the child's diet daily from 12 to 24 months of age. In terms of protein, specific counselling related to plant- or animal-based sources was not given. Most of the counselling material used, for example brochures and paper-pencil tasks, was especially developed for the project due to the lack of ready-made materials for children.

Other lifestyle factors related to cardiometabolic diseases, such as smoking and sedentary lifestyle, were also discussed and parents were encouraged to change the child's habits towards healthy lifestyle. If a parent was a smoker, the possibilities of the child to be exposed to tobacco smoke were discussed. When the children were 5 years old, the families received a booklet about the adverse health effects of smoking. (Kallio et al., 2006) Counselling aimed at prevention of active smoking began when intervention children were eight years old. Topics of the counselling covered e.g., adverse health effects of both active and passive tobacco smoke exposure, the development of addiction, and how to avoid passive tobacco smoke exposure. Suggestions on related topics, for example how to refuse an offered tobacco, were discussed. A physically active lifestyle was also encouraged but it was not a structured, continuous part of the intervention.

4.5 Dietary data and dietary target score

Food consumption was recorded using a 3-day food record at the ages of 8, 13, and 18 months, while a 4-day food record (consecutive days, at least one weekend day included) was applied from the age of 2 to 20 years to account for greater variation in the diet. (Matthews et al., 2019; Simell et al., 2009) Parents or other caregivers were responsible for filling out the food records during early childhood. When the child began attending day care, and subsequently school, also establishment's personnel were asked to assist the child in completing the food records. As the child aged, they were given more responsibility in completing the food records, however parents were still advised to check the records and assist the child. Throughout the study, the child and parents or other caregivers were given written and verbal instructions on how to fill out the food records. A detailed food picture booklet was used to assist in the estimation of the amounts of food or drink beginning from the age of 13 years. During study visits a dietitian checked the food records for completeness and accuracy and, if necessary, added missing details after discussion with the child or parents. The food and nutrient intakes were analysed with a Micro Nutrica® program, developed by the Research and Development Centre of the Social Insurance Institution, Turku, Finland. (Hakala et al., 1996) The program calculates 66 nutrients of over 4000 foods and dishes. Data bank of the program is flexible, permitting continuous updating and additions of new single or composite foods based on foods and drinks reported by the participants. A single dietary technician analysed all food records and updated the data bank throughout the study.

The dietary target score was created to reflect achievement of the key STRIP dietary intervention goals. (Laitinen et al., 2018) Quality of dietary fat was assessed using two separate targets: ratio of saturated to monounsaturated and polyunsaturated fatty acids (SFA / MUFA + PUFA) < 1:2, and as an intake of SFA < 10 percentage of total daily energy intake. Fibre intake reflected whole-grain product, fruit, and vegetable consumption, which were secondary goals of the intervention. Dietary fibre target was defined as being at the top age-specific quintile ($\geq 80^{th}$ age-specific percentile). This criterion was chosen as very few participants met the goal set by Nordic Nutrition Recommendations of ≥ 3g/MJ. (Laitinen et al., 2018; Nordic Council of Ministers, 2014) As further reflection of the dietary carbohydrate quality, desired sucrose intake (E%) was defined as being in the lowest age-specific quintile ($\leq 20^{th}$ age-specific percentile). This criterion was used in the absence of a consensus recommendation on sucrose intake. To form the dietary target score, participants were given 1 point for meeting each of the four targets: 1) SFA / (MUFA + PUFA) < 1:2, 2) SFA < 10 E%, 3) dietary fibre $\geq 80^{th}$ age-specific percentile, and 4) sucrose $\leq 20^{th}$ age-specific percentile. Score range was 0–4 points.

The current Nordic Nutrition Recommendations or American dietary recommendations do not set an upper limit to dietary cholesterol intake whereas, for

example, the Finnish guideline for treatment of dyslipidemia does. (Blomhoff et al., 2023; Dyslipidemiat. Käypä hoito -suositus., 2022; Lichtenstein et al., 2021) Low intake of dietary cholesterol was targeted in the STRIP intervention based on the nutrition recommendations effective during the launch of STRIP intervention. (Lapinleimu et al., 1995) Thus, a secondary dietary target score with additional target for dietary cholesterol was formed with the range of 0–5 points. Percentage of the STRIP study participants having dietary cholesterol < 300 mg per day, as recommended for example in the Dietary Guidelines for Americans, ranged between 65–91% at different time points. (Benjamin, 2011) Since majority of participants reached this goal, the additional target for dietary cholesterol was defined as being in the lowest age-specific quintile.

4.6 Self-reported physical activity and tobacco smoke exposure

Leisure-time physical activity (LTPA) comprising of recreational and organized physical activity or sports outside school hours was assessed with a self-administered questionnaire starting from the age of 13 years, where frequency, duration, and intensity of habitual LTPA was reported in multiple-choice questions. For the habitual LTPA intensity, the choices were: never sweating and becoming breathless, some sweating and becoming breathless, and heavy sweating and becoming breathless. For the frequency of LTPA, the choices were less than once a month, once a month, 2 to 3 times a month, once a week, 2 to 6 times a week, and once a day. For the average duration of a LTPA bout, 4 choices were given: < 20, 20 to 40, 40 to 60, and > 60 minutes. Leisure-time physical activity was then calculated by multiplying the mean frequency, duration, and intensity (multiple of the resting metabolic rate; MET) of weekly LTPA and expressed as MET h/wk. (Pahkala et al., 2011) For intensity of LTPA, coefficient values of 4, 6, and 10 corresponding to light, moderate, and vigorous physical activity were used in the calculation. The questionnaire has been widely used in studies involving children, adolescents, and adults. (Mansikkaniemi et al., 2012) It correlates moderately well with objectively assessed physical activity (accelerometers: r = 0.26–0.40; pedometers: r = 0.30–0.39) in young adults, and with maximal exercise capacity (r = 0.49–0.53). (Mansikkaniemi et al., 2012; Yang et al., 2006) One hour of brisk walking per week corresponds approximately to a level of 5 MET h/wk, and a level of 30 MET h/wk corresponds to about an hour of moderate intensity exercise every day of the week or, for example, running three hours per week. (Butte et al., 2018)

Participants were queried with a detailed multiple-choice questionnaire about their smoking habits and attitudes towards smoking. Children in the intervention group answered the questionnaire first at the age of 9.5 years and children in the

control group at their 10-year study visit, and annually thereafter. Participants were also asked whether either of their parents smoked. From the age of 13 years onwards, those who reported having smoked more than one cigarette during their lifetime were additionally asked about their current smoking status and whether they smoked daily, weekly, less frequently than weekly, or if they had ceased smoking. The participants were informed that their answers were confidential.

4.7 Anthropometric, pubertal, cotinine and other biochemical measures

Height was measured by a wall-mounted Harpenden stadiometer with ear canal and corner of the eye in the same horizontal plane to the nearest 0.1 cm (Holtain, Crymych, Great Britain) and weight with an electronic scale to the nearest 0.1 kg (Soehnle S10; Soehnle, Murrhardt, Germany). Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Two three-level categorical variable were used to represent parental socio-economic status that was based on the highest education and occupation level obtained when the study participants were aged 9 years.

Pubertal status was classified using Tanner staging (M1–M5 for females, G1–G5 for males) beginning at the age of 9 years. (Tanner, 1962) Breast tissue diameter and pubic hair development were estimated visually. Testicular length was measured with a ruler.

Serum cotinine analyses were performed in the Joint Clinical Biochemistry Laboratory of the University of Turku, Turku University Central Hospital and Wallac Oy, in Turku, Finland. Fasting serum samples for cotinine measurement were drawn annually from the study participants beginning at the age of 8 years, after which serum was separated, and stored at -70°C until analysed. Cotinine was extracted into dichloroethane from 0.2 mL of serum to which 0.2 mL of 5-methylcotinine had been added with the method described by Feyerabend and Russel. (Feyerabend & Russell, 1990) The concentrated extract (2.0 µL) was then injected into a Hewlett Packard free fatty acid phase silica capillary column (Agilent Technologies, Palo Alto, CA) in a Shimadzu model GC-17 gas chromatograph (Shimadzu Corp, Kyoto, Japan), equipped with a nitrogen-sensitive flame-thermionic detector. (Kallio et al., 2007) The analytical sensitivity of the method was 0.16 ng/mL. The method was validated by cotinine analyses of 20 serum samples both in the Joint Clinical Biochemistry Laboratory and in ABS Laboratories (Medical Toxicology Unit, London, UK). The correlation coefficient of these determinations was 0.994.

Fasting venous blood samples were applied to provide data on triglyceride, Lp(a), C-reactive protein (CRP), total and high-density lipoprotein cholesterol as

well as on insulin concentrations. The analyses were performed at the laboratory of the National Public Health Institute in Turku, Finland. The results were regularly crosschecked by Labquality, Helsinki, Finland. Serum total cholesterol and triglyceride concentrations were analysed with a fully enzymatic cholesterol oxidase-p-aminophenazone method (Merck, Darmstadt, Germany), with an automatic AU400 analyser (Olympus, Hamburg, Germany). Serum HDL cholesterol concentration was measured after precipitation of low-density lipoprotein and very-low-density lipoprotein with dextran sulfate 500,000. LDL cholesterol concentration was obtained by using the Friedewald formula. (Niinikoski et al., 2012) Serum insulin was measured with a microparticle enzyme immunoassay (insulin IMX system reagent; Abbott, Chicago, IL), or chemiluminescent microparticle immunoassay (ARCHITECT insulin assay; Abbott) between 7 and 13 years. From the age of 15 years onwards, serum insulin was measured by radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden). (Oranta et al., 2013) As an estimation of insulin resistance, HOMA-IR (fasting insulin mU/mL x [fasting glucose (mmol/L)/22.5]) measures were used. (Matthews et al., 1985)

Lp(a) concentration was measured with a solid phase immunoradiometric assay with a direct sandwich technique (Pharmacia/Mercordia, Uppsala, Sweden). (März et al., 1993) Lp(a) measures were available for analyses at other time points except at the age of 19 years. High-sensitivity CRP was assayed by a turbidimetric immunoassay with sensitivity of 0.06 mg/L, and it was available for analyses at other age points except at 9 years of age.

4.8 Statistical methods

Metabolic measures with skewed distributions were log(x+1)-transformed prior to analyses. The assumptions of linearity and homoscedasticity of the linear mixed effect models were checked by visual inspection of the residual plots, and the normality of residuals assumption was visually inspected from histogram plot of residuals. To ascertain that the results were not affected by influential data points, analyses were repeated in a data set in which metabolic measures of over and under three standard deviations (SD) from the mean had been removed. To check for absence of collinearity for the variables of interest, *performance* package was used. (Lüdecke et al., 2021)

To facilitate comparison of effect sizes across metabolites, all metabolic measures were scaled to standard deviation units. Statistical analyses were conducted by using R software and *nlme* package. (Pinheiro et al., 2023; R Core Team, 2019)

Multiple testing correction with Bonferroni adjustment for 75 independent tests gives p-value threshold of 0.0007. Since the metabolic measures in part correlate, a principal component analysis was additionally performed showing 23 metabolic

measures explaining 95% of the observed variance in the data set, and thus p-value < 0.002 gives evidence of an effect or an association.

4.8.1 Dietary counselling and metabolic profile (I)

Analyses were conducted both sex-combined and stratified by sex since the STRIP study has previously reported pronounced sex interactions in the lipid lowering effect of the intervention. (Niinikoski et al., 2012) For each measure of metabolite concentration or dietary intake, a linear mixed-effect model for repeated measures was fitted with the STRIP study group, sex, and age as fixed effects and an intercept for subjects as a random effect. Analyses with study group * sex interaction term included in the model were also performed. The reported effect sizes of the intervention hereby correspond to the average difference in SD-scaled metabolite concentration due to the intervention. Age * study group interaction was included in all dietary intake analyses.

4.8.2 Dietary targets and metabolic profile (II)

Two binary dietary target variables indicating participants meeting either none of the dietary targets or at least one of the dietary targets or at least two of the dietary targets were used in the analyses. For secondary dietary target score including targeted cholesterol intake, similar criteria were applied.

To study possible sex interactions with the dietary target score a linear mixed effects model for each measure of metabolite concentration and the dietary target score was formed with dietary target score, age, sex, and sex * dietary target score as fixed effect and an intercept for subject as a random effect. Two metabolite measures showed significant interaction between sex and dietary target score. Thus, analyses with binary dietary target variables were conducted sex-combined by fitting a linear mixed-effects model for repeated measures with binary dietary target variable, sex, and age as fixed effects and an intercept for subject as a random effect. The effect sizes reported correspond to the average difference in SD-scaled metabolite concentration due to achieving at least one or at least two dietary targets compared to achieving none of the dietary targets and are from pooled analyses across the 6 time points.

4.8.3 Physical activity and metabolic profile (III)

For each measure of metabolite concentration, a linear mixed-effects model for repeated measures was fitted with continuous physical activity variable (MET h/wk), sex, and age as fixed effects and an intercept for subject as a random effect (model

1). To gain more insight on whether diet or BMI confounded the results, multivariable analyses were performed with the previously mentioned variables in the model and additionally adjusting for BMI, and the dietary intakes of protein, carbohydrates, and fatty acids (SFA, MUFA, and PUFA) as percentages of total daily energy intake (model 2). Additional adjustments for pubertal status and STRIP study group were performed on model 1 and the results remained essentially unchanged, thus these data are not shown. Reported metabolic measures are from pooled analyses across the 4 time points, and the effect sizes reported correspond to the average difference in SD-scaled metabolic concentration due to one unit increase (= 1 MET h/wk) in physical activity during adolescence.

The STRIP study has previously reported differing results between the sexes on physical activity and metabolic risk factors in adolescents. (Pahkala et al., 2012) Thus, sex-stratified analyses with similar adjustments were conducted to study possible differences in the association of physical activity with metabolic profile between males and females in adolescence.

4.8.4 Tobacco smoke exposure and metabolic profile (IV)

To analyse the associations of passive tobacco smoke exposure with metabolic profile, those reporting smoking at least once a week were excluded from the analyses and a binary exposure variable was created to indicate either no exposure to tobacco smoke (serum cotinine concentrations < 1 ng/mL) or strong passive tobacco smoke exposure (serum cotinine concentrations of at least 10 ng/mL). For each measure of metabolite concentration, a linear mixed-effects model for repeated measures was then fitted with the exposure variable, sex, and age as fixed effects and an intercept for subject as a random effect (model 1). Additionally, analyses stratified by sex were also performed. In further multivariable analyses, a similar statistical model with the abovementioned variables was fitted with additional adjustments for BMI, and categorical control variables for STRIP study group allocation, dietary target score, pubertal status, and parental socio-economic status (model 2).

The associations of daily smoking on serum metabolic profile were analysed using a categorical variable indicating those who self-reported no smoking and had serum cotinine concentrations < 1 ng/mL, and those who reported smoking at least one cigarette daily. For each metabolite measure, a linear mixed-effect model for repeated measures was fitted with the binary variable, age, and sex as fixed effects and an intercept for subject as a random effect. Similar multivariable analyses with additional control variables as those mentioned for passive tobacco smoke exposure were also performed.

The effect sizes reported correspond to the average difference in SD-scaled metabolic concentration between participants with passive tobacco smoke exposure and participants not exposed to tobacco smoke, or between participants with daily tobacco smoking and participants not exposed to tobacco smoke.

To examine whether parental smoking was confounding the associations between passive tobacco smoke exposure and metabolic profile, a categorical variable was created based on child's reporting on their parent's smoking status. Smoking status of the parents were divided into three categories: neither parent smoked and the serum cotinine concentration of the child was < 1 ng/mL (n = 1474, for all applied age points), at least one parent smoked but the serum cotinine concentration of the child was < 1 ng/mL (n = 466), and at least one parent was a smoker and the serum cotinine concentration of the child was ≥ 1 ng/mL (n = 142). For each metabolic measure where an association with passive tobacco smoke exposure was observed ($p < 0.05$) a linear mixed-effects model was then fitted with parental smoking status, age and sex as fixed effects and an intercept for subject as a random effect.

There is significant overlap between the serum cotinine concentrations of active tobacco smokers and those passively exposed to tobacco smoke, and various cotinine concentration values have been used to differentiate between the two. In recent years this value has decreased, and it has been attributed to increased regulatory oversight on smoking. (Jarvis et al., 2008; Makadia et al., 2017) In Finland, the proportion of children experimenting with smoking and the number of active smokers among adolescents as well as parental smoking has been on decline from the start of this century. (Kinnunen et al., 2015; Rainio et al., 2009) Similar trends have been observed in other countries. (Kim, 2016). To reflect this change in tobacco smoke exposure during the study period, which was from 1998 to 2011, and as additional analyses, cotinine concentration values of at least 3 ng/mL and at least 15 ng/mL were used to form secondary exposure variables. Similar statistical analyses as for serum cotinine value of at least 10 ng/mL were then conducted examining also the possible dose-response associations of passive tobacco smoke exposure with different metabolic measures.

4.9 Ethics

The STRIP study has been approved by the Ethics Committee of Turku University and Turku University Central Hospital. At the beginning of the study, written informed consent was obtained from the parents, and twice from adolescents at 15 and 18 years of age.

5 Results

5.1 Characteristics of the subjects

Basic characteristics of the study subjects are shown in Table 2 with the STRIP study groups combined. The number of participants achieving various key dietary targets of the STRIP intervention are shown in Figure 4.

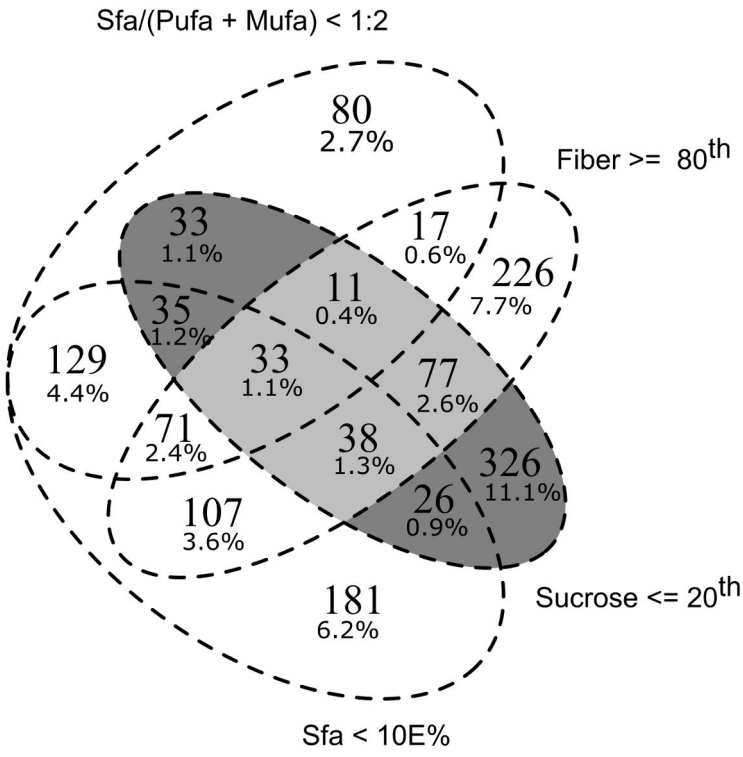

Figure 4. Number and percentage of STRIP participants achieving various key targets of the dietary intervention across six time points from 9 to 19 years of age.

Table 2. Characteristics of the study participants at 9 and 19 years of age (intervention and control group participants combined). Data are mean (SD) or percentage.

