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FULL RESEARCH PAPER
Smoking

Association of tobacco smoke exposure with metabolic profile from childhood to early adulthood: the Special Turku Coronary Risk Factor Intervention Project

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Aims To investigate the associations between passive tobacco smoke exposure and daily smoking with a comprehensive metabolic profile, measured repeatedly from childhood to adulthood.

Methods and results Study cohort was derived from the Special Turku Coronary Risk Factor Intervention Project (STRIP). Smoking status was obtained by questionnaire, while serum cotinine concentrations were measured using gas chromatography. Metabolic measures were quantified by nuclear magnetic resonance metabolomics at 9 ($n = 539$), 11 ($n = 536$), 13 ($n = 525$), 15 ($n = 488$), 17 ($n = 455$), and 19 ($n = 409$) years. Association of passive tobacco smoke exposure with metabolic profile compared participants who reported less-than-weekly smoking and had serum cotinine concentration <1 ng/mL (no exposure) with those whose cotinine concentration was ≥10 ng/mL (passive tobacco smoke exposure). Associations of daily smoking with metabolic profile in adolescence were analysed by comparing participants reporting daily smoking with those reporting no tobacco use and having serum cotinine concentrations <1 ng/mL. Passive tobacco smoke exposure was directly associated with the serum ratio of monounsaturated fatty acids to total fatty acids [$\beta = 0.34$ standard deviation (SD), (0.17–0.51), $P < 0.0001$] and inversely associated with the serum ratios of polyunsaturated fatty acids. Exposure to passive tobacco smoke was directly associated with very-low-density lipoprotein particle size [$\beta = 0.28$ SD, (0.12–0.45), $P = 0.001$] and inversely associated with HDL particle size {$\beta = -0.21$ SD, [−0.34 to −0.07], $P = 0.003$}. Daily smokers exhibited a similar metabolic profile to those exposed to passive tobacco smoke. These results persisted after adjusting for body mass index, STRIP study group allocation, dietary target score, pubertal status, and parental socio-economic status.

Conclusion Both passive and active tobacco smoke exposures during childhood and adolescence are detrimentally associated with circulating metabolic measures indicative of increased cardio-metabolic risk.

Lay summary A substantial proportion of children are affected by tobacco smoke exposure worldwide, and early life exposure to passive tobacco smoke may be even more harmful than active smoking in terms of cardiovascular disease risk. Our study suggests the following:

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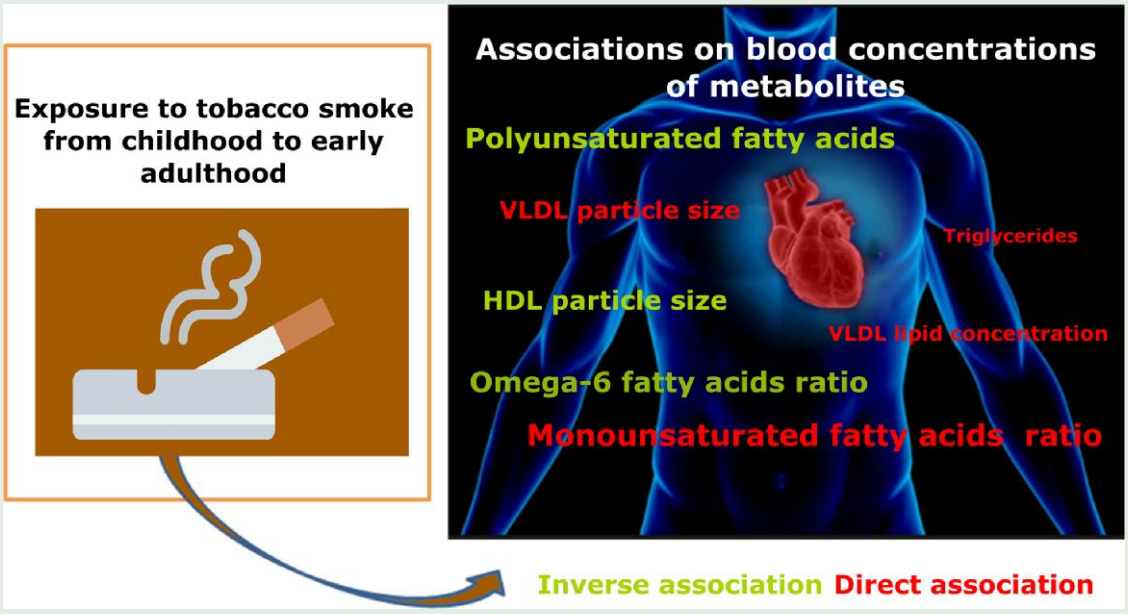
- Passive tobacco smoke exposure during childhood is associated with metabolic measures indicative of increased cardio-metabolic risk and that the association profile is similar with active daily smoking during adolescence.
- Reducing both active and passive tobacco smoke exposures during childhood and adolescence could reduce the risk of future cardio-metabolic disease.