	9 years		19 years	
	Females (n=268)	**Males (n=286)**	**Females (n=211)**	**Males (n=206)**
Height [cm]	136 (6)	136 (6)	167 (6)	181 (6)
Weight [kg]	31 (6)	31 (5)	63 (12)	74 (12)
BMI [kg/m^2]	17.0 (2.5)	16.5 (2.1)	22.5 (4.0)	22.5 (3.4)
Overweight [%]	19	13	15	19
Systolic blood pressure [mmHg]	100.7 (8.0)	100.2 (6.5)	115.6 (11.6)	128.0 (12.8)
Diastolic blood pressure [mmHg]	57.9 (6.0)	57.8 (5.8)	65.1 (7.1)	66.1 (8.0)
Dietary energy intake [kcal]	1584 (299)	1773 (320)	1619 (389)	2236 (641)
Dietary fat intake [E%]	30.7 (4.9)	30.9 (5.1)	31.6 (5.4)	32.6 (6.4)
Dietary protein intake [E%]	16.4 (2.7)	16.2 (2.5)	17.7 (3.7)	18.9 (3.6)
Dietary carbohydrate intake [E%]	52.9 (5.1)	52.9 (5.5)	49.8 (5.8)	46.8 (6.8)
Serum total cholesterol [mmol/L]	4.78 (0.82)	4.53 (0.69)	4.56 (0.77)	4.09 (0.73)
LDL cholesterol [mmol/L]	3.11 (0.71)	2.84 (0.58)	2.59 (0.66)	2.45 (0.63)
HDL cholesterol [mmol/L]	1.31 (0.24)	1.37 (0.26)	1.46 (0.29)	1.17 (0.24)
Serum total triglycerides [mmol/L]	0.74 (0.28)	0.65 (0.31)	1.12 (0.50)	1.03 (0.58)
Physical activity [MET, h/wk]	NA	NA	21.7 (20.0)	26.6 (24.5)
Smoking [%]*	NA	NA	14.9	18.9

MET, metabolic equivalent; NA, not available
*Those who smoked at least once a week.

5.2 Intervention effect on dietary fat intake

Intervention effect on different dietary fatty acids is presented in Figure 5. Children in the STRIP dietary counselling intervention group had lower SFA intake compared with children in the control group throughout the intervention. However, the difference of approximately 3 E% in early childhood decreased towards the end of the intervention at 20 years of age (mean difference in daily E% between groups: -2.0 [95% confidence interval (CI): -1.7, -2.3] in males and -1.8 [-1.5, -2.1] in females). The intervention also resulted in higher intakes of PUFAs for both sexes (mean daily difference in E% 0.5 [0.4, 0.6] for males and 0.6 [0.5, 0.8] for females), but this decreased towards adolescence. Consequently, the intervention children had a higher ratio of unsaturated to SFA in their diet compared with control children throughout the intervention. For males and females, no consistent differences between the study groups were observed in the dietary intake of MUFAs.

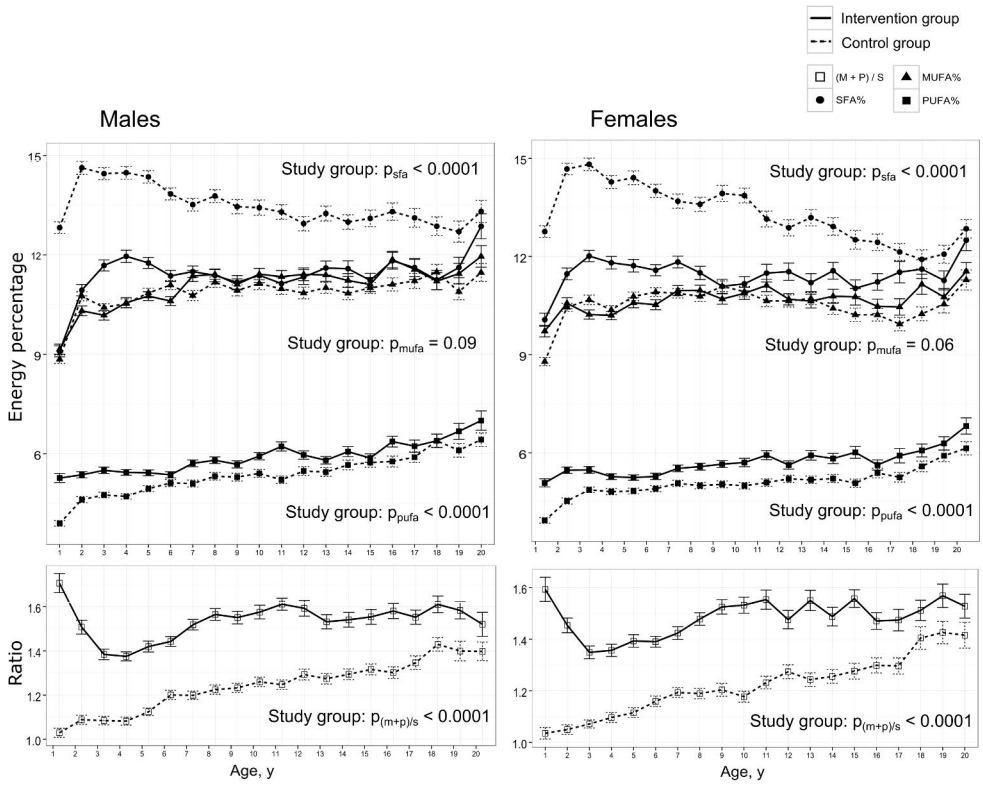

Figure 5. Dietary intake of fatty acids in the intervention and control group of males and females. Values are mean (SE) dietary intake in energy percentage of saturated, monounsaturated, and polyunsaturated fatty acids from age 1 to 20 years. The dietary intake is also shown for the sum of MUFA+PUFA to SFA (M + P / S).

5.3 Effect of dietary intervention and association of risk factors on serum metabolic profile

5.3.1 Fatty acid measures

The dietary counselling given in the STRIP study yielded most prominent differences between the intervention group and the control group in serum fatty acid measures (Figure 6, Study I). For males, the serum ratio of PUFAs to total fatty acids was higher in the intervention group compared to the control group (0.30 SD [0.14–0.40], corresponding to a 1.0% [0.53–1.49] higher serum ratio of PUFAs). Higher serum ratios were observed for both total omega-3 and omega-6 fatty acids, but the effects tended to be stronger for omega-6. Similar effects were observed for the serum ratios of the main constituents of these PUFAs to total fatty acids, namely DHA (an omega-3 fatty acid) and linoleic acid (LA, an omega-6 fatty acid). No

differences between the study groups were found in the absolute serum concentrations of these polyunsaturated fatty acid metabolic measures. In females, the difference in the serum ratio of PUFAs to total fatty acids between the intervention and control groups was weaker than in males, but the association pattern was similar which is reflected as a strong combined analysis result. Serum MUFAs were lower among the intervention group compared to controls. There was a significant ($p < 0.05$) interaction between the STRIP study group and sex for the serum ratios of MUFAs and DHA to total fatty acids. Serum SFAs were lower in the intervention group compared to the control group.

Attaining to the dietary targets of the STRIP study, regardless of study group, was associated with similar deviations in the serum fatty acid profile when compared with the effects of the intervention (Figure 6, Study II). Generally, where the most prominent associations were observed, higher number of targets met was associated with greater differences in serum fatty acid measures. Achieving the dietary targets was directly associated with the serum ratio of total omega-3 fatty acids to total fatty acids, and serum ratio of PUFAs to total fatty acids whereas a weaker direct association for the serum ratio of total omega-6 fatty acids to total fatty acids was observed. There was an inverse association between the dietary targets achieved and the serum concentration of MUFAs and the serum ratio of SFAs to total fatty acids. Attaining the dietary targets was directly associated with the number of double bonds per fatty acid.

For LTPA, the most distinct direct associations with the serum fatty acid profile were seen for the ratios of circulating total omega-6 fatty acids and PUFAs to total fatty acids (Figure 7, Study III). There was a distinct inverse association between LTPA and the serum ratio of MUFAs to total fatty acids while only a weak inverse association between LTPA and serum concentration of MUFAs was seen. LTPA was directly associated with serum concentrations of total omega-3 fatty acids with no association found for DHA. No association was found either between LTPA and serum SFA concentrations or the total serum concentration of fatty acids. LTPA was directly associated with the number of double bonds per fatty acid. These associations mostly remained when adjusting for BMI and macronutrient intakes.

Passive tobacco smoke exposure was strongly and directly associated with the serum ratio of MUFAs to total fatty acids ($\beta = 0.34$ SD [95% confidence interval (CI): 0.17-0.51], $p<0.0001$), while no association was observed between passive tobacco smoke exposure and the absolute serum concentration of MUFAs (Figure 7, Study IV). The most prominent inverse associations were observed between passive tobacco smoke exposure and the serum ratios of omega-6 fatty acids and PUFAs to total fatty acids. Other PUFA-related measures and the number of double bonds per fatty acid followed a similar pattern, except no association was observed between

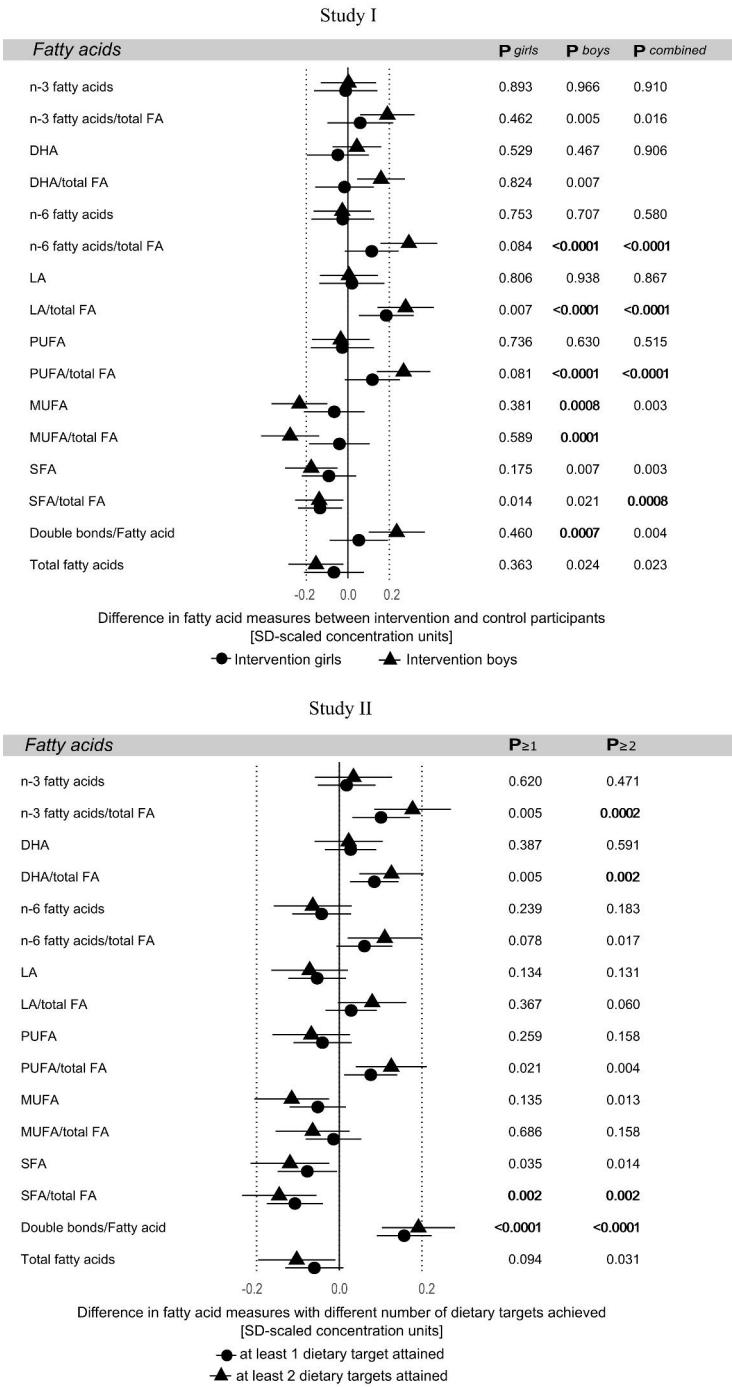

Figure 6. Differences in fatty acid measures between the intervention group and the control group (Study I) and achieving zero dietary targets with respect to achieving ≥1 or ≥2 of the targets (Study II). Error bars indicate 95% confidence intervals. Statistically significant values are bolded.

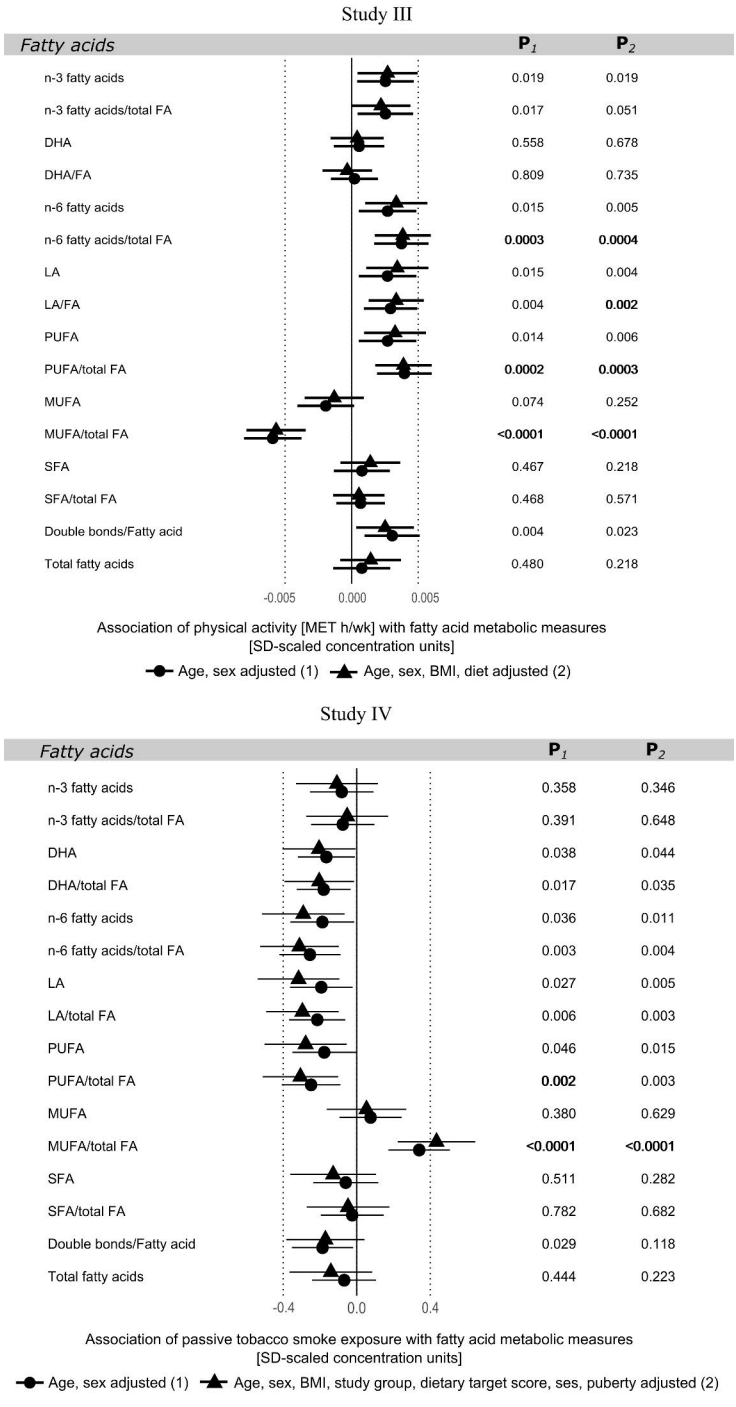

Figure 7. Associations of physical activity and passive tobacco smoke exposure with fatty acid measures (Study III and Study IV). Error bars indicate 95% confidence intervals. Statistically significant values are bolded.

passive tobacco smoke exposure and serum total omega-3 fatty acid concentration. Neither were associations observed between passive tobacco smoke exposure and serum SFA concentration, or serum total concentration of fatty acids. All the above-mentioned associations remained similar after adjustment for potentially confounding factors.

5.3.2 Lipoprotein measures

5.3.2.1 Clinical lipoprotein measures

The effects of the dietary intervention on the clinical lipid measures shown in Figure 8 (Study I) reveal that the concentrations of total cholesterol, non-HDL cholesterol, and LDL cholesterol were reduced for both sexes whereas the concentrations of triglycerides and ApoB were reduced for the intervention males only. No differences were found between the concentrations of HDL cholesterol, Lp(a), and ApoA-1 between the study groups.

In line with the effects of the dietary intervention, achieving the dietary targets of the STRIP study (Figure 8, Study II) was inversely associated with serum concentrations of total cholesterol, non-HDL cholesterol, LDL-C, and ApoB while no association was found between dietary targets attained and serum concentration of HDL-C. Only weak associations were seen between the dietary targets and other clinical lipoprotein measures.

Contrary to the previous results from a more dietary perspective, leisure-time physical activity was directly associated with HDL-C, ApoA-1 and inversely associated with serum concentration of triglycerides whereas no associations were found between physical activity and serum concentrations of total cholesterol, non-HDL cholesterol, LDL-C, or ApoB (Figure 9, Study III).

Weak inverse associations between passive tobacco smoke exposure and serum concentrations of total cholesterol, non-HDL-cholesterol, LDL-C, HDL-C, and ApoA-1 were observed with the concentration of ApoB following a similar pattern (Figure 9, Study IV). There was a tendency for a direct association between passive tobacco smoke and serum concentration of triglycerides.

5.3.2.2 Lipoprotein particles and subclasses

The dietary counselling given in the STRIP study resulted in reduced VLDL particle concentration and size in the intervention males whereas no effects for VLDL lipids were observed for females. Overall, the lipoprotein subclass profiling showed reduced lipid concentrations (i.e., combined concentration of cholesterol, phospholipid, and triglycerides) in VLDL subclasses in the intervention males and reduced lipid

concentrations in the IDL and LDL subclasses in the intervention group. In the intervention males, the total lipid concentrations in most VLDL subclasses were significantly lower. Accordingly, the concentration of remnant-cholesterol (= VLDL cholesterol + IDL cholesterol) was lower in the intervention males. There were no differences found in serum triglyceride concentration between the intervention and control females and this might be reflected in VLDL subclasses since triglycerides are their main constituents. This is further substantiated by no differences found in the triglyceride concentrations of VLDL particles between the intervention and control females. For both sexes, no robust differences were observed in the lipid concentration of HDL subclasses, although a tendency of lower lipid concentration of small HDL particles was evident in the intervention males compared to controls.

Achieving the dietary targets of the intervention was inversely associated with VLDL and LDL particle concentrations and directly associated with the size of LDL particles. In lipoprotein subclass profiling, there was a weak inverse association between achieving the dietary targets and the total lipid concentrations in VLDL and IDL lipoprotein subclasses. Achieving the dietary targets was inversely associated with total lipid concentrations of LDL subclasses as well as medium and small HDL subclasses. There was a significant interaction ($p = 0.01$) between the dietary target score and sex observed for the total lipid concentration of small VLDL subclass with males showing an inverse association with dietary targets achieved whereas no association was found for females.

Physical activity was inversely associated with VLDL particle concentration and size, and directly associated with HDL particle concentration and size while no association was found for LDL particles. LTPA was inversely associated with the total lipid concentrations of all VLDL subclasses except for very small VLDL subclass. Specifically, an inverse association between LTPA and the triglyceride concentration of VLDL particles was observed. There was a direct association between physical activity and the total lipid concentrations of very large and large HDL subclasses.

An inverse association was observed between passive tobacco smoke exposure and the number of LDL particles, while no association was found for VLDL or HDL particle concentration. In contrast, there was a direct association between passive tobacco smoke exposure and VLDL particle size, and an inverse association with HDL particle size whereas no association was observed for the size of LDL particles. In line with the previous observation on serum triglycerides, a direct association between passive tobacco smoke exposure and total lipid concentrations of large and medium VLDL subclasses was observed, with most other VLDL subclasses showing a similar tendency. Conversely, weak inverse associations were observed between passive tobacco smoke exposure and total lipid concentrations of intermediate-density lipoproteins, LDL subclasses, and very large and large HDL subclasses.

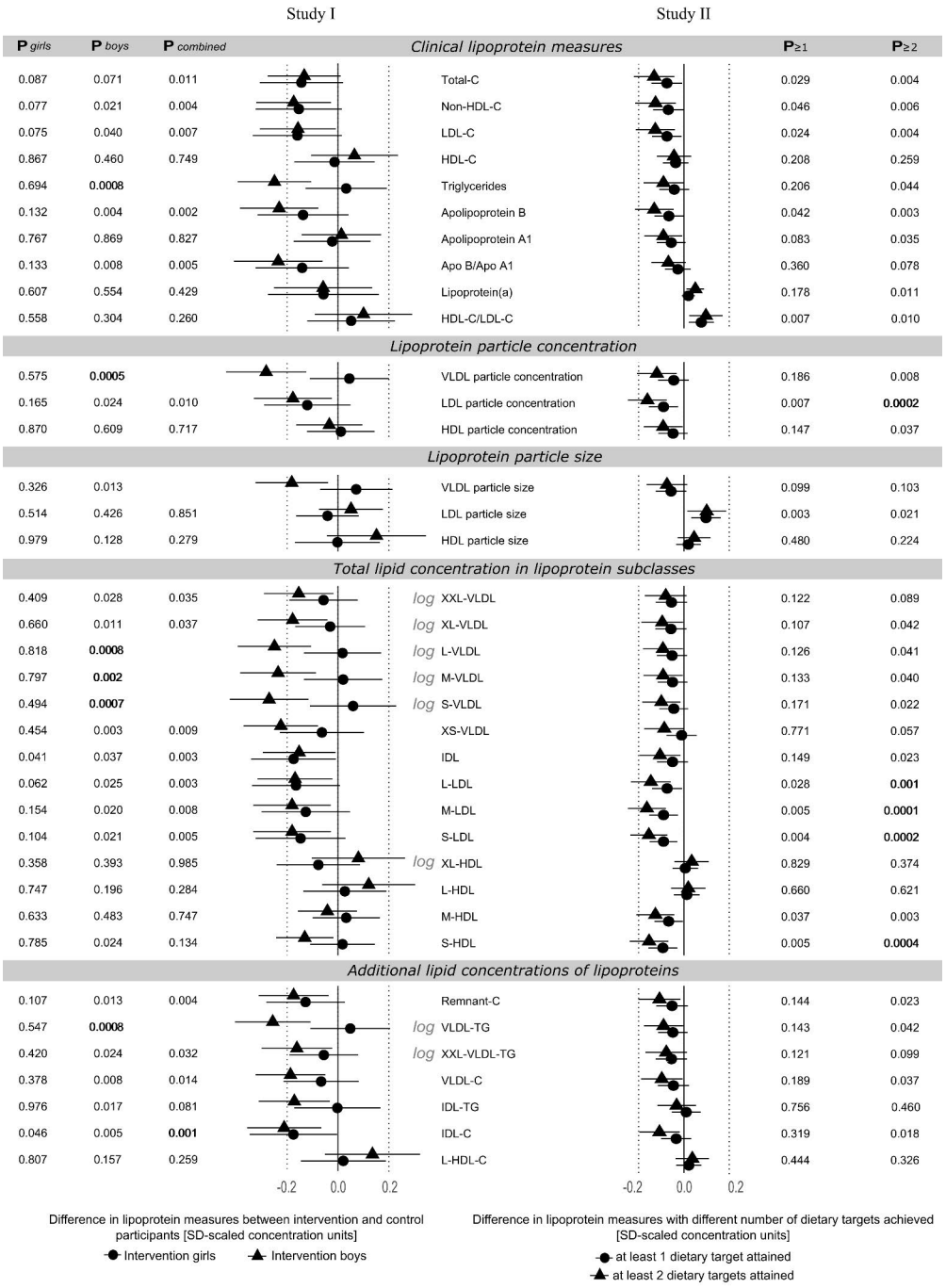

Figure 8. Intervention effects on lipoprotein measures (Study I) and differences in lipoprotein measures between achieving STRIP dietary targets compared to achieving none of the dietary targets (Study II). Error bars indicate 95% confidence intervals. Statistically significant values are bolded.

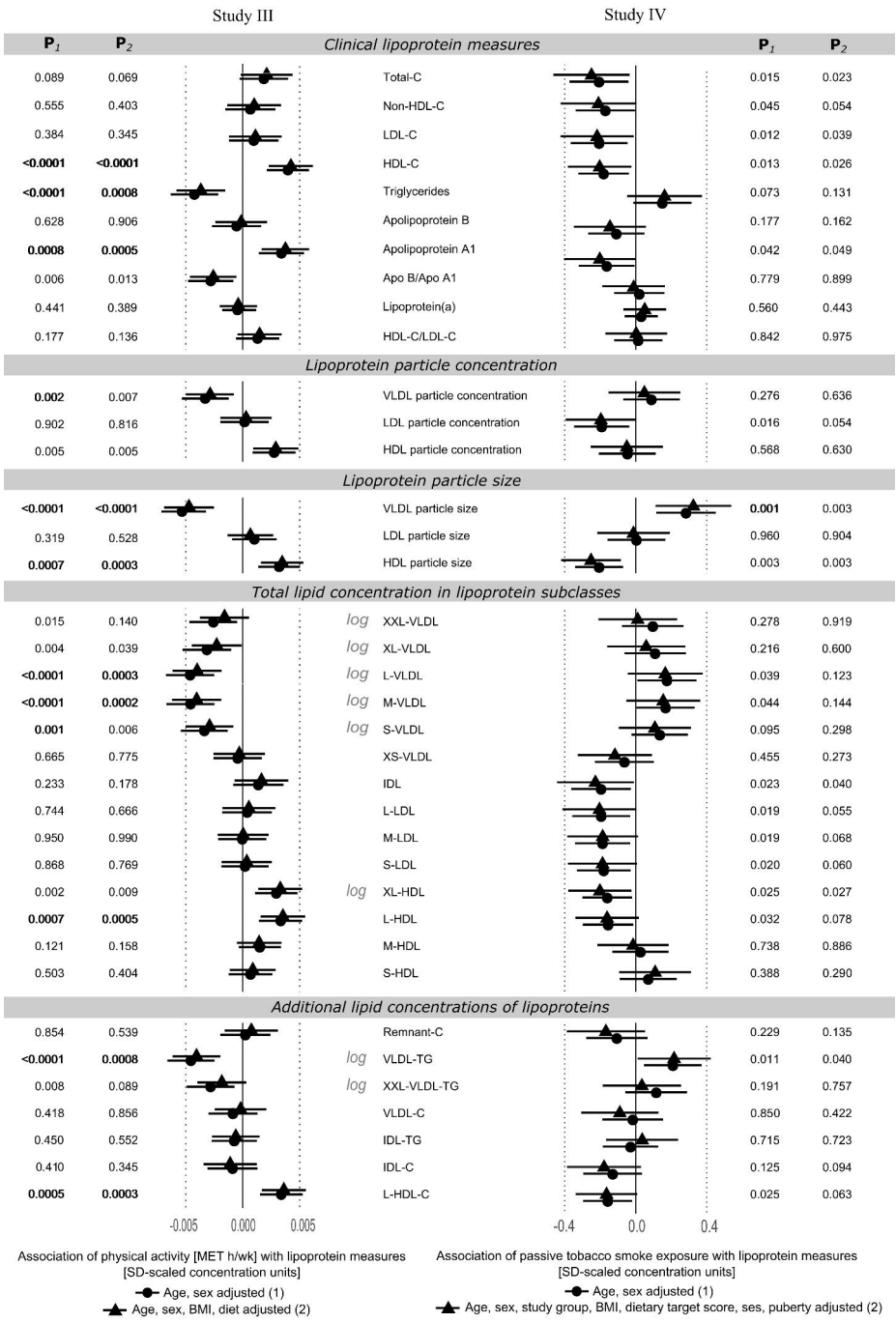

Figure 9. Association of physical activity with lipoprotein measures (Study III) and association of passive tobacco smoke exposure with lipoprotein measures (Study IV). Error bars indicate 95% confidence intervals. Statistically significant values are bolded.

5.3.3 Amino acids and other metabolic measures

The effects of the dietary intervention on serum concentrations of amino acids were modest and broadly similar for males and females (Figure 10, Study I). The most robust effect was found for higher concentrations of glutamine in the intervention group compared to the control children. Furthermore, intervention males had lower serum concentrations of glycerol, and a tendency towards lower concentrations of branched-chain amino acids and glycoprotein acetylation, an inflammatory biomarker, than the control males. (Ritchie et al., 2015) The STRIP study has previously reported improved insulin sensitivity in the intervention group compared

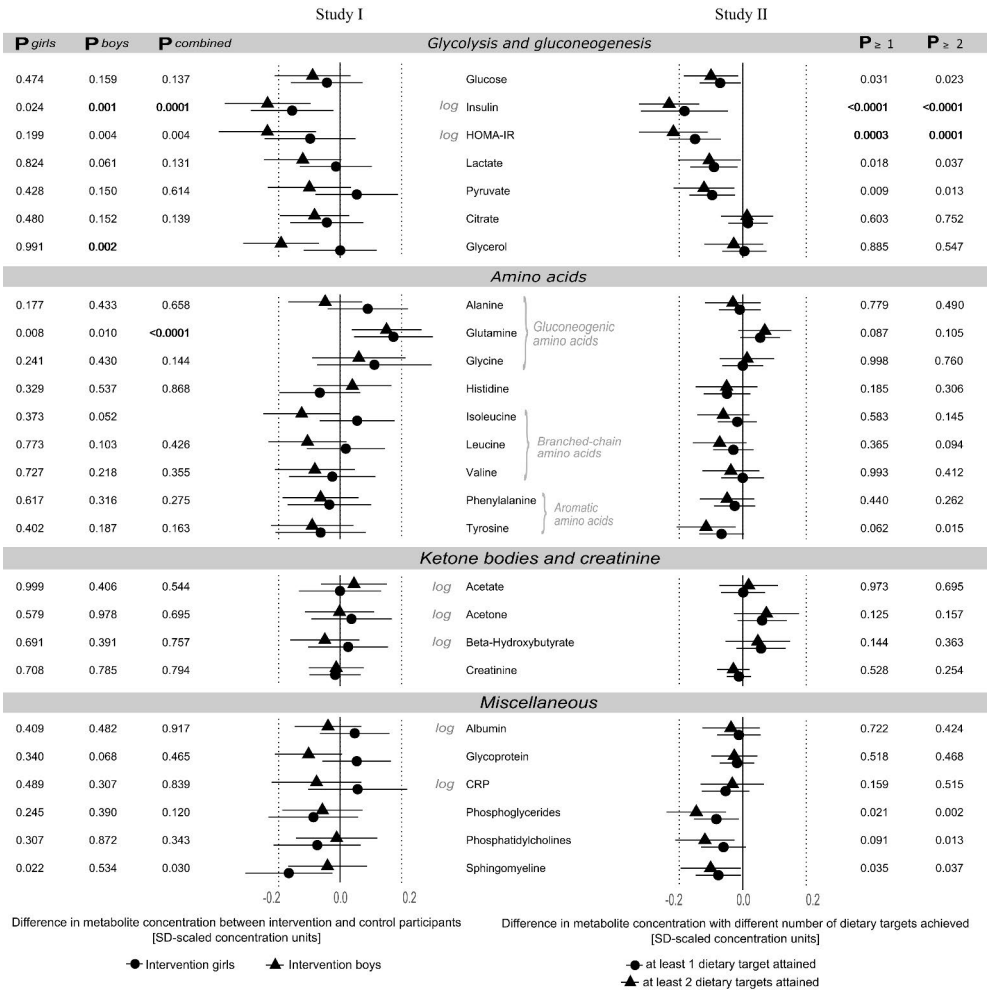

Figure 10. Effect of dietary intervention on (Study I) and association of dietary targets with (Study II) serum metabolic measures. Error bars indicate 95% confidence intervals. Statistically significant values are bolded.

with the control group as assessed by serum concentration of insulin and homeostasis model of insulin resistance (HOMA-IR). (Oranta et al., 2013) The current results confirm these previous findings as intervention led to reduced insulin and HOMA-IR measures.

There was an inverse association observed between the main dietary targets achieved and serum concentrations of glucose, lactate, and pyruvate (Figure 10, Study II). No associations were found between the dietary targets and serum amino acid concentrations except for an inverse association observed for serum

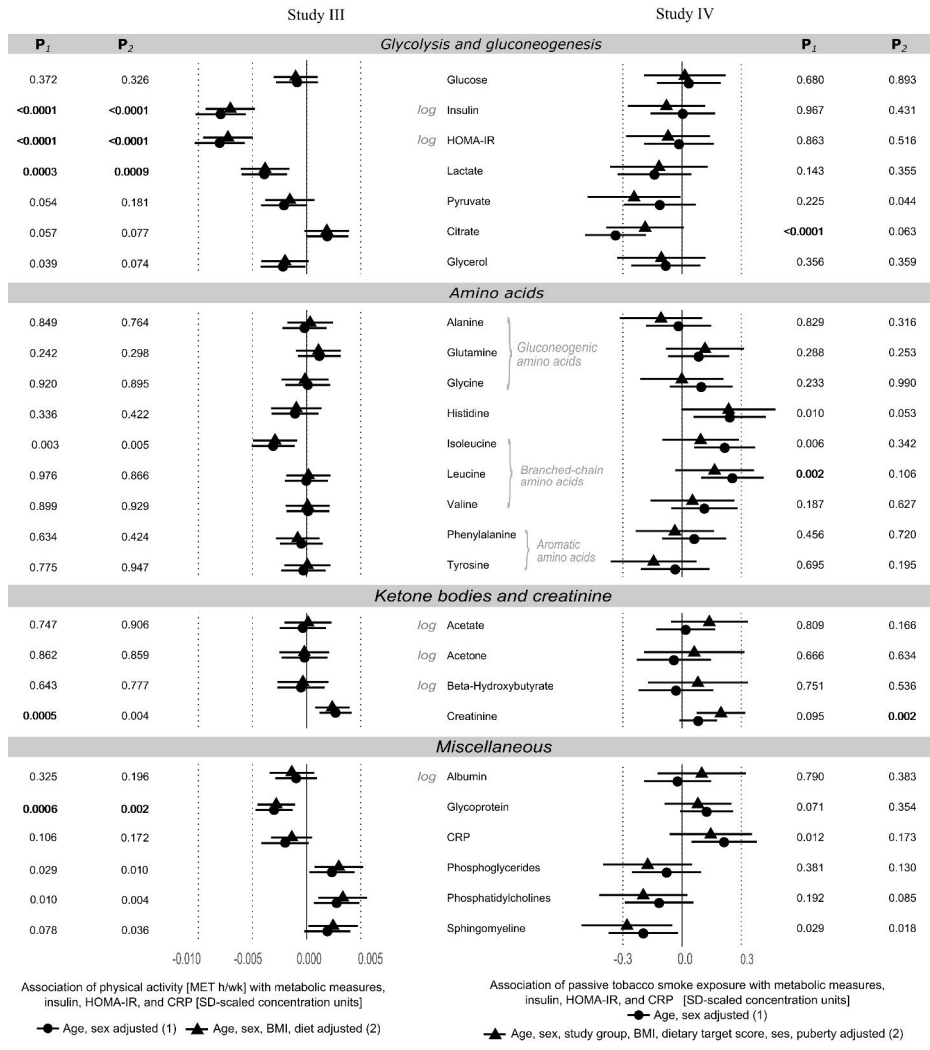

Figure 11. Association of physical activity (Study III) and passive tobacco smoke exposure (Study IV) on metabolic measures. Error bars indicate 95% confidence intervals. Statistically significant values are bolded.

concentration of tyrosine. There was an inverse association found as well between dietary targets and the serum concentrations of phosphoglycerides, phosphatidylcholines, and sphingomyelins. A significant interaction (p = 0.02) between dietary target score and sex was observed for glycoprotein acetylation with females showing an inverse association with dietary targets whereas no association was found for males. All these associations were modest. Serum concentration of insulin and HOMA-IR were inversely associated with the dietary targets, confirming the previous findings from the STRIP study. (Laitinen et al., 2018)

Similarly to previous results, LTPA was inversely associated with insulin and HOMA-IR measures whereas glucose showed only a similar tendency (Figure 11, Study III). LTPA was also inversely associated with the serum concentrations of lactate, isoleucine, and glycoprotein acetylation, while a direct association was found with the concentration of creatinine. There was a weak inverse association of LTPA with serum concentration of glycerol, and a direct association with serum concentrations of citrate, and phospholipid-related metabolic measures. The results mostly remained after adjusting for BMI and macronutrients.

No association was observed between passive tobacco smoke exposure and serum concentration of glucose or insulin (Figure 11, Study IV). An inverse association was observed for serum concentration of citrate, and there was a direct association with the concentration of histidine, isoleucine, and leucine. Weaker direct associations were observed between passive tobacco smoke exposure and serum concentration of creatinine, glycoprotein acetylation, and CRP. These associations were generally diluted after adjustment for potential confounders, except a stronger direct association seen between passive tobacco smoke exposure and serum concentration of creatinine after the adjustments.

5.3.4 Associations of active smoking with metabolic profile

5.3.4.1 Daily smoking during adolescence

The associations of daily tobacco smoking with serum metabolic profile are shown in Figure 12. Adolescents who smoked tobacco daily showed inverse associations with the serum ratios of omega-3 and omega-6 fatty acids, LA, and PUFAs to total fatty acids with a similar pattern observed for the number of double bonds per fatty acids and the serum concentration of total omega-3 fatty acids. Like passive tobacco smoke exposure, the strongest direct association was observed between daily smoking and the ratio of MUFAs to total fatty acids (β = 0.57SD [95% confidence interval (CI): 0.37-0.77], p < 0.0001). There was also a direct association between daily smoking and serum concentration of MUFAs, but it was diluted after adjusting

for possible confounding factors of STRIP study group, BMI, dietary target score, pubertal status, and socioeconomic status. No associations were observed between daily smoking and SFAs, or total fatty acid concentration, with the associations further attenuated after adjusting for potential confounders.

Daily smoking generally showed similar differences in the lipoprotein measures with passive tobacco smoke exposure but with stronger direct associations observed for triglycerides, VLDL particle size, and all but the very small VLDL subclass. Adjusting for potential confounders markedly diluted these associations. Unlike exposure to passive tobacco smoke, daily smoking was weakly associated with serum concentrations of glucose, insulin, and HOMA-IR, and like passive tobacco smoke exposure, there was an inverse association between daily smoking and serum concentration of citrate. Direct associations were observed between daily smoking and serum concentrations of isoleucine, and CRP. Again, adjusting for confounders diluted most of these associations.

5.3.4.2 Parental smoking during childhood

Metabolic measures where an association with passive tobacco smoke exposure was observed ($p < 0.05$) were further analysed with respect to parents' smoking status and child's cotinine concentration and the results are shown in Figure 13. The associations were mostly similar to the associations seen between passive tobacco smoke exposure and the serum metabolic profile. Thus, a parent who smoked resulted in the child having a similar serum metabolic profile than children who were strongly exposed to passive tobacco smoke, regardless of the child's cotinine concentration.