Graphical Abstract



Keywords Passive tobacco smoke exposure • Active smoking • Metabolic profile • Children • Adolescents

Introduction

Tobacco smoke exposure, including both active and passive smoking exposures, is a well-established and preventable risk factor for atherosclerosis.[1,2] The harmful effects of tobacco smoke exposure are evident in childhood, and despite global efforts to reduce exposure, a substantial proportion of children remain affected.[3,4] Early-life exposure to passive tobacco smoke can be as harmful, or even more so, than active smoking in terms of cardiovascular disease risk due to the increased concentrations of certain toxic chemicals,[5,6] with implications persisting into adulthood.[7]

The Special Turku Coronary Risk Factor Intervention Project (STRIP) aimed to minimize children's exposure to environmental cardiovascular risk factors from infancy to early adulthood.[8] The primary focus of the intervention was on diet, promoting heart-healthy dietary habits by targeting the quality of dietary fat from infancy. The prevention of other environmental atherosclerosis risk factors, such as smoking, was introduced later in the intervention protocol. While the STRIP intervention has been shown to have favourable effects on diet,[9,10] its impact on smoking habits, except at age 20 years, has not been observed.[11] Nonetheless, the intervention has positively influenced various cardio-metabolic outcomes.[10,12] Moreover, participants exposed to tobacco smoke, as determined by serum cotinine, have shown functional and structural arterial changes indicative of early atherosclerosis at ages 11 and 13 years,[13,14] increased apolipoprotein B and triglyceride concentrations at age 13 years,[14] and a higher risk of developing overweight due to parental smoking.[15]

Adults exposed to passive tobacco smoke have been shown to have an increased risk of cardiovascular disease, with possible explanations including adverse effects on pro-inflammatory markers and lipid profiles.[5,16] However, findings in children and adolescents are scarce and conflicting.[11,17] For example, some studies report associations between passive tobacco smoke exposure and lipid profile,[17,18] while others have not found such associations.[11] Consequently, it remains unclear how exposure to passive tobacco smoke during childhood and adolescence affects a comprehensive profile of circulating metabolic measures and whether daily smoking during adolescence has different associations with these measures. High-throughput nuclear magnetic resonance (NMR) metabolomics enables the simultaneous quantification of various circulating metabolic markers, including serum fatty acids, amino acids, and lipoprotein subclasses. Nuclear magnetic resonance is widely used to identify novel fatty acid and non-lipid biomarkers for cardiovascular disease risk and Type 2 diabetes.[19,20] This study aims to assess how passive tobacco smoke exposure from childhood to early adulthood and daily smoking during adolescence are associated with a detailed serum metabolic profile measured repeatedly in 539 participants.

Methods

Study design and participants

The STRIP study is a randomized, prospective intervention trial aiming to reduce the exposure to known environmental atherosclerosis risk factors from infancy to early adulthood. The study design, recruitment of study participants, dietary intervention, and data collection methods have been detailed previously.[8] Briefly, families of healthy 5-month-old infants, born between July 1989 and December 1991, were recruited by nurses during routine visits to well-baby clinics in the city of Turku, Finland. At the age of 7 months, 1062 infants (56.2% of the eligible age cohort) and their

families commenced the study and were randomized to either the dietary intervention ($n = 540$; 256 girls) or control ($n = 522$; 256 girls) group. A further 45 children born between March and July of 1989, recruited and randomized (intervention $n = 22$, control $n = 23$) to test the study protocols and serve as a pilot group, were included in the current study. Both the intervention and control groups attended regular clinic visits that were led by a paediatrician or a study nurse and a dietitian.

The STRIP intervention group had study visits at 1- to 3-month intervals until the child was aged 2 years, whereas the control group had study visits biannually. After the age of 2 years, children in both groups had biannual visits, and when the control group reached age 7 years, they had annual visits. Study visits continued until participants reached the age of 20 years.