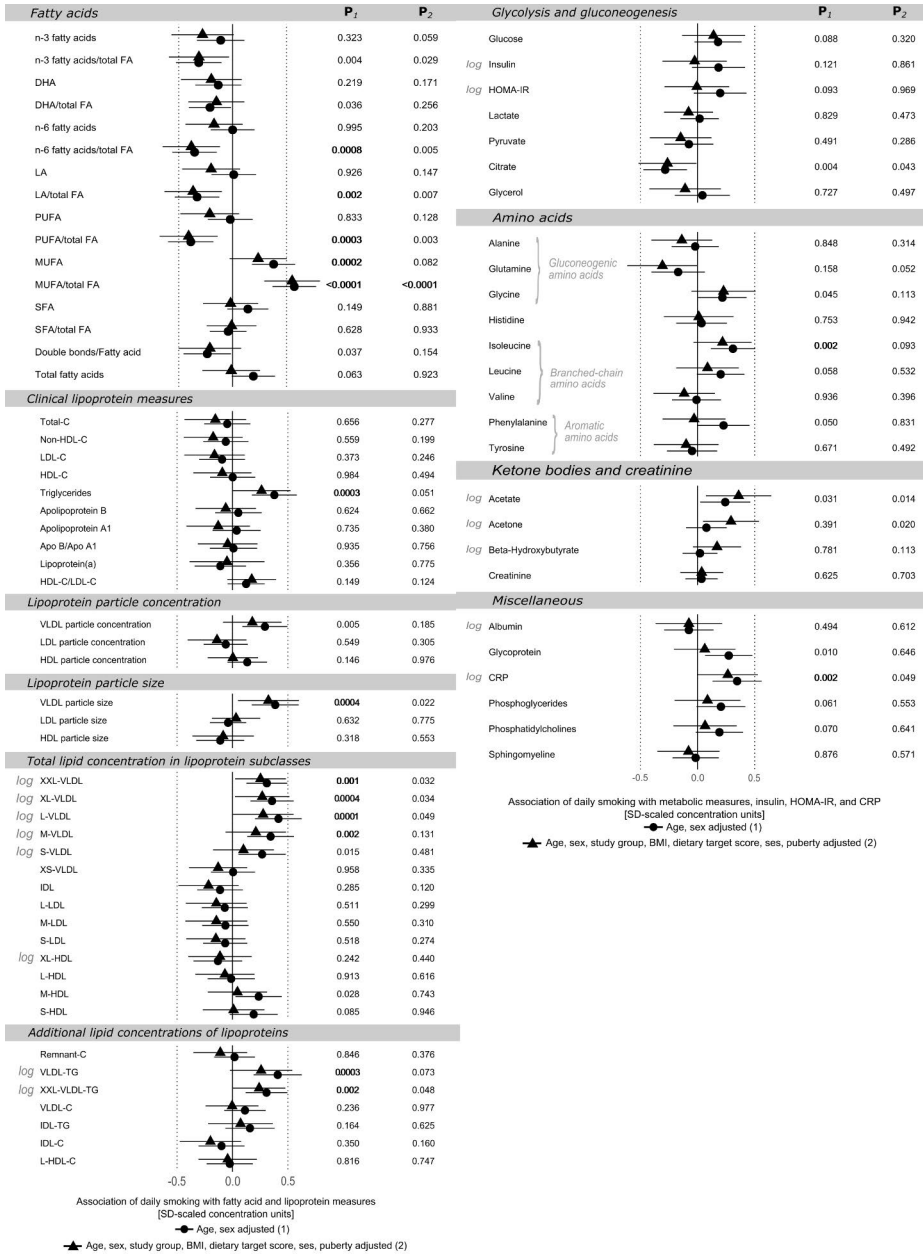

Figure 12. Association of daily smoking with serum metabolic profile during adolescence. Error bars indicate 95% confidence intervals. Effect estimates are SD-scaled differences in metabolic measures between daily smokers and non-smokers. Metabolic measures are from pooled analyses across three time points. Statistically significant values are bolded.

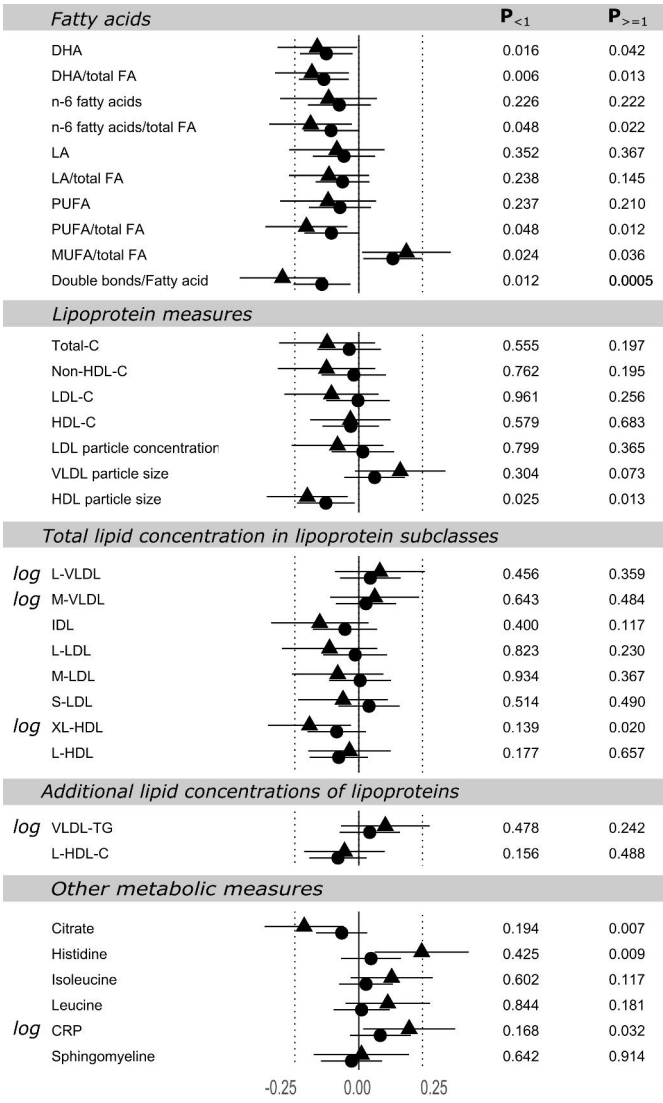

Figure 13. Differences in serum metabolic measures between study participants reporting at least one tobacco smoking parent (serum cotinine < 1 ng/mL denoted by black dot, ≥ 1 ng/mL denoted by black triangle) and participants without parental smoking and serum cotinine < 1 ng/mL. Effect estimates are SD-scaled differences between those with parental smoking and those without parental smoking. Statistical models were adjusted with age and sex. Error bars indicate 95% confidence intervals. Metabolic measures are from pooled analyses across the 6 time points. Statistically significant values are bolded.

5.3.5 Additional analyses

When a cholesterol intake target (≤ 20th age-specific percentile) was added to the dietary target score, the associations observed between achieving ≥ 1 or ≥ 2 of the dietary targets remained essentially unchanged. These results can be found in the original publication II. For example, the strong inverse association between dietary targets achieved and serum ratio of SFAs to total fatty acids and a direct association seen for the number of double bonds per fatty acid remained after including the cholesterol target.

Sex-stratified results between LTPA and the metabolic measures mostly followed a similar pattern as seen in the sex-combined results. The PUFA-related measures were directly associated with males whereas females showed only a similar tendency in metabolic measures evaluating their ratio to total fatty acids. LTPA was inversely associated with serum concentrations of MUFAs in women, while both sexes showed a strong inverse association with the ratio of MUFAs to total fatty acids. These results remained after adjusting for possible confounding factors, and the results are presented in the original publication III.

Additional cotinine concentration cutoffs of 3 ng/mL and 15 ng/mL used to indicate passive tobacco smoke exposure presented a similar metabolic profile as in the analyses for cotinine concentrations of ≥ 10 ng/mL. The observed associations were generally weaker for concentrations ≥ 3 ng/mL, and stronger for concentrations ≥ 15 ng/mL, an indication of a possible dose-response relationship as well. These results can be found in the original publication IV.

6 Discussion

The present study shows that repeated dietary counselling intervention from childhood to early adulthood resulted in greater dietary intake of PUFAs and lower dietary intake of SFAs which were reflected in the serum ratios of these fatty acids to total fatty acid concentration. These results extend the previous findings from the STRIP study, demonstrating the lowering of serum LDL-C in the intervention group, to VLDL and IDL cholesterol and further corroborates it by lipoprotein subclass profiling. (Niinikoski et al., 2012) This study also shows that adherence to the dietary targets of the intervention, regardless of study group allocation, was beneficially associated with especially serum fatty acid measures and that achieving a higher number of the dietary targets generally resulted in more pronounced associations. Physical activity in adolescence was also beneficially associated with several metabolic measures. For instance, there was a direct association between physical activity and most serum PUFA-related measures and HDL-C while an inverse association was seen for serum ratio of MUFAs to total fatty acids. On the other hand, exposure to passive tobacco smoke was directly associated with the ratio of MUFAs to total fatty acids and inversely associated with serum PUFA-related measures. The results on physical activity and passive tobacco smoke exposure largely persisted after adjustments for potential confounders. Active smoking during adolescence was associated with similar metabolic profile as passive tobacco smoke exposure from childhood to early adulthood.

6.1 Study design and participants

Currently, the STRIP study has continued for over 30 years. It presents a unique collection of data on dietary, anthropometric, lifestyle, biochemical, and metabolomic measures obtained repeatedly from infancy to adulthood in a relatively large number of participants. An evident strength of the study is its intervention design, as no other study in the world has introduced dietary counselling in infancy and continued it for 20 years.

Limitations of the study include a potential selection bias during the initial recruitment phase as families who participated in the study might have been more interested in health-related matters. This might have been reflected even in the lower

prevalence of active smoking in the study cohort than in the general adolescent population. Additionally, the intervention effect might have been diluted as the control children and their families received regularly information on, for example, their serum cholesterol concentrations.

With an exceptionally long intervention phase spanning over two decades there is unevitably loss to follow-up. Most common reasons for not remaining in the study were unsufficient time, birth of a younger sibling, child's recurring infections, moving away from the Turku area, and reluctance to blood sampling. The baseline characteristics of those continuing in the study and those lost to follow-up have been repeatedly compared and no systematic differences have been found in key characteristics such as weight, total cholesterol, blood pressure, SFA intake, or physical activity. (Pahkala et al., 2020; Simell et al., 2009) Additionally, detailed loss-to-follow up analyses regarding the components of the metabolic syndrome and the STRIP study group have shown that the proportion of premature discontinuance has been higher in the intervention group than in the control group, but none of the components of metabolic syndrome associated with loss-to-follow up, nor were there any STRIP study group-by-metabolic syndrome component interactions, indicating that the greater loss to follow-up in the intervention group was not modified by the components of the metabolic syndrome. (Nupponen et al., 2015) There are also limited data on hormonal levels and body composition not allowing for a detailed analyses into the observed sex differences. One potential limitation as well is the transferability of the results. As a single country study with all participants being White, the findings might not be transferrable to other areas of the world.

6.2 Methodological considerations

The key methodological considerations in this study are related to the quantification of the metabolites and assessment of the lifestyle factors. The metabolic platform used in this study is well established and highly automated with respect to the experimentation as well analyses of the spectral data, and all metabolic measures are obtained from a single serum or plasma sample. (Soininen et al., 2015) The absolute quantification of the metabolites enables direct comparison of data from different studies and platforms. However, with respect to the entire metabolome, only a small amount of it is captured by NMR. Still, the platform used in this study has been applied extensively in large epidemiological cohorts and biobanks in order to attain biomarker candidates by alleviating the confounding effects inherent in small studies. (Julkunen et al., 2023; Nagana Gowda & Raftery, 2023; Tikkanen et al., 2021; Vojinovic et al., 2020) Thus, as a strength of this study was a detailed and substantiated metabolic profiling method applied on a large number of healthy children repeated up to six times.

Lifestyle factors were assessed by subjective methods and this may introduce limitations. Data on the dietary intakes were collected by food records which are considered a golden standard when assessing dietary intake outside of laboratory conditions. (Willett, 1998) Food records have been applied by other studies examining the diet of children and adolescents as well. (Eloranta et al., 2021; Handakas et al., 2022) However, food records have limitations related to possible misrepresentation of dietary intakes, such as they can be inadequately filled or they might influence the foods consumed on the days the record is kept. Still, some evidence exists that the possible misrepresentation is not systematically associated to macronutrients or for instance, unhealthy foods. (Hirvonen et al., 1997; Lillegaard & Andersen, 2005) Additionally, the programs used in analysing the intake of nutrients and food consumption may lack accurate food and nutrient data affecting the results. In the STRIP study, the families and children were repeatedly instructed in keeping food records with written instructions and drawings and by a special food picture booklet provided to help the families with the recording. This diligence in completing the records accompanied with a continously updated database was used to minimize these errors. Additionally, day-care and school personnel were asked to collaborate by giving information about nutrient composition on catering menus and services. As the children grew they were repeatedly taught and motivated to keep the food records themselves. The food records were reviewed by a nutritionist for completeness and accuracy at each study visit, and the records were analysed by the same experienced dietary technician throughout the study. The database of the Micro Nutrica® program was continously updated. In this study, also the use of age-point specific cut-points for fibre, sucrose and cholesterol intake limits the ability of an individual to achieve a high score. Further, the data collection period in this study completed in 2011 and thus, the dietary data in this study may not fully reflect the diet of contemporary Finnish children and adolescents.

The use of self-reported physical activity is another limitation in this study, which might have hampered the estimation of the total amount of leisure-time physical activity, for instance as the reported typical intensity or duration of one physical activity bout gives a somewhat rough estimate of the behaviour. Furthermore, physical activity during leisure-time in contrast to all waking hours was assessed. Other studies have applied e.g., accelometer data or a combined movement and heart rate sensors to try to overcome these limitations. (Bell et al., 2018; Eloranta et al., 2021) Similarly the use of self-reported data on smoking status can be subject to recall or reporting bias.

The main limitation of using serum cotinine as a measure of tobacco smoke exposure is that it reflects the exposure during the past few days and it is not representative of a longer term exposure. However, there are no objective methods to quantify long-term exposure to tobacco smoke. Still, the longitudinal design of

this study to some extent reflects the participant's long term exposure as it has been suggested in a World Health Organization Tobacco Free Initiative Background paper that frequent measurement of cotinine can provide insight into the longitudinal tobacco smoke exposure of children. (Jarvis et al., 2001)

Overall, the methods used to assess the anthropometric measures of weight and height as well as evaluation of pubertal status and the biochemical measures were well standardized and generalizable between the study follow-ups.

6.3 Results

6.3.1 Fatty acid profile

The repeated dietary counselling given in the STRIP study resulted in increased dietary intake of PUFAs and decreased intake of SFAs which were reflected in the serum fatty acid profile as increased ratios of both total omega-3 and omega-6 PUFAs to total fatty acids and decreased SFA measures. Contrasting the dietary intake, the ratio of MUFAs to total fatty acids was decreased in the intervention males. Though this study cannot separate whether this is due to increased dietary intake of PUFAs or decreased intake of SFAs, it can be speculated that the lower serum ratio of MUFAs to total fatty acids is due to reduced SFA intake as desaturation of SFAs is a primary source of serum MUFAs. (Mahendran et al., 2013) Generally, dietary intakes of SFAs and MUFAs correlate in the Western diet. (Sundström et al., 2001) Additionally, adherence to the dietary targets of the intervention and concomitantly to the dietary recommendations regarding fat and carbohydrate quality, regardless of study group allocation, resulted in similar associations in fatty acid measures as the intervention. Direct associations were observed especially for the ratios of total omega-3 fatty acids and PUFAs to total fatty acids, and an inverse association with SFAs. Achieving a higher number of the dietary targets generally resulted in stronger associations. The results from these two studies are in contrast with observations from the cross-sectional and prospective ALSPAC study examining the associations between children's consumption of ultra-processed foods with metabolic profile which showed inverse associations with the ratio of total omega-3 fatty acids and a direct association for the ratio of MUFAs to total fatty acids. (Handakas et al., 2022) Further, the fatty acid profiles observed here comply with the key findings from a study assessing how adherence to the Alternative Healthy Eating Index was associated with metabolic profile reflecting not only healthy diet but also reduced future cardiovascular disease risk. (Akbaraly et al., 2018)

Physical activity during adolescence was also beneficially associated especially with serum omega-6 PUFAs while showing a strong inverse association for the ratio

of MUFAs to total fatty acids. No association was seen between physical activity and serum SFAs. Long-term physical activity has been associated with a less saturated fatty acid profile in adults and generally studies have linked physical activity or exercise with a less saturated fatty acid profile. (Kelly et al., 2020; Kujala et al., 2013) On the other hand, being passively exposed to tobacco smoke presented a nearly opposite fatty acid profile compared to being physically active with direct associations seen between passive tobacco smoke exposure and the ratio of MUFAs to total fatty acids and inverse associations observed for most PUFA-related measures. Previous studies on actively smoking adults have observed similar associations, especially an increase in the ratio of MUFAs to total fatty acids, though they have mostly been cross-sectional and with small sample sizes. (Muller et al., 2014; Titz et al., 2016) The mechanisms behind associations between physical activity and the fatty acid profile observed here are likely to be multiple and related to oxidation and desaturation of fatty acids while a possible mechanism for the observed direct association between passive tobacco smoke exposure and the ratio of MUFAs to total fatty acids is due to altered endogenous fatty acid desaturation. (Hodson & Fielding, 2013; Kujala et al., 2013) Interestingly, it has been recently proposed that the measures of fatty acid ratios to total fatty acids might be markers of systemic inflammation rather than composition of diet. (Julkunen et al., 2023) The results observed here for physical activity and passive tobacco smoke exposure remained after adjusting for possible confounders, including dietary measures.

6.3.2 Lipoprotein profile

The STRIP study has previously shown that the dietary intervention led to reduced serum total cholesterol, LDL-C, triglyceride, and ApoB concentrations. (Niinikoski et al., 2012) The current results substantiate the benefits of the intervention on the more detailed lipoprotein measures with reduced lipid concentrations in LDL and IDL particles for both sexes and in VLDL subclasses in the intervention males, especially in small and medium-sized VLDL. Consistently, the cholesterol carried in the VLDL and IDL particles, i.e., remnant cholesterol, was lower in the intervention males. Contrary to the intervention where only intervention males showed effect on VLDL measures, achieving the dietary targets of the intervention was inversely associated with VLDL particle concentration with a weaker inverse association observed for VLDL particle size. This was reflected in the total lipid concentrations of VLDL subclasses. Generally, the clinical lipoprotein measures observed in these studies are in line with reduced cardiovascular disease risk, while the role of lipoprotein particle size and subclasses is unclear. (Zhao et al., 2021) Still, similar inverse associations for VLDL particle size, and the lipid concentrations in VLDL, IDL, and LDL subclasses were found among those who adhered to the

Alternative Healthy Eating Index and these, except for IDL, showed direct association with future cardiovascular disease risk as well. (Akbaraly et al., 2018) Additionally, and contrary to adherence to the dietary targets of the STRIP intervention, the FinnTwin12 study found that a "junk food" pattern (with high intakes of e.g., French fries, hamburgers, pizza, and salty snacks) was directly associated with concentrations of triglycerides and small HDL particles while VLDL subclass distribution was shifted towards larger particles and LDLs toward smaller particles. (Bogl et al., 2013) In the FinnTwin12 study habitual fish intake inversely associated with VLDL particle size and directly with HDL particle size, and similar deviations were found for the intervention males and those achieving the dietary targets of the STRIP intervention.

Physical activity was directly associated, for example, with HDL particle concentration and cholesterol whereas an inverse association was seen for concentration of VLDL particles. These associations are linked with reduced cardiovascular disease risk in adults. (Holmes et al., 2018; MacKey et al., 2012)

Similar to this study, reduced concentrations of VLDL particles and increased concentrations of very large and large HDL particles have been reported previously in studies examining the associations between long-term physical activity in adults and metabolic profile, along with a direct association with HDL-cholesterol. (Fukai et al., 2016; Kujala et al., 2013) In children, a cross-sectional study using accelerometer measured physical activity data showed inverse associations with most ApoB-containing lipoprotein subclasses and triglyceride measures, and direct associations with HDL-C concentration, particle size, and larger HDL subclasses. (Jones et al., 2019) The ALSPAC study used repeated measures of accelerometer data and metabolic profile assessed at the age of 15 years. (Bell et al., 2018) It found higher total activity to associate, for example, directly with HDL-C and inversely with VLDL particles and triglycerides. Our results on adolescents with repeated measures of both physical activity and metabolic data mostly agree with these findings except for no association for IDL or LDL subclasses (ApoB-containing lipoprotein subclasses). Overall, the results seen in this study are in line with modifications generally seen in lipoprotein metabolism due to exercise induced increase of lipoprotein lipase concentration and activity leading to reduced concentration of triglycerides and increased concentration of HDL-C. (Franczyk et al., 2023; Sulague et al., 2022)

Contrary to physical activity, exposure to passive tobacco smoke was directly associated with VLDL particle size with a weak direct association seen for serum triglyceride concentration as well, and inversely associated with HDL particle size, pointing to an unfavourable cardiometabolic risk profile. (Arsenault et al., 2009; Mora et al., 2009) On clinical lipoprotein measures, adult smokers have shown direct associations with triglycerides and inverse association with HDL-C, possibly

through increased lipolysis in adipose tissue due to nicotine leading to release of free fatty acids and resulting in increased hepatic synthesis of triglycerides and VLDL. (Freeman et al., 1998; Jain & Ducatman, 2018; Z. Wang et al., 2017) Similar associations, albeit weak, were seen in this study with passive tobacco smoke exposure during childhood and these seemed to be reflected in other VLDL and HDL measures as well. For children and adolescents studies are generally limited and the results conflicting. Considering passive tobacco smoke exposure, one study found direct associations with serum total and LDL-C concentrations and an inverse association with serum HDL-C concentration while another found a direct association with serum triglyceride concentration but not with LDL, HDL, or total cholesterol concentrations. (Ebrahimi et al., 2019; Merianos et al., 2018) While the results presented here align with some of these findings on children, it is noteworthy that these previous studies were cross-sectional and used different definitions for passive tobacco smoke exposure.

6.3.3 Other metabolic measures

The intervention effects on other metabolic measures, such as amino acids, were mostly weak, except for increased concentrations of glutamine observed in the intervention group. Glutamine, the most abundant and versatile amino acid in the body, has been directly associated with reduced risk for diabetes-related complications and cardiovascular disease events. (Cruzat et al., 2018; Julkunen et al., 2023; Ritchie et al., 2015) For intervention males, tendency for lower concentrations in gluconeogenesis-related metabolites and some branched-chain amino acids was observed. Generally, the effects of the dietary intervention were stronger in males than in females on some metabolic measures (especially triglycerides and VLDL subclasses), though no differences were found in the dietary intake of fatty acids between the sexes. Possible reasons for this discrepancy are differences in body composition, males having a more pronounced intervention effect on consumption of favoured foods, and sex hormone concentrations.

Achieving the dietary targets of the intervention has also been previously shown to inversely associate with fasting serum glucose and insulin concentrations, and HOMA-IR. (Laitinen et al., 2018) This study is in line with previous results while also showing weak inverse associations between achieving the dietary targets and concentrations of other glycolysis-related measures, tyrosine, and phospholipid-measures. Tyrosine has been directly associated with risk for cardiovascular events and subclinical atherosclerosis. (Julkunen et al., 2023; Würtz et al., 2012) Similarly to the intervention effect on males, achieving the dietary targets showed a tendency towards inverse association for isoleucine and leucine whereas inverse association with glycoprotein acetylation was observed only in the intervention males.

Branched-chain amino acids and glycoprotein acetylation have been directly associated with risk for type 2 diabetes as well as cardiovascular disease risk (Jiang et al., 2020; Julkunen et al., 2023; Tibuakuu et al., 2019) Similarly to these associations observed here with adherence to the dietary targets, attaining to the Alternative Healthy Eating Index was inversely associated with amino acids such as isoleucine and leucine which also showed direct association with future cardiovascular disease risk. (Akbaraly et al., 2018)

Physical activity was inversely associated with most glycolysis-related measures as well as with isoleucine, and glycoprotein acetylation indicating reduced risk for type 2 diabetes and cardiovascular disease. (Tibuakuu et al., 2019; Wurtz et al., 2013) A direct association was seen between physical activity and the concentrations of creatinine and phospholipid-measures. The latter association might reflect the fact that phospholipids are the main constituents of HDL particles. Physical activity has been positively associated with kidney function and in young adults more aerobic or strength exercise has been associated with higher concentrations of creatinine. (Fragala et al., 2017; Hawkins et al., 2011) Similarly to these results, previous studies on long-term physical activity have shown inverse associations with isoleucine, glucose, and glycoprotein acetylation. (Fukai et al., 2016; Kujala et al., 2013) Passive tobacco smoke exposure showed mostly weak associations with amino acids and glycolysis and gluconeogenesis-related metabolites, especially after adjusting for possibly confounding factors. An association between passive tobacco smoke exposure and creatinine was seen after adjusting for confounding factors. Active tobacco smoke exposure has been directly associated with a risk for chronic kidney disease with various possible mechanisms indicated. (Xia et al., 2017)

6.3.4 Active smoking

Daily smoking during adolescence was associated with mostly similar deviations in the metabolic profile when compared with passive tobacco smoke exposure. The most prominent direct associations were seen, for example, for the ratio of MUFAs to total fatty acids, triglycerides, VLDL particle size as well as isoleucine and CRP, while inverse associations were seen especially for the ratio of omega-6 fatty acids to total fatty acids and adjusting for confounding factors diluted most of these associations except for fatty acid measures. These associations observed for fatty acids were in line with greater cardiovascular disease risk. (Wurtz et al., 2015) Generally, the observed associations for daily smokers were stronger than those observed for passive tobacco smoke exposure even though the daily smoking cohort consisted of only three time points.

Children exposed to parental active smoking were associated with similar deviations in the metabolic profile when compared to children who were passively

exposed. These associations were generally stronger when in addition to a smoking parent the child's serum cotinine concentration was elevated. The fatty acid profile of children exposed to parental smoking was towards greater cardiovascular disease risk. Most profound inverse associations were seen for the number of double bonds per fatty acid and concentration of citrate whereas direct associations were seen for histidine and CRP. Of these, exposure to parental smoking has been associated with CRP previously. (D. Wang et al., 2017) Additionally, exposure to parental smoking has been associated with increased risk of atherosclerotic plaque in adulthood, with poor smoking hygiene (child's cotinine concentration elevated) showing even greater risk. (West et al., 2015)

6.3.5 Clinical and future research considerations

As cardiovascular diseases are rooted in childhood, and there is evidence that having optimal risk factor levels at 50 years of age is associated with very low risk for future cardiovascular disease and markedly longer survival, tackling the lifestyle risk factors early in life is warranted. (Lloyd-Jones et al., 2006) For children, lifestyle counselling is the foundation for promoting cardiovascular health at the population level. (Raitakari et al., 2022) The results of this thesis reveal the notable metabolic effects of an infancy-onset dietary counselling intervention continued until early adulthood and imply that attaining even one dietary target is favourable with regards to cardiometabolic disease risk. Specifically, these results give evidence to the current recommendations advocating unsaturated fats, consumption of fibre-rich foods, and low intake of sucrose and SFA. In addition to dietary aspects, these results show that physical activity during adolescence seems to be beneficially linked with metabolic profile with regards to cardiometabolic disease risk even when diet and body mass index are taken into account. Thus, these results support promoting physically active lifestyle during adolescence.

The results also suggest that passive tobacco smoke exposure during childhood is associated with a risk-prone metabolic profile indicative of increased cardiometabolic risk and that the profile is similar with daily smoking during adolescence. Thus, efforts to reduce tobacco smoke exposure during childhood are justified. To even lower all-cause mortality, attempts to promote healthy lifestyle through diet and physical activity and to reduce tobacco smoke exposure should be made at several levels of society and they should include different approaches. (Gallienne et al., 2018) In Finland only around 36% of 7- to 15-year-old children reached their daily activity goal of one hour of moderate or moderate-to-vigorous intensity of physical activity. (Kokko & Martin, 2023) According to Finnish Institute for Health and Welfare, the functional ability of Finnish adults has been declining in recent years due to, for example, cardiovascular diseases, poor diet, physical

inactivity, smoking, and mental health problems. (Kestilä et al., 2023) Though the prevalence of cigarette smoking among Finnish adolescents has been on decline, a recent study examining the use of e-cigarettes reported between 8% to 14% of 15 – 16-year-olds having used such products within the last month. (Scheffels et al., 2023) The cardiovascular consequences of e-cigarettes are not fully understood, but data are beginning to accumulate showing cardiac function and cardiometabolic issues. (Mears et al., 2023) Thus, strategies to promote healthy lifestyle are needed and improving cardiometabolic wellbeing on population level is current.

Various operators should be involved. Well-baby clinics are central in identifying families benefiting from interventions, such as counselling, regarding e.g., dietary matters or smoking. Day-cares and schools are essential in providing and educating on healthy dietary choices and promoting physical activity. Health education given in schools should inform about the adverse effects of smoking and passive smoking. School nurses are central in recognising children with adverse risk factors during regular check-ups and the local health care system needs to provide low-level and family-based services to introduce better lifestyle choices. Third sector operators could also provide, for example, inclusive low-cost physical activities locally. Additionally, legislative measures, such as taxation, can be used to promote consumption of healthy foods while supporting smoking reduction and promoting smoke-free environments.

The participants in the STRIP study are currently young adults, and during future follow-ups information on cardiovascular disease events will provide data that can be used to better understand the risks associated with e.g. their metabolic profiles.

7 Conclusions

In summary, the present study shows the benefits of repeated dietary counselling intervention or attaining to the dietary targets of the intervention from childhood to adulthood as well as being physically active during adolescence, while noting the drawbacks of tobacco smoke exposure. Specifically,

1. Repeated dietary counselling intervention from infancy to early adulhood resulted in reduced dietary intake of saturated fat and increased intake of polyunsaturated fat yielding favourable effects on multiple fatty acid measures and lipoprotein subclass lipids, particularly in males.

2. Achieving the dietary targets of the intervention, reflecting nutrition recommendations, is favourably associated with the metabolic profile from childhood to early adulthood. Achieving more dietary targets enhanced the metabolic profile even further while supporting the notion that achieving even one of these dietary targets results in a more favourable profile with regards to cardiometabolic disease risk.

3. Physical activity during adolescence is beneficially associated with metabolic profile, and these associations seem to be independent of diet and body mass index. These findings support efforts to promote physically active lifestyle among adolescents to improve current cardiometabolic health.

4. Passive tobacco smoke exposure during chilhdood is associated with a metabolic profile indicative of increased cardiometabolic risk and this association profile is similar with active daily smoking during adolescence. These findings suggest that reducing both active and passive tobacco smoke exposure during childhood and adolescence could reduce future cardiometabolic disease risk.

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References

Abela, G. S., Aziz, K., Vedre, A., Pathak, D. R., Talbott, J. D., & DeJong, J. (2009). Effect of cholesterol crystals on plaques and intima in arteries of patients with acute coronary and cerebrovascular syndromes. *The American Journal of Cardiology*, *103*(7), 959–968.

Akbaraly, T., Würtz, P., Singh-Manoux, A., Shipley, M. J., Haapakoski, R., Lehto, M., et al. (2018). Association of circulating metabolites with healthy diet and risk of cardiovascular disease: analysis of two cohort studies. *Scientific Reports*, *8*(1), 8620.

American Diabetes Association. (2021). 8. Obesity Management for the Treatment of Type 2 Diabetes: Standards of Medical Care in Diabetes-2021. *Diabetes Care*, *44*(Suppl 1), s100–110.

Appel, L. J., Moore, T. J., Obarzanek, E., Vollmer, W. M., Svetkey, L. P., Sacks, F. M., et al. (1997). A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *The New England Journal of Medicine*, *336*(16), 1117–1124.

Arnett, D. K., Blumenthal, R. S., Albert, M. A., Buroker, A. B., Goldberger, Z. D., Hahn, E. J., et al. (2019). 2019 ACC/AHA Guideline on the Primary Prevention of Cardiovascular Disease: Executive Summary: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Journal of the American College of Cardiology*, *74*(10), 1376–1414.

Arsenault, B. J., Lemieux, I., Després, J. P., Gagnon, P., Wareham, N. J., Stroes, E. S. G., et al. (2009). HDL particle size and the risk of coronary heart disease in apparently healthy men and women: the EPIC-Norfolk prospective population study. *Atherosclerosis*, *206*(1), 276–281.

Bahls, M., Leitzmann, M. F., Karch, A., Teumer, A., Dörr, M., Felix, S. B., et al. (2021). Physical activity, sedentary behavior and risk of coronary artery disease, myocardial infarction and ischemic stroke: a two-sample Mendelian randomization study. *Clinical Research in Cardiology*, *110*(10), 1564–1573.

Balling, M., Afzal, S., Varbo, A., Langsted, A., Davey Smith, G., & Nordestgaard, B. G. (2020). VLDL Cholesterol Accounts for One-Half of the Risk of Myocardial Infarction Associated With apoB-Containing Lipoproteins. *Journal of the American College of Cardiology*, *76*(23), 2725–2735.

Barnoya, J., & Glantz, S. A. (2005). Cardiovascular effects of secondhand smoke: nearly as large as smoking. *Circulation*, *111*(20), 2684–2698.

Basu, D., & Bornfeldt, K. E. (2020). Hypertriglyceridemia and Atherosclerosis: Using Human Research to Guide Mechanistic Studies in Animal Models. *Frontiers in Endocrinology*, *11*, 504.

Belardo, D., Michos, E. D., Blankstein, R., Blumenthal, R. S., Ferdinand, K. C., Hall, K., et al. (2022). Practical, Evidence-Based Approaches to Nutritional Modifications to Reduce Atherosclerotic Cardiovascular Disease: An American Society For Preventive Cardiology Clinical Practice Statement. *American Journal of Preventive Cardiology*, *10*, 100323.

Bell, J. A., Hamer, M., Richmond, R. C., Timpson, N. J., Carslake, D., & Smith, G. D. (2018). Associations of device-measured physical activity across adolescence with metabolic traits: Prospective cohort study. *PLoS Medicine*, *15*(9), e1002649.

Benjamin, R. M. (2011). Dietary guidelines for Americans, 2010: the cornerstone of nutrition policy. *Public Health Reports*, *126*(3), 310–311.

Berenson, G. S., Srinivasan, S. R., Bao, W., Newman, W. P., Tracy, R. E., & Wattigney, W. A. (1998). Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. *The New England Journal of Medicine*, *338*(23), 1650–1656.

Björkegren, J. L. M., Hägg, S., Talukdar, H. A., Foroughi Asl, H., Jain, R. K., Cedergren, C., et al. (2014). Plasma cholesterol-induced lesion networks activated before regression of early, mature, and advanced atherosclerosis. *PLoS Genetics*, *10*(2), e1004201.

Blomhoff, R., Andersen, R., Arnesen, E. K., Christensen, J. J., Eneroth, H., Erkkola, M., et al. (2023). *Nordic Nutrition Recommendations 2023*. Copenhagen: Nordic Council of Ministers.

Bogl, L. H., Pietiläinen, K. H., Rissanen, A., Kangas, A. J., Soininen, P., Rose, R. J., et al. (2013). Association between habitual dietary intake and lipoprotein subclass profile in healthy young adults. *Nutrition, Metabolism, and Cardiovascular Diseases*, *23*(11), 1071–1078.

Borén, J., Matikainen, N., Adiels, M., & Taskinen, M. R. (2014). Postprandial hypertriglyceridemia as a coronary risk factor. *Clinica Chimica Acta*, *431*, 131–142.

Borén, J., & Williams, K. J. (2016). The central role of arterial retention of cholesterol-rich apolipoprotein-B-containing lipoproteins in the pathogenesis of atherosclerosis: a triumph of simplicity. *Current Opinion in Lipidology*, *27*(5), 473–483.

Brandts, J., & Ray, K. K. (2023). Novel and future lipid-modulating therapies for the prevention of cardiovascular disease. *Nature Reviews. Cardiology, 20*(9), 600-616.

Budoff, M. (2016). Triglycerides and Triglyceride-Rich Lipoproteins in the Causal Pathway of Cardiovascular Disease. *The American Journal of Cardiology*, *118*(1), 138–145.

Butte, N. F., Watson, K. B., Ridley, K., Zakeri, I. F., McMurray, R. G., Pfeiffer, K. A., et al. (2018). A Youth Compendium of Physical Activities: Activity Codes and Metabolic Intensities. *Medicine and Science in Sports and Exercise*, *50*(2), 246–256.

Carr, S. S., Hooper, A. J., Sullivan, D. R., & Burnett, J. R. (2019). Non-HDL-cholesterol and apolipoprotein B compared with LDL-cholesterol in atherosclerotic cardiovascular disease risk assessment. *Pathology*, *51*(2), 148–154.

Chalfant, C., & Del Poeta, M. (2010). *Sphingolipids as Signaling and Regulatory Molecules* (1st ed.). Springer: New York, NY.

Chen, W., Yun, M., Fernandez, C., Li, S., Sun, D., Lai, C. C., et al. (2015). Secondhand smoke exposure is associated with increased carotid artery intima-media thickness: the Bogalusa Heart Study. *Atherosclerosis*, *240*(2), 374–379.

Cheng, S., Shah, S. H., Corwin, E. J., Fiehn, O., Fitzgerald, R. L., Gerszten, R. E., et al. (2017). Potential Impact and Study Considerations of Metabolomics in Cardiovascular Health and Disease: A Scientific Statement From the American Heart Association. *Circulation. Cardiovascular Genetics*, *10*(2), e000032.

Cheng, W., Zhang, Z., Cheng, W., Yang, C., Diao, L., & Liu, W. (2018). Associations of leisure-time physical activity with cardiovascular mortality: A systematic review and meta-analysis of 44 prospective cohort studies. *European Journal of Preventive Cardiology*, *25*(17), 1864–1872.

Chiavaroli, L., Viguiliouk, E., Nishi, S. K., Mejia, S. B., Rahelić, D., Kahleová, H., et al. (2019). DASH Dietary Pattern and Cardiometabolic Outcomes: An Umbrella Review of Systematic Reviews and Meta-Analyses. *Nutrients*, *11*(2), 338.

Choi, S. H., & Stommel, M. (2017). Impact of Age at Smoking Initiation on Smoking-Related Morbidity and All-Cause Mortality. *American Journal of Preventive Medicine*, *53*(1), 33–41.

Choi, Y., Larson, N., Steffen, L. M., Schreiner, P. J., Gallaher, D. D., Duprez, D. A., et al. (2021). Plant-Centered Diet and Risk of Incident Cardiovascular Disease During Young to Middle Adulthood. *Journal of the American Heart Association*, *10*(16), e020718.

Cohen, J. C., Boerwinkle, E., Mosley, T. H., & Hobbs, H. H. (2006). Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *The New England Journal of Medicine*, *354*(12), 1264–1272.

Cruzat, V., Rogero, M. M., Keane, K. N., Curi, R., & Newsholme, P. (2018). Glutamine: Metabolism and Immune Function, Supplementation and Clinical Translation. *Nutrients*, *10*(11), 1564.

da Silva, G. C. R., Tebar, W. R., Saraiva, B. T. C., Farah, B. Q., Vanderlei, L. C. M., Ferrari, G., et al. (2023). Association of Early Sports Practice with Cardiovascular Risk Factors in Community-Dwelling Adults: A Retrospective Epidemiological Study. *Sports Medicine - Open*, *9*(1), 15.

Dai, H., Much, A. A., Maor, E., Asher, E., Younis, A., Xu, Y., et al. (2022). Global, regional, and national burden of ischaemic heart disease and its attributable risk factors, 1990-2017: results from the Global Burden of Disease Study 2017. *European Heart Journal. Quality of Care & Clinical Outcomes*, *8*(1), 50–60.

Dawber, T. R., & Kannel, W. B. (1966). The Framingham study. An epidemiological approach to coronary heart disease. *Circulation*, *34*(4), 553–555.

Deelen, J., Kettunen, J., Fischer, K., van der Spek, A., Trompet, S., Kastenmuller, G., et al. (2019). A metabolic profile of all-cause mortality risk identified in an observational study of 44,168 individuals. *Nature Communications*, *10*(1), 3346.

Di Angelantonio, E., Sarwar, N., Perry, P., Kaptoge, S., Ray, K. K., Thompson, A., et al. (2009). Major lipids, apolipoproteins, and risk of vascular disease. *JAMA*, *302*(18), 1993–2000.

Di Pietro, P., Izzo, C., Abate, A. C., Iesu, P., Rusciano, M. R., Venturini, E., et al. (2023). The Dark Side of Sphingolipids: Searching for Potential Cardiovascular Biomarkers. *Biomolecules, 13*(1), 168.

Diab, A., Dastmalchi, L. N., Gulati, M., & Michos, E. D. (2023). A Heart-Healthy Diet for Cardiovascular Disease Prevention: Where Are We Now? *Vascular Health and Risk Management*, *19*, 237–253.

Ding, M., Zeleznik, O. A., Guasch-Ferre, M., Hu, J., Lasky-Su, J., Lee, I. M., et al. (2019). Metabolome-Wide Association Study of the Relationship Between Habitual Physical Activity and Plasma Metabolite Levels. *American Journal of Epidemiology*, *188*(11), 1932–1943.

Domanski, M. J., Tian, X., Wu, C. O., Reis, J. P., Dey, A. K., Gu, Y., et al. (2020). Time Course of LDL Cholesterol Exposure and Cardiovascular Disease Event Risk. *Journal of the American College of Cardiology*, *76*(13), 1507–1516.

Duewell, P., Kono, H., Rayner, K. J., Sirois, C. M., Vladimer, G., Bauernfeind, F. G., et al. (2010). NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals. *Nature*, *464*(7293), 1357–1361.

Duprez, D. A., Otvos, J., Sanchez, O. A., Mackey, R. H., Tracy, R., & Jacobs, D. R. (2016). Comparison of the Predictive Value of GlycA and Other Biomarkers of Inflammation for Total Death, Incident Cardiovascular Events, Noncardiovascular and Noncancer Inflammatory-Related Events, and Total Cancer Events. *Clinical Chemistry*, *62*(7), 1020–1031.

Dyslipidemiat. Käypä hoito -suositus. (2022). *Suomalaisen Lääkäriseuran Duodecimin ja Suomen Sisätautilääkärien Yhdistys ry:n asettama työryhmä*. Helsinki: Suomalainen Lääkäriseura Duodecim. Saatavilla internetissä: www.käypähoito.fi.

Ebrahimi, M., Aghdam, M. H., Qorbani, M., Abbaspour Kaboodan, F., Shafiee, G., Khatami, F., et al. (2019). Passive smoking and cardiometabolic risk factors in Iranian children and adolescents: CASPIAN-V study. *Journal of Diabetes and Metabolic Disorders*, *18*(2), 401–408.

El Harchaoui, K., Arsenault, B. J., Franssen, R., Després, J. P., Hovingh, G. K., Stroes, E. S. G., et al. (2009). High-density lipoprotein particle size and concentration and coronary risk. *Annals of Internal Medicine*, *150*(2), 84–93.

Eloranta, A. M., Lindi, V., Schwab, U., Kiiskinen, S., Venäläinen, T., Lakka, H. M., et al. (2014). Dietary factors associated with metabolic risk score in Finnish children aged 6-8 years: the PANIC study. *European Journal of Nutrition*, *53*(6), 1431–1439.

Eloranta, A. M., Sallinen, T., Viitasalo, A., Lintu, N., Väistö, J., Jalkanen, H., et al. (2021). The effects of a 2-year physical activity and dietary intervention on plasma lipid concentrations in children: the PANIC Study. *European Journal of Nutrition*, *60*(1), 425–434.

Erkkilä, A. T., Schwab, U. S., Lehto, S., De Mello, V. D., Kangas, A. J., Soininen, P., et al. (2014). Effect of fatty and lean fish intake on lipoprotein subclasses in subjects with coronary heart disease: a controlled trial. *Journal of Clinical Lipidology*, *8*(1), 126–133.

Ference, B. A., Ginsberg, H. N., Graham, I., Ray, K. K., Packard, C. J., Bruckert, E., et al. (2017). Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *European Heart Journal*, *38*(32), 2459–2472.

Ference, B. A., Yoo, W., Alesh, I., Mahajan, N., Mirowska, K. K., Mewada, A., et al. (2012). Effect of long-term exposure to lower low-density lipoprotein cholesterol beginning early in life on the risk of coronary heart disease: a Mendelian randomization analysis. *Journal of the American College of Cardiology*, *60*(25), 90–98.

Fernandes, R. A., Christofaro, D. G. D., Casonatto, J., Codogno, J. S., Rodrigues, E. Q., Cardoso, M. L., et al. (2011). Prevalence of dyslipidemia in individuals physically active during childhood, adolescence and adult age. *Arquivos Brasileiros de Cardiologia*, *97*(4), 317–323.

Ferraro, R. A., Fischer, N. M., Xun, H., & Michos, E. D. (2020). Nutrition and physical activity recommendations from the United States and European cardiovascular guidelines: a comparative review. *Current Opinion in Cardiology*, *35*(5), 508–516.

Feyerabend, C., & Russell, M. A. (1990). A rapid gas-liquid chromatographic method for the determination of cotinine and nicotine in biological fluids. *The Journal of Pharmacy and Pharmacology*, *42*(6), 450–452.

Fragala, M. S., Bi, C., Chaump, M., Kaufman, H. W., & Kroll, M. H. (2017). Associations of aerobic and strength exercise with clinical laboratory test values. *PloS One*, *12*(10), e0180840.

Franczyk, B., Gluba-Brzózka, A., Ciałkowska-Rysz, A., Ławiński, J., & Rysz, J. (2023). The Impact of Aerobic Exercise on HDL Quantity and Quality: A Narrative Review. *International Journal of Molecular Sciences*, *24*(5), 4653.

Freeman, D. J., Caslake, M. J., Griffin, B. A., Hinnie, J., Tan, C. E., Watson, T. D. G., et al. (1998). The effect of smoking on post-heparin lipoprotein and hepatic lipase, cholesteryl ester transfer protein and lecithin:cholesterol acyl transferase activities in human plasma. *European Journal of Clinical Investigation*, *28*(7), 584–591.

Fukai, K., Harada, S., Iida, M., Kurihara, A., Takeuchi, A., Kuwabara, K., et al. (2016). Metabolic Profiling of Total Physical Activity and Sedentary Behavior in Community-Dwelling Men. *PloS One*, *11*(10), e0164877.

Funk, S. D., Yurdagul, A., & Orr, A. W. (2012). Hyperglycemia and endothelial dysfunction in atherosclerosis: lessons from type 1 diabetes. *International Journal of Vascular Medicine*, *2012*, 569654.

Gaggini, M., Gorini, F., & Vassalle, C. (2022). Lipids in Atherosclerosis: Pathophysiology and the Role of Calculated Lipid Indices in Assessing Cardiovascular Risk in Patients with Hyperlipidemia. *International Journal of Molecular Sciences*, *24*(1), 75.

Gall, S., Huynh, Q. L., Magnussen, C. G., Juonala, M., Viikari, J. S. A., Kähönen, M., et al. (2014). Exposure to parental smoking in childhood or adolescence is associated with increased carotid intima-media thickness in young adults: evidence from the Cardiovascular Risk in Young Finns study and the Childhood Determinants of Adult Health Study. *European Heart Journal*, *35*(36), 2484–2491.

Gallienne, C., Wang, L., Khan, T., & Sievenpiper, J. (2018). Low risk lifestyle behaviours and all cause mortality: a systematic review and meta-analysis of prospective cohort studies. *Canadian Journal of Cardiology*, *34*(10), S41.

Ganda, O. P. (2023). Triglyceride-rich lipoproteins, remnant-cholesterol, and atherosclerotic cardiovascular disease. *Current Opinion in Lipidology*, *34*(3), 105-113.

Ganna, A., Salihovic, S., Sundström, J., Broeckling, C. D., Hedman, Å. K., Magnusson, P. K. E., et al. (2014). Large-scale metabolomic profiling identifies novel biomarkers for incident coronary heart disease. *PLoS Genetics*, *10*(12), e1004801.

Georgiopoulos, G., Oikonomou, D., Pateras, K., Masi, S., Magkas, N., Delialis, D., et al. (2021). A Bayesian meta-analysis on early tobacco exposure and vascular health: From childhood to early adulthood. *European Journal of Preventive Cardiology*, *28*(12), 1315–1322.

German, C. A., Baum, S. J., Ferdinand, K. C., Gulati, M., Polonsky, T. S., Toth, P. P., et al. (2022). Defining preventive cardiology: A clinical practice statement from the American Society for Preventive Cardiology. *American Journal of Preventive Cardiology*, *12*, 100432.

Glantz, S. A., & Parmley, W. W. (1991). Passive smoking and heart disease. Epidemiology, physiology, and biochemistry. *Circulation*, *83*(1), 1–12.

Goldstein, J. L., & Brown, M. S. (2015). A century of cholesterol and coronaries: from plaques to genes to statins. *Cell*, *161*(1), 161–172.

Gu, F., Derkach, A., Freedman, N. D., Landi, M. T., Albanes, D., Weinstein, S. J., et al. (2016). Cigarette smoking behaviour and blood metabolomics. *International Journal of Epidemiology*, *45*(5), 1421–1432.

Guthold, R., Stevens, G. A., Riley, L. M., & Bull, F. C. (2020). Global trends in insufficient physical activity among adolescents: a pooled analysis of 298 population-based surveys with 1·6 million participants. *The Lancet. Child & Adolescent Health*, *4*(1), 23–35.

Haapala, E. A., Leppänen, M. H., Lehti, M., Lintu, N., Tompuri, T., Viitasalo, A., et al. (2022). Cross-sectional associations between cardiorespiratory fitness and NMR-derived metabolic biomarkers in children - the PANIC study. *Frontiers in Endocrinology*, *13*, 954418.

Hakala, P., Marniemi, J., Knuts, L.-R., Kumpulainen, J., Tahvonen, R., & Plaami, S. (1996). Calculated vs. analysed nutrient composition of weight reduction diets. *Food Chemistry*, *57(1)*, 71–75.

Handakas, E., Chang, K., Khandpur, N., Vamos, E. P., Millett, C., Sassi, F., et al. (2022). Metabolic profiles of ultra-processed food consumption and their role in obesity risk in British children. *Clinical Nutrition*, *41*(11), 2537–2548.

Hawkins, M. S., Sevick, M. A., Richardson, C. R., Fried, L. F., Arena, V. C., & Kriska, A. M. (2011). Association between physical activity and kidney function: National Health and Nutrition Examination Survey. *Medicine and Science in Sports and Exercise*, *43*(8), 1457–1464.

Hirvonen, T., Männistö, S., Roos, E., & Pietinen, P. (1997). Increasing prevalence of underreporting does not necessarily distort dietary surveys. *European Journal of Clinical Nutrition*, *51*(5), 297–301.

Hodson, L., & Fielding, B. A. (2013). Stearoyl-CoA desaturase: rogue or innocent bystander? *Progress in Lipid Research*, *52*(1), 15–42.

Holmes, M. V., & Ala-Korpela, M. (2019). What is “LDL cholesterol”? *Nature Reviews. Cardiology*, *16*(4), 197–198.

Holmes, M. V., Millwood, I. Y., Kartsonaki, C., Hill, M. R., Bennett, D. A., Boxall, R., et al. (2018). Lipids, Lipoproteins, and Metabolites and Risk of Myocardial Infarction and Stroke. *Journal of the American College of Cardiology*, *71*(6), 620–632.

Hooper, L., Martin, N., Jimoh, O. F., Kirk, C., Foster, E., & Abdelhamid, A. S. (2020). Reduction in saturated fat intake for cardiovascular disease. *The Cochrane Database of Systematic Reviews*, *8*(8), CD011737.

Hu, X., Fan, Y., Li, H., Zhou, R., Zhao, X., Sun, Y., et al. (2021). Impacts of Cigarette Smoking Status on Metabolomic and Gut Microbiota Profile in Male Patients With Coronary Artery Disease: A Multi-Omics Study. *Frontiers in Cardiovascular Medicine*, *8:766739*.

Huang, J. Y., Larose, T. L., Luu, H. N., Wang, R., Fanidi, A., Alcala, K., et al. (2020). Circulating markers of cellular immune activation in prediagnostic blood sample and lung cancer risk in the Lung Cancer Cohort Consortium (LC3). *International Journal of Cancer*, *146*(9), 2394–2405.

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (Ed.). (2004). *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 83*. IARC.

Jacobs, D. R., Woo, J. G., Sinaiko, A. R., Daniels, S. R., Ikonen, J., Juonala, M., et al. (2022). Childhood Cardiovascular Risk Factors and Adult Cardiovascular Events. *The New England Journal of Medicine*, *386*(20), 1877–1888.

Jain, R. B., & Ducatman, A. (2018). Associations between smoking and lipid/lipoprotein concentrations among US adults aged ≥20 years. *Journal of Circulating Biomarkers*, *7*, 1849454418779310.

Jarvis, M. J., Fidler, J., Mindell, J., Feyerabend, C., & West, R. (2008). Assessing smoking status in children, adolescents and adults: cotinine cut-points revisited. *Addiction*, *103*(9), 1553–1561.

Jarvis, M. J., World Health Organization (WHO). (2001). *Children's Exposure to Passive Smoking: Survey Methodology and Monitoring Trends, 1999.*

Järvisalo, M. J., Jartti, L., Näntö-Salonen, K., Irjala, K., Rönnemaa, T., Hartiala, J. J., et al. (2001). Increased aortic intima-media thickness: a marker of preclinical atherosclerosis in high-risk children. *Circulation*, *104*(24), 2943–2947.

Jiang, Y., Zhang, K., Zhu, Z., Cui, M., An, Y., Wang, Y., et al. (2020). Associations between serum metabolites and subclinical atherosclerosis in a Chinese population: the Taizhou Imaging Study. *Aging*, *12*(15), 15302–15313.

Jones, P. R., Rajalahti, T., Resaland, G. K., Aadland, E., Steene-Johannessen, J., Anderssen, S. A., et al. (2019). Associations of physical activity and sedentary time with lipoprotein subclasses in Norwegian schoolchildren: The Active Smarter Kids (ASK) study. *Atherosclerosis*, *288*, 186–193.

Julkunen, H., Cichońska, A., Tiainen, M., Koskela, H., Nybo, K., Mäkelä, V., et al. (2023). Atlas of plasma NMR biomarkers for health and disease in 118,461 individuals from the UK Biobank. *Nature Communications*, *14*(1), 604.

Juonala, M., Ellul, S., Lawlor, D. A., Santos Ferreira, D. L., Carlin, J. B., Cheung, M., et al. (2019). A Cross-Cohort Study Examining the Associations of Metabolomic Profile and Subclinical Atherosclerosis in Children and Their Parents: The Child Health CheckPoint Study and Avon Longitudinal Study of Parents and Children. *Journal of the American Heart Association*, *8*(14), e011852.

Juonala, M., Magnussen, C. G., Venn, A., Dwyer, T., Burns, T. L., Davis, P. H., et al. (2010). Influence of age on associations between childhood risk factors and carotid intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study, the Childhood Determinants of Adult Health Study, the Bogalusa Heart Study, and the Muscatine Study for the International Childhood Cardiovascular Cohort (i3C) Consortium. *Circulation*, *122*(24), 2514–2520.

Juonala, M., Viikari, J. S. A., & Raitakari, O. T. (2013). Main findings from the prospective Cardiovascular Risk in Young Finns Study. *Current Opinion in Lipidology*, *24*(1), 57–64.

Juonala, M., Wu, F., Sinaiko, A., Woo, J. G., Urbina, E. M., Jacobs, D., et al. (2020). Non-HDL Cholesterol Levels in Childhood and Carotid Intima-Media Thickness in Adulthood. *Pediatrics*, *145*(4), 1–9.

Kallio, K., Jokinen, E., Hämäläinen, M., Kaitosaari, T., Volanen, I., Viikari, J., et al. (2006). Impact of repeated lifestyle counselling in an atherosclerosis prevention trial on parental smoking and children's exposure to tobacco smoke. *Acta Paediatrica*, *95*(3), 283–290.

Kallio, K., Jokinen, E., Raitakari, O. T., Hämäläinen, M., Siltala, M., Volanen, I., et al. (2007). Tobacco smoke exposure is associated with attenuated endothelial function in 11-year-old healthy children. *Circulation*, *115*(25), 3205-3212.

Kaluarachchi, M. R., Boulangé, C. L., Garcia-Perez, I., Lindon, J. C., & Minet, E. F. (2016). Multiplatform serum metabolic phenotyping combined with pathway mapping to identify biochemical differences in smokers. *Bioanalysis*, *8*(19), 2023–2043.

Karvinen, S., Waller, K., Silvennoinen, M., Koch, L. G., Britton, S. L., Kaprio, J., et al. (2015). Physical activity in adulthood: genes and mortality. *Scientific Reports*, *5*, 18259.

Kelly, R. S., Kelly, M. P., & Kelly, P. (2020). Metabolomics, physical activity, exercise and health: A review of the current evidence. *Biochimica et Biophysica Acta. Molecular Basis of Disease*, *1866*(12), 165936.

Kestilä, L., Karvonen, S., Jauhiainen, S., & Mikkola, H. (2023). *Väestön terveys- ja hyvinvointikatsaus 2023: tavoitteena sosiaalisesti kestävä Suomi*. Terveyden ja hyvinvoinnin laitos THL, Helsinki.

Keys, A., Menotti, A., Aravanis, C., Blackburn, H., Djordevič, B. S., Buzina, R., et al. (1984). The seven countries study: 2,289 deaths in 15 years. *Preventive Medicine*, *13*(2), 141–154.

Kim, D. S., Li, Y. K., Bell, G. A., Burt, A. A., Vaisar, T., Hutchins, P. M., et al. (2016). Concentration of Smaller High-Density Lipoprotein Particle (HDL-P) Is Inversely Correlated With Carotid

Intima Media Thickening After Confounder Adjustment: The Multi Ethnic Study of Atherosclerosis (MESA). *Journal of the American Heart Association*, *5*(5), e002977.

Kim, S. (2016). Overview of Cotinine Cutoff Values for Smoking Status Classification. *International Journal of Environmental Research and Public Health*, *13*(12), 1236.

Kinnunen, J. M., Pere, L., Lidfors, P., Ollila, H., & Rimpelä, A. (2015). *Nuorten terveystapatutkimus 2015. Nuorten tupakkatuotteiden ja päihteiden käyttö 1977–2015.* STM raportteja Ja muistioita 2005, 31. Sosiaali- ja terveysministeriö, Helsinki.

Kinnunen, J. M., Pere, L., Raisamo, S., Katainen, A., Myöhänen, A., Lahti, L., et al. (2019). *Nuorten terveystapatutkimus 2019. Nuorten tupakkatuotteiden ja päihteiden käyttö sekä rahapelaaminen*. Sosiaali- ja terveysministeriön raportteja ja muistioita 2019:56. Sosiaali- ja terveysministeriö.

Kokko, S., & Martin, L. (2023). *Lasten ja nuorten liikuntakäyttäytyminen Suomessa, LIITU-tutkimuksen tuloksia 2022*. Valtion liikuntaneuvoston julkaisuja 2023:1.

Koponen, P., Borodulin, K., Lundqvist, A., Sääksjärvi, K., & Koskinen, S. (2018). *Terveys, toimintakyky ja hyvinvointi Suomessa: FinTerveys 2017 -tutkimus*.

Koskinen, J. S., Kytö, V., Juonala, M., Viikari, J. S. A., Nevalainen, J., Kähönen, M., et al. (2020). Childhood risk factors and carotid atherosclerotic plaque in adulthood: The Cardiovascular Risk in Young Finns Study. *Atherosclerosis*, *293*, 18–25.

Kozákova, M., Palombo, C., Morizzo, C., Nolan, J. J., Konrad, T., & Balkau, B. (2010). Effect of sedentary behaviour and vigorous physical activity on segment-specific carotid wall thickness and its progression in a healthy population. *European Heart Journal*, *31*(12), 1511–1519.

Kujala, U. M. (2018). Is physical activity a cause of longevity? It is not as straightforward as some would believe. A critical analysis. *British Journal of Sports Medicine*, *52*(14), 914–918.

Kujala, U. M., Mäkinen, V. P., Heinonen, I., Soininen, P., Kangas, A. J., Leskinen, T. H., et al. (2013). Long-term leisure-time physical activity and serum metabolome. *Circulation*, *127*(3), 340–348.

Laitinen, T. T., Nuotio, J., Juonala, M., Niinikoski, H., Rovio, S., Viikari, J. S. A., et al. (2018). Success in achieving the targets of the 20-year infancy-onset dietary intervention: Association with insulin sensitivity and serum lipids. *Diabetes Care*, *41*(10), 2236–2244.

Laitinen, T. T., Nuotio, J., Rovio, S. P., Niinikoski, H., Juonala, M., Magnussen, C. G., et al. (2020). Dietary Fats and Atherosclerosis From Childhood to Adulthood. *Pediatrics*, *145*(4), e20192786.

Lapinleimu, H., Viikari, J., Jokinen, E., Salo, P., Routi, T., Leino, A., et al. (1995). Prospective randomised trial in 1062 infants of diet low in saturated fat and cholesterol. *Lancet*, *345*(8948), 471–476.

Larsson, S. C., Mason, A. M., Bäck, M., Klarin, D., Damrauer, S. M., Michaëlsson, K., et al. (2020). Genetic predisposition to smoking in relation to 14 cardiovascular diseases. *European Heart Journal*, *41*(35), 3304–3310.

Lawler, P. R., Akinkuolie, A. O., Harada, P., Glynn, R. J., Chasman, D. I., Ridker, P. M., et al. (2017). Residual Risk of Atherosclerotic Cardiovascular Events in Relation to Reductions in Very-Low-Density Lipoproteins. *Journal of the American Heart Association*, *6*(12), e007402.

Lear, S. A., Hu, W., Rangarajan, S., Gasevic, D., Leong, D., Iqbal, R., et al. (2017). The effect of physical activity on mortality and cardiovascular disease in 130 000 people from 17 high-income, middle-income, and low-income countries: the PURE study. *Lancet, 390*(10113), 2643–2654.

Lee, D. H., Rezende, L. F. M., Ferrari, G., Aune, D., Keum, N. N., Tabung, F. K., et al. (2021). Physical activity and all-cause and cause-specific mortality: assessing the impact of reverse causation and measurement error in two large prospective cohorts. *European Journal of Epidemiology*, *36*(3), 275–285.

Lewis, G. D., Asnani, A., & Gerszten, R. E. (2008). Application of metabolomics to cardiovascular biomarker and pathway discovery. *Journal of the American College of Cardiology*, *52*(2), 117–123.

Li, J., Guasch-Ferré, M., Chung, W., Ruiz-Canela, M., Toledo, E., Corella, D., et al. (2020). The Mediterranean diet, plasma metabolome, and cardiovascular disease risk. *European Heart Journal*, *41*(28), 2645–2656.

Lichtenstein, A. H., Appel, L. J., Vadiveloo, M., Hu, F. B., Kris-Etherton, P. M., Rebholz, C. M., et al. (2021). 2021 Dietary Guidance to Improve Cardiovascular Health: A Scientific Statement From the American Heart Association. *Circulation*, *144*(23), e472–487.

Lillegaard, I. T. L., & Andersen, L. F. (2005). Validation of a pre-coded food diary with energy expenditure, comparison of under-reporters v. acceptable reporters. *The British Journal of Nutrition*, *94*(6), 998–1003.

Lin, J. P., O'Donnell, C. J., Schwaiger, J. P., Cupples, L. A., Lingenhel, A., Hunt, S. C., et al. (2006). Association between the UGT1A1*28 allele, bilirubin levels, and coronary heart disease in the Framingham Heart Study. *Circulation*, *114*(14), 1476–1481.

Lindström, J., Louheranta, A., Mannelin, M., Rastas, M., Salminen, V., Eriksson, J., et al. (2003). The Finnish Diabetes Prevention Study (DPS): Lifestyle intervention and 3-year results on diet and physical activity. *Diabetes Care*, *26*(12), 3230–3236.

Lindström, J., Peltonen, M., Eriksson, J. G., Ilanne-Parikka, P., Aunola, S., Keinänen-Kiukaanniemi, et al. (2013). Improved lifestyle and decreased diabetes risk over 13 years: long-term follow-up of the randomised Finnish Diabetes Prevention Study (DPS). *Diabetologia*, *56*(2), 284–293.

Lloyd-Jones, D. M., Leip, E. P., Larson, M. G., D'Agostino, R. B., Beiser, A., Wilson, P. W. F., et al. (2006). Prediction of lifetime risk for cardiovascular disease by risk factor burden at 50 years of age. *Circulation*, *113*(6), 791–798.

Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P., & Makowski, D. (2021). performance: An R Package for Assessment, Comparison and Testing of Statistical Models. *Journal of Open Source Software*, *6*(60), 3139.

Luepker, R. V., Perry, C. L., McKinlay, S. M., Nader, P. R., Parcel, G. S., Stone, E. J., et al. (1996). Outcomes of a field trial to improve children's dietary patterns and physical activity. The Child and Adolescent Trial for Cardiovascular Health. CATCH collaborative group. *JAMA*, *275*(10), 768-76.

Ma, S., Xia, M., & Gao, X. (2021). Biomarker Discovery in Atherosclerotic Diseases Using Quantitative Nuclear Magnetic Resonance Metabolomics. *Frontiers in Cardiovascular Medicine*, *8*, 681444.

Mach, F., Baigent, C., Catapano, A. L., Koskina, K. C., Casula, M., Badimon, L., et al. (2019). 2019 ESC/EAS guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. *Atherosclerosis*, *290*(1), 140–205.

MacKey, R. H., Greenland, P., Goff, D. C., Lloyd-Jones, D., Sibley, C. T., & Mora, S. (2012). High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (multi-ethnic study of atherosclerosis). *Journal of the American College of Cardiology*, *60*(6), 508–516.

MacMahon, S., Duffy, S., Rodgers, A., Tominaga, S., Chambless, L., De Backer, G., et al. (2007). Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet*, *370*(9602), 1829–1839.

Magnussen, C. G., Venn, A., Thomson, R., Juonala, M., Srinivasan, S. R., Viikari, et al. (2009). The association of pediatric low- and high-density lipoprotein cholesterol dyslipidemia classifications and change in dyslipidemia status with carotid intima-media thickness in adulthood evidence from the cardiovascular risk in Young Finns study, the Bogalusa Heart study, and the CDAH (Childhood Determinants of Adult Health) study. *Journal of the American College of Cardiology*, *53*(10), 860–869.

Mahendran, Y., Cederberg, H., Vangipurapu, J., Kangas, A. J., Soininen, P., Kuusisto, J., et al. (2013). Glycerol and fatty acids in serum predict the development of hyperglycemia and type 2 diabetes in Finnish men. *Diabetes Care*, *36*(11), 3732–3738.

Makadia, L. D., Roper, P. J., Andrews, J. O., & Tingen, M. S. (2017). Tobacco Use and Smoke Exposure in Children: New Trends, Harm, and Strategies to Improve Health Outcomes. In *Current Allergy and Asthma Reports*, 17(8):55.

Manninen, S. M., Lankinen, M. A., de Mello, V. D., Laaksonen, D. E., Schwab, U. S., & Erkkilä, A. T. (2018). Intake of Fatty Fish Alters the Size and the Concentration of Lipid Components of HDL Particles and Camelina Sativa Oil Decreases IDL Particle Concentration in Subjects with Impaired Glucose Metabolism. *Molecular Nutrition & Food Research*, *62*(10), e1701042.

Mansikkaniemi, K., Juonala, M., Taimela, S., Hirvensalo, M., Telama, R., Huupponen, R., et al. (2012). Cross-sectional associations between physical activity and selected coronary heart disease risk factors in young adults. The Cardiovascular Risk in Young Finns Study. *Annals of Medicine*, *44*(7), 733–744.

Martínez-González, M. A., Salas-Salvadó, J., Estruch, R., Corella, D., Fitó, M., & Ros, E. (2015). Benefits of the Mediterranean Diet: Insights From the PREDIMED Study. *Progress in Cardiovascular Diseases*, *58*(1), 50–60.

März, W., Siekmeier, R., Groß, E., & Groß, W. (1993). Determination of lipoprotein(a): enzyme immunoassay and immunoradiometric assay compared. *Clinica Chimica Acta*, *214*(2), 153–163.

Matthews, D. R., Hosker, J. P., Rudenski, A. S., Naylor, B. A., Treacher, D. F., & Turner, R. C. (1985). Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, *28*(7), 412–419.

Matthews, L. A., Rovio, S. P., Jaakkola, J. M., Niinikoski, H., Lagström, H., Jula, A., et al. (2019). Longitudinal effect of 20-year infancy-onset dietary intervention on food consumption and nutrient intake: the randomized controlled STRIP study. *European Journal of Clinical Nutrition*, *73*(6), 937–949.

McGarrah, R. W., & White, P. J. (2023). Branched-chain amino acids in cardiovascular disease. *Nature Reviews. Cardiology*, *20*(2), 77–89.

McGill, H. C., Alex McMahan, C., Malcom, G. T., Oalmann, M. C., & Strong, J. P. (1997). Effects of serum lipoproteins and smoking on atherosclerosis in young men and women. The PDAY Research Group. Pathobiological Determinants of Atherosclerosis in Youth. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *17*(1), 95–106.

Mears, M. J., Hookfin, H. L., Bandaru, P., Vidal, P., Stanford, K. I., & Wold, L. E. (2023). Electronic Nicotine Delivery Systems and Cardiovascular /Cardiometabolic Health. *Circulation Research*, *132*(9), 1168–1180.

Mendis, S., Puska, P., Norrving, B., World Health Organization, World Heart Federation. et al. (2011). *Global atlas on cardiovascular disease prevention and control.* World Health Organization.

Merianos, A. L., Jandarov, R. A., Khoury, J. C., & Mahabee-Gittens, E. M. (2018). Tobacco Smoke Exposure Association with Lipid Profiles and Adiposity Among U.S. Adolescents. *The Journal of Adolescent Health*, *62*(4), 463.

Meucci, M., Baldari, C., Guidetti, L., Alley, J. R., Cook, C., & Collier, S. R. (2017). Metabolomic Shifts Following Play-Based Activity in Overweight Preadolescents. *Current Pediatric Reviews*, *13*(2), 144-151.

Mihaleva, V. V., Korhonen, S. P., Van Duynhoven, J., Niemitz, M., Vervoort, J., & Jacobs, D. M. (2014). Automated quantum mechanical total line shape fitting model for quantitative NMR-based profiling of human serum metabolites. *Analytical and Bioanalytical Chemistry*, *406*(13), 3091–3102.

Mikkilä, V., Räsänen, L., Raitakari, O. T., Pietinen, P., & Viikari, J. (2005). Consistent dietary patterns identified from childhood to adulthood: the cardiovascular risk in Young Finns Study. *The British Journal of Nutrition*, *93*(6), 923–931.

Mikola, H., Pahkala, K., Niinikoski, H., Rönnemaa, T., Viikari, J. S. A., Jula, A., et al. (2017). Cardiometabolic Determinants of Carotid and Aortic Distensibility From Childhood to Early Adulthood. *Hypertension*, *70*(2), 452–460.

Ministry of Social Affairs and Health. (2005). *Recommendations for physical activity in early childhood education.* Handbooks of the Ministry of Social Affairs and Health, Helsinki.

Mora, S., Otvos, J. D., Rifai, N., Rosenson, R. S., Buring, J. E., & Ridker, P. M. (2009). Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation*, *119*(7), 931–939.

Mora, S., Szklo, M., Otvos, J. D., Greenland, P., Psaty, B. M., Goff, D. C., et al. (2007). LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis*, *192*(1), 211–217.

Morton, K., Heindl, B., Clarkson, S., & Bittner, V. (2022). Primordial Prevention of Atherosclerotic Cardiovascular Disease: A REVIEW OF THE LITERATURE. *Journal of Cardiopulmonary Rehabilitation and Prevention*, *42*(6), 389–396.

Mozaffarian, D. (2016). Dietary and Policy Priorities for Cardiovascular Disease, Diabetes, and Obesity: A Comprehensive Review. *Circulation*, *133*(2), 187–225.

Muller, D. C., Degen, C., Scherer, G., Jahreis, G., Niessner, R., & Scherer, M. (2014). Metabolomics using GC-TOF-MS followed by subsequent GC-FID and HILIC-MS/MS analysis revealed significantly altered fatty acid and phospholipid species profiles in plasma of smokers. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, *966*, 117–126.

Mutharasan, R. K., Thaxton, C. S., Berry, J., Daviglus, M. L., Yuan, C., Sun, J., et al. (2017). HDL efflux capacity, HDL particle size, and high-risk carotid atherosclerosis in a cohort of asymptomatic older adults: the Chicago Healthy Aging Study. *Journal of Lipid Research*, *58*(3), 600–606.

Nagana Gowda, G. A., & Raftery, D. (2023). NMR Metabolomics Methods for Investigating Disease. *Analytical Chemistry*, *95*(1), 83–99.

Newman, A. B., Dodson, J. A., Church, T. S., Buford, T. W., Fielding, R. A., Kritchevsky, S., et al. (2016). Cardiovascular Events in a Physical Activity Intervention Compared With a Successful Aging Intervention: The LIFE Study Randomized Trial. *JAMA Cardiology*, *1*(5), 568–574.

Niinikoski, H., Pahkala, K., Ala-Korpela, M., Viikari, J., Rönnemaa, T., Lagström, H., et al. (2012). Effect of repeated dietary counseling on serum lipoproteins from infancy to adulthood. *Pediatrics*, *129*(3), e704-13.

Nordic Council of Ministers. (2014). Nordic Nutrition Recommendations 2012. Integrating Nutrition and Physical Activity. *Nord*, *5*(11), 1–3.

Nupponen, M., Pahkala, K., Juonala, M., Magnussen, C. G., Niinikoski, H., Rönnemaa, T., et al. (2015). Metabolic syndrome from adolescence to early adulthood: effect of infancy-onset dietary counseling of low saturated fat: the Special Turku Coronary Risk Factor Intervention Project (STRIP). *Circulation*, *131*(7), 605–613

Obarzanek, E., Kimm, S. Y. S., Barton, B. A., Van Horn, L., Kwiterovich, P. O., Simons-Morton, D. G., et al. (2001). Long-term safety and efficacy of a cholesterol-lowering diet in children with elevated low-density lipoprotein cholesterol: seven-year results of the Dietary Intervention Study in Children (DISC). *Pediatrics*, *107*(2), 256–264.

Official Statistics of Finland (OSF). (2022, December 9). *Causes of death [online publication]*. Reference Period: 2021. ISSN=1799-5078. Helsinki: Statistics Finland[Referenced: 7.5.2023].

O'Keefe, J. H., Cordain, L., Harris, W. H., Moe, R. M., & Vogel, R. (2004). Optimal low-density lipoprotein is 50 to 70 mg/dl: Lower is better and physiologically normal. *Journal of the American College of Cardiology*, *43*(11), 2142–2146.

Okekunle, A. P., Asowata, J. O., Adedokun, B., & Akpa, O. M. (2022). Secondhand smoke exposure and dyslipidemia among non-smoking adults in the United States. *Indoor Air*, *32*(1), e12914.

Oranta, O., Pahkala, K., Ruottinen, S., Niinikoski, H., Lagström, H., Viikari, J. S., et al. (2013). Infancy-onset dietary counseling of low-saturated-fat diet improves insulin sensitivity in healthy adolescents 15-20 years of age: the Special Turku Coronary Risk Factor Intervention Project (STRIP) study. *Diabetes Care*, *36*(10), 2952–2959.

Ottosson, F., Emami Khoonsari, P., Gerl, M. J., Simons, K., Melander, O., & Fernandez, C. (2021). A plasma lipid signature predicts incident coronary artery disease. *International Journal of Cardiology*, *331*, 249–254.

Otvos, J. D., Mora, S., Shalaurova, I., Greenland, P., MacKey, R. H., & Goff, D. C. (2011). Clinical implications of discordance between low-density lipoprotein cholesterol and particle number. *Journal of Clinical Lipidology*, *5*(2), 105–113.

Pahkala, K., Heinonen, O. J., Lagström, H., Hakala, P., Hakanen, M., Hernelahti, M., et al. (2012). Clustered metabolic risk and leisure-time physical activity in adolescents: effect of dose? *British Journal of Sports Medicine*, *46*(2), 131–137.

Pahkala, K., Heinonen, O. J., Simell, O., Viikari, J. S., Rönnemaa, T., Niinikoski, H., et al. (2011). Association of physical activity with vascular endothelial function and intima-media thickness. *Circulation*, *124*(18), 1956–1963.

Pahkala, K., Laitinen, T. T., Niinikoski, H., Kartiosuo, N., Rovio, S. P., Lagström, H., et al. (2020). Effects of 20-year infancy-onset dietary counselling on cardiometabolic risk factors in the Special Turku Coronary Risk Factor Intervention Project (STRIP): 6-year post-intervention follow-up. *The Lancet Child and Adolescent Health*, *4*(5), 359-369.

Pan, L., Chen, L., Lv, J., Pang, Y., Guo, Y., Pei, P., et al. (2022). Association of Red Meat Consumption, Metabolic Markers, and Risk of Cardiovascular Diseases. *Frontiers in Nutrition*, *9*, 833271.

Pappa, E., Elisaf, M. S., Kostara, C., Bairaktari, E., & Tsimihodimos, V. K. (2020). Cardioprotective Properties of HDL: Structural and Functional Considerations. *Current Medicinal Chemistry*, *27*(18), 2964–2978.

Pinheiro, J., Bates, D., & R Core Team. (2023). *nlme: Linear and Nonlinear Mixed Effects Models.* (3.1-162).

Poitras, V. J., Gray, C. E., Borghese, M. M., Carson, V., Chaput, J. P., Janssen, I., et al. (2016). Systematic review of the relationships between objectively measured physical activity and health indicators in school-aged children and youth. *Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquee, Nutrition et Metabolisme*, *41*(6 Suppl 3), S197–S239.

Pool, L. R., Aguayo, L., Brzezinski, M., Perak, A. M., Davis, M. M., Greenland, P., et al. (2021). Childhood Risk Factors and Adulthood Cardiovascular Disease: A Systematic Review. *The Journal of Pediatrics*, *232*, 118-126.

Public Health England. (2019). *Saturated fats and health: SACN report*. The Scientific Advisory Committee on Nutrition (SACN).

R Core Team. (2019). *R: A language and environment for statistical computing.* https://www.R-project.org/

Raghuveer, G., Hartz, J., Lubans, D. R., Takken, T., Wiltz, J. L., Mietus-Snyder, M., et al. (2020). Cardiorespiratory Fitness in Youth: An Important Marker of Health: A Scientific Statement From the American Heart Association. *Circulation*, *142*(7), e101–118.

Rainio, S., Pere, L., Lindfors, P., Lavikainen, H., Saarni, L., & Rimpelä, A. (2009). *Nuorten terveystapatutkimus 2009. Nuorten tupakkatuotteiden ja päihteiden käyttö 1977—2009. Sosiaali- ja terveysministeriön selvityksiä 2009:47*. Sosiaali- ja terveysministeriö.

Raitakari, O. T., Pahkala, K., & Magnussen, C. G. (2022). Prevention of atherosclerosis from childhood. *Nature Reviews. Cardiology*, *19*(8), 543–554.

Raitakari, O. T., Juonala, M., Kähönen, M., Taittonen, L., Laitinen, T., Mäki-Torkko, et al. (2003). Cardiovascular risk factors in childhood and carotid artery intima-media thickness in adulthood: the Cardiovascular Risk in Young Finns Study. *Jama*, *290*(17), 2277–2283.

Raitakari, O. T., Mäkinen, V. P., McQueen, M. J., Niemi, J., Juonala, M., Jauhiainen, M., et al. (2013). Computationally estimated apolipoproteins B and A1 in predicting cardiovascular risk. *Atherosclerosis*, *226*(1), 245–251.

Resaland, G. K., Aadland, E., Nilsen, A. K. O., Bartholomew, J. B., Andersen, L. B., & Anderssen, S. A. (2018). The effect of a two-year school-based daily physical activity intervention on a clustered CVD risk factor score-The Sogndal school-intervention study. *Scandinavian Journal of Medicine & Science in Sports*, *28*(3), 1027–1035.

Rigotti, N. A., & Clair, C. (2013). Managing tobacco use: the neglected cardiovascular disease risk factor. *European Heart Journal*, *34*(42), 3259–3267.

Ritchie, S. C., Würtz, P., Nath, A. P., Abraham, G., Havulinna, A. S., Fearnley, L. G., et al. (2015). The Biomarker GlycA Is Associated with Chronic Inflammation and Predicts Long-Term Risk of Severe Infection. *Cell Systems*, *1*(4), 293–301.

Roberts, L. D., & Gerszten, R. E. (2013). Toward new biomarkers of cardiometabolic diseases. *Cell Metabolism*, *18*(1), 43–50.

Rohatgi, A., Khera, A., Berry, J. D., Givens, E. G., Ayers, C. R., Wedin, K. E., et al. (2014). HDL cholesterol efflux capacity and incident cardiovascular events. *The New England Journal of Medicine*, *371*(25), 2383–2393.

Röhrl, C., & Stangl, H. (2013). HDL endocytosis and resecretion. *Biochimica et Biophysica Acta*, *1831*(11), 1626.

Rosenson, R. S., Davidson, M. H., Hirsh, B. J., Kathiresan, S., & Gaudet, D. (2014). Genetics and causality of triglyceride-rich lipoproteins in atherosclerotic cardiovascular disease. *Journal of the American College of Cardiology*, *64*(23), 2525–2540.

Roth, G. A., Mensah, G. A., Johnson, C. O., Addolorato, G., Ammirati, E., Baddour, L. M., et al. (2020). Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. *Journal of the American College of Cardiology*, *76*(25), 2982–3021.

Ruuth, M., Nguyen, S. D., Vihervaara, T., Hilvo, M., Laajala, T. D., Kondadi, P. K., et al. (2018). Susceptibility of low-density lipoprotein particles to aggregate depends on particle lipidome, is modifiable, and associates with future cardiovascular deaths. *European Heart Journal*, *39*(27), 2562–2573.

Sandström, B., Lyhne, N., Pedersen, J. I., Aro, A., Thorsdottir, I., & Becker, W. (1996). Nordic nutrition recommendations 1996. *Scandinavian Journal of Food and Nutrition*, *40*, 161–165.

Sato, S., Makita, S., Uchida, R., Ishihara, S., & Majima, M. (2008). Physical activity and progression of carotid intima-media thickness in patients with coronary heart disease. *Journal of Cardiology*, *51*(3), 157–162.

Sattelmair, J., Pertman, J., Ding, E. L., Kohl, H. W., Haskell, W., & Lee, I. M. (2011). Dose response between physical activity and risk of coronary heart disease: a meta-analysis. *Circulation*, *124*(7), 789–795.

Scheffels, J., Tokle, R., Linnansaari, A., Rasmussen, S. K. B., & Pisinger, C. (2023). E-cigarette use in global digital youth culture. A qualitative study of the social practices and meaning of vaping among 15–20-year-olds in Denmark, Finland, and Norway. *International Journal of Drug Policy*, *111*, 103928.

Schooling, C. M., & Zhao, J. V. (2023). Insights into Causal Cardiovascular Risk Factors from Mendelian Randomization. *Current Cardiology Reports*, *25*(2), 67–76.

Schulze, M. B., Minihane, A. M., Saleh, R. N. M., & Risérus, U. (2020). Intake and metabolism of omega-3 and omega-6 polyunsaturated fatty acids: nutritional implications for cardiometabolic diseases. *The Lancet. Diabetes & Endocrinology*, *8*(11), 915–930.

Sellem, L., Eichelmann, F., Jackson, K. G., Wittenbecher, C., Schulze, M. B., & Lovegrove, J. A. (2023). Replacement of dietary saturated with unsaturated fatty acids is associated with beneficial effects on lipidome metabolites: a secondary analysis of a randomized trial. *The American Journal of Clinical Nutrition*, 117(6), 1248-1261.

Selph, S., Patnode, C., Bailey, S. R., Pappas, M., Stoner, R., & Chou, R. (2020). Primary Care-Relevant Interventions for Tobacco and Nicotine Use Prevention and Cessation in Children and Adolescents: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA*, *323*(16), 1599–1608.

Shah, S. H., Kraus, W. E., & Newgard, C. B. (2012). Metabolomic profiling for the identification of novel biomarkers and mechanisms related to common cardiovascular diseases: form and function. *Circulation*, *126*(9), 1110–1120.

Shin, H. K., Kim, Y. K., Kim, K. Y., Lee, J. H., & Hong, K. W. (2004). Remnant lipoprotein particles induce apoptosis in endothelial cells by NAD(P)H oxidase-mediated production of superoxide and

cytokines via lectin-like oxidized low-density lipoprotein receptor-1 activation: prevention by cilostazol. *Circulation*, *109*(8), 1022–1028.

Sievenpiper, J. L., & Dworatzek, P. D. N. (2013). Food and dietary pattern-based recommendations: an emerging approach to clinical practice guidelines for nutrition therapy in diabetes. *Canadian Journal of Diabetes*, *37*(1), 51–57.

Simell, O., Niinikoski, H., Rönnemaa, T., Raitakari, O. T., Lagström, H., Laurinen, M., et al. (2009). Cohort profile: The STRIP study (Special Turku coronary risk factor intervention project), an infancy-onset dietary and life-style intervention trial. *International Journal of Epidemiology*, *38*(3), 650-655.

Skålén, K., Gustafsson, M., Knutsen Rydberg, E., Hultén, L. M., Wiklund, O., Innerarity, T. L., et al. (2002). Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. *Nature*, *417*(6890), 750–754.

Soininen, P., Kangas, A. J., Würtz, P., Suna, T., & Ala-Korpela, M. (2015). Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circulation.Cardiovascular Genetics*, *8*(1), 192–206.

Soininen, P., Kangas, A. J., Würtz, P., Tukiainen, T., Tynkkynen, T., Laatikainen, R., et al. (2009). High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. *The Analyst*, *134*(9), 1781–1785.

Song, W., Guan, J., He, P., Fan, S., Zhi, H., & Wang, L. (2020). Mediating effects of lipids on the association between smoking and coronary artery disease risk among Chinese. *Lipids in Health and Disease*, *19*(1), 149.

Stewart, R. A. H., Wallentin, L., Benatar, J., Danchin, N., Hagström, E., Held, C., et al. (2016). Dietary patterns and the risk of major adverse cardiovascular events in a global study of high-risk patients with stable coronary heart disease. *European Heart Journal*, *37*(25), 1993–2001.

Stocker, R., Yamamoto, Y., McDonagh, A. F., Glazer, A. N., & Ames, B. N. (1987). Bilirubin is an antioxidant of possible physiological importance. *Science*, *235*(4792), 1043–1046.

Sulague, R. M., Suan, N. N. M., Mendoza, M. F., & Lavie, C. J. (2022). The associations between exercise and lipid biomarkers. *Progress in Cardiovascular Diseases*, *75*, 59–68.

Sundström, J., Lind, L., Vessby, B., Andrén, B., Aro, A., & Lithell, H. O. (2001). Dyslipidemia and an unfavorable fatty acid profile predict left ventricular hypertrophy 20 years later. *Circulation*, *103*(6), 836–841.

Tabas, I., Williams, K. J., & Borén, J. (2007). Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. *Circulation*, *116*(16), 1832–1844.

Tanner, J. M. (1962). *Growth at Adolescence (2nd ed.).* Thomas: Springfield, Ill..

Taskinen, M. R., & Borén, J. (2015). New insights into the pathophysiology of dyslipidemia in type 2 diabetes. *Atherosclerosis*, *239*(2), 483–495.

The Diabetes and Nutrition Study Group (DNSG) of the European Association for the Study of Diabetes (EASD). (2023). Evidence-based European recommendations for the dietary management of diabetes. *Diabetologia*, *66*(6), 965–985.

Thomsen, M., Varbo, A., Tybjærg-Hansen, A., & Nordestgaard, B. G. (2014). Low nonfasting triglycerides and reduced all-cause mortality: a mendelian randomization study. *Clinical Chemistry*, *60*(5), 737–746.

Tibuakuu, M., Fashanu, O. E., Zhao, D., Otvos, J. D., Brown, T. T., Haberlen, S. A., et al. (2019). GlycA, a novel inflammatory marker, is associated with subclinical coronary disease. *AIDS*, *33*(3), 547–557.

Tikkanen, E., Jägerroos, V., Holmes, M. V., Sattar, N., Ala-Korpela, M., Jousilahti, P., et al. (2021). Metabolic Biomarker Discovery for Risk of Peripheral Artery Disease Compared With Coronary Artery Disease: Lipoprotein and Metabolite Profiling of 31 657 Individuals From 5 Prospective Cohorts. *Journal of the American Heart Association*, *10*(23), e021995.

Titz, B., Luettich, K., Leroy, P., Boue, S., Vuillaume, G., Vihervaara, T., et al. (2016). Alterations in Serum Polyunsaturated Fatty Acids and Eicosanoids in Patients with Mild to Moderate Chronic

Obstructive Pulmonary Disease (COPD). *International Journal of Molecular Sciences*, *17*(9), 1583.

Tong, T. Y. N., Koulman, A., Griffin, J. L., Wareham, N. J., Forouhi, N. G., & Imamura, F. (2020). A Combination of Metabolites Predicts Adherence to the Mediterranean Diet Pattern and Its Associations with Insulin Sensitivity and Lipid Homeostasis in the General Population: The Fenland Study, United Kingdom. *The Journal of Nutrition*, *150*(3), 568–578.

Trajkovska, K. T., & Topuzovska, S. (2017). High-density lipoprotein metabolism and reverse cholesterol transport: strategies for raising HDL cholesterol. *Anatolian Journal of Cardiology*, *18*(2), 149.

Trichopoulou, A., Costacou, T., Bamia, C., & Trichopoulos, D. (2003). Adherence to a Mediterranean diet and survival in a Greek population. *The New England Journal of Medicine*, *348*(26), 2599–2608.

Tzoulaki, I., Castagné, R., Boulangé, C. L., Karaman, I., Chekmeneva, E., Evangelou, E., et al. (2019). Serum metabolic signatures of coronary and carotid atherosclerosis and subsequent cardiovascular disease. *European Heart Journal*, *40*(34), 2883–2896.

U.S. Department of Health and Human Services. (2018). *Physical activity guidelines for americans. (2nd ed.).* U.S. Department of Health and Human Services.

Ussher, J. R., Elmariah, S., Gerszten, R. E., & Dyck, J. R. B. (2016). The Emerging Role of Metabolomics in the Diagnosis and Prognosis of Cardiovascular Disease. *Journal of the American College of Cardiology*, *68*(25), 2850–2870.

van der Steeg, W. A., Holme, I., Boekholdt, S. M., Larsen, M. L., Lindahl, C., Stroes, E. S. G., et al. (2008). High-density lipoprotein cholesterol, high-density lipoprotein particle size, and apolipoprotein A-I: significance for cardiovascular risk: the IDEAL and EPIC-Norfolk studies. *Journal of the American College of Cardiology*, *51*(6), 634–642.

van Leeuwen, E. M., Emri, E., Merle, B. M. J., Colijn, J. M., Kersten, E., Cougnard-Gregoire, A., et al. (2018). A new perspective on lipid research in age-related macular degeneration. *Progress in Retinal and Eye Research*, *67*, 56–86.

van Oort, S., Beulens, J. W. J., van Ballegooijen, A. J., Burgess, S., & Larsson, S. C. (2021). Cardiovascular risk factors and lifestyle behaviours in relation to longevity: a Mendelian randomization study. *Journal of Internal Medicine*, *289*(2), 232–243.

Visseren, F. L. J., Mach, F., Smulders, Y. M., Carballo, D., Koskinas, K. C., Bäck, M., et al. (2022). 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice: Developed by the Task Force for cardiovascular disease prevention in clinical practice with representatives of the European Society of Cardiology and 12 medical societies With the special contribution of the European Association of Preventive Cardiology (EAPC). *Revista Espanola de Cardiologia (English Ed.)*, *75*(5), 429.

Vojinovic, D., Kalaoja, M., Trompet, S., Fischer, K., Shipley, M. J., Li, S., et al. (2020). Association of circulating metabolites in plasma or serum and risk of stroke: Meta-analysis from seven prospective cohorts. *Neurology*, *96*(8), e1110–e1123.

Wahlqvist, Mark. (2005). Finnish Nutrition Recommendations – Integrating Nutrition and Physical Activity. *Scandinavian Journal of Nutrition*. 49. 180-180.

Wang, D., Juonala, M., Viikari, J. S. A., Wu, F., Hutri-Kähönen, N., Raitakari, O. T., et al. (2017). Exposure to Parental Smoking in Childhood is Associated with High C-Reactive Protein in Adulthood: The Cardiovascular Risk in Young Finns Study. *Journal of Atherosclerosis and Thrombosis*, *24*(12), 1231–1241.

Wang, F., Zheng, J., Yang, B., Jiang, J., Fu, Y., & Li, D. (2015). Effects of Vegetarian Diets on Blood Lipids: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Journal of the American Heart Association*, *4*(10), e002408.

Wang, Z., Wang, D., & Wang, Y. (2017). Cigarette Smoking and Adipose Tissue: The Emerging Role in Progression of Atherosclerosis. *Mediators of Inflammation*, *2017*, 3102737.

West, H. W., Juonala, M., Gall, S. L., Kähönen, M., Laitinen, T., Taittonen, L., et al. (2015). Exposure to parental smoking in childhood is associated with increased risk of carotid atherosclerotic plaque in adulthood: the Cardiovascular Risk in Young Finns Study. *Circulation*, *131*(14), 1239–1246.

Willett, W. (1998). *Nutritional epidemiology* (2nd ed.). Oxford University Press.

Williams, K. J., & Tabas, I. (1995). The Response-to-Retention Hypothesis of Early Atherogenesis. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *15*(5), 551.

Williams, P. T., Zhao, X. Q., Marcovina, S. M., Otvos, J. D., Brown, B. G., & Krauss, R. M. (2014). Comparison of four methods of analysis of lipoprotein particle subfractions for their association with angiographic progression of coronary artery disease. *Atherosclerosis*, *233*(2), 713–720.

Wisniewski, B. L., Shrestha, C. L., Zhang, S., Thompson, R., Gross, M., Groner, J. A., et al. (2020). Metabolomics profiling of tobacco exposure in children with cystic fibrosis. *Journal of Cystic Fibrosis*, *19*(5), 791–800.

Würtz, P., Havulinna, A. S., Soininen, P., Tynkkynen, T., Prieto-Merino, D., Tillin, T., et al. (2015). Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. *Circulation*, *131*(9), 774–785.

Würtz, P., Raiko, J. R., Magnussen, C. G., Soininen, P., Kangas, A. J., Tynkkynen, T., et al. (2012). High-throughput quantification of circulating metabolites improves prediction of subclinical atherosclerosis. *European Heart Journal*, *33*(18), 2307–2316.

Würtz, P., Soininen, P., Kangas, A. J., Rönnemaa, T., Lehtimäki, T., Kähönen, M., et al. (2013). Branched-chain and aromatic amino acids are predictors of insulin resistance in young adults. *Diabetes Care*, *36*(3), 648–655.

Xia, J., Wang, L., Ma, Z., Zhong, L., Wang, Y., Gao, Y., et al. (2017). Cigarette smoking and chronic kidney disease in the general population: a systematic review and meta-analysis of prospective cohort studies. *Nephrology, Dialysis, Transplantation*, *32*(3), 475–487.

Xia, M., Zhong, Y., Peng, Y., & Qian, C. (2022). Olive oil consumption and risk of cardiovascular disease and all-cause mortality: A meta-analysis of prospective cohort studies. *Frontiers in Nutrition*, *9*, 1041203.

Yang, X., Telama, R., Viikari, J., & Raitakari, O. T. (2006). Risk of obesity in relation to physical activity tracking from youth to adulthood. *Medicine and Science in Sports and Exercise*, *38*(5), 919–925.

Zakhar, J., Amrock, S. M., & Weitzman, M. (2016). Passive and Active Tobacco Exposure and Children's Lipid Profiles. *Nicotine & Tobacco Research*, *18*(5), 982–987.

Zhang, Y., Pletcher, M. J., Vittinghoff, E., Clemons, A. M., Jacobs, D. R., Allen, N. B., et al. (2021). Association Between Cumulative Low-Density Lipoprotein Cholesterol Exposure During Young Adulthood and Middle Age and Risk of Cardiovascular Events. *JAMA Cardiology*, *6*(12), 1406–1413.

Zhao, Q., Wang, J., Miao, Z., Zhang, N. R., Hennessy, S., Small, D. S., et al. (2021). A Mendelian randomization study of the role of lipoprotein subfractions in coronary artery disease. *ELife*, *10*, e58361.

Zhu, H., Abdullah, A. S., He, J., Xi, J., Mao, Y., Feng, Y., et al. (2021). Untargeted Urinary Metabolomics and Children's Exposure to Secondhand Smoke: The Influence of Individual Differences. *International Journal of Environmental Research and Public Health*, *18*(2), 1–16.

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