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 parents and from adolescents at 15 and 18 years of age.

Counselling

The intervention families received individualized lifestyle counselling focusing mainly on diet with suggestions made based on the child's food records and designed to meet the Nordic Dietary Recommendations.[21] The main dietary aim was to replace saturated fat with unsaturated fat and simultaneously reduce the intake of cholesterol. Additionally, the intervention group received counselling on ways to reduce salt intake and how to promote the use of wholegrain products, vegetables, and fruits. Other lifestyle factors related to cardio-metabolic diseases such as smoking and sedentary lifestyle were also discussed, with parents encouraged to modify the child's habits towards a healthier lifestyle. If a parent was a smoker, the possibilities of the child to be exposed to tobacco smoke were discussed. When the intervention children were 5 years old, the families received a booklet about the adverse health effects of smoking.[22] Counselling was provided to parents until the child was aged 7 years. Thereafter, more information was progressively given directly to the child. Counselling aimed at the prevention of active smoking began when intervention children were aged 8 years, using materials mostly developed for the project as ready-made materials for children were sparse.[8] Topics of the counselling covered the adverse health effects of both active and passive smoking, development of addiction, and how to avoid passive tobacco smoke exposure. Suggestions on related topics such as how to refuse offered tobacco were discussed. The control children received only basic health education given at well-baby clinics and school health care. They received no detailed counselling related to diet or tobacco smoking.

Cotinine determination and self-reported smoking

Fasting serum samples for cotinine measurement were drawn annually from the study participants beginning from the age of 8 years 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-methyl cotinine had been added with the method described by Feyerabend and Russell.[23] The concentrated extract was then injected into a Hewlett Packard free fatty acid phase silica capillary column (Agilent Technologies, Palo Alto, CA, USA) in a Shimadzu model GC-17 gas chromatograph (Shimadzu Corp, Kyoto, Japan), equipped with a nitrogen-sensitive flame-thermionic detector.[13] The analytical sensitivity of the method was 0.16 ng/mL.

Participants reported their smoking habits and attitudes towards smoking annually during the study visits starting from age 9 years. Participants were also asked whether either of their parents smoked. From age 13 years, 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.

Passive tobacco smoke exposure and daily smoking

For the analyses on passive tobacco smoke exposure, we excluded participants reporting tobacco smoking at least once a week. Study participants providing both metabolic data and cotinine concentrations at the same age point at age 9 ($n = 539$), 11 ($n = 536$), 13 ($n = 524$), 15 ($n = 475$), 17 ($n = 419$), or 19 ($n = 360$) years, representing 82–97% of the total study participants, were included in the analyses. Cotinine concentrations <1 ng/mL were considered as not having been exposed to tobacco smoke and cotinine concentrations of at least 1 ng/mL as having been exposed to passive tobacco smoke.

There is a significant overlap between the serum cotinine concentrations of active tobacco smokers and those passively exposed to tobacco smoke, and various cut-offs have been used to differentiate between them, with the recent drop in the cut-offs attributed to increased regulatory oversight on smoking.[24,25] Thus, we chose cotinine concentrations ≥10 ng/mL as an indication of strong passive tobacco smoke exposure. We additionally used cotinine concentrations of ≥3 and ≥15 ng/mL in sensitivity analyses to explore possible dose–response associations between passive tobacco smoke exposure and metabolic profile.

To explain the metabolic profile associated with daily smoking in adolescence, we included study participants reporting at least daily smoking to those reporting no tobacco use and had serum cotinine concentrations <1 ng/mL, with serum cotinine and metabolic measures assessed at the same age point at age 15 ($n = 383$, with 13 daily smokers), 17 ($n = 275$, 36), or 19 ($n = 210$, 49) years.

The number of participants at different age points and in these two study settings are shown in Supplementary material online, *Table S1*.

Metabolite quantification

A high-throughput NMR metabolomics platform was used to quantify 75 serum lipid and metabolite measures. This platform enables the quantification of clinical lipoprotein measures, and total lipid, cholesterol, esterified cholesterol, free cholesterol, triglyceride, phospholipid, and particle concentrations of 14 lipoprotein subclasses. Further, it offers the quantification of numerous fatty acids, amino acids, ketone bodies, and gluconeogenesis-related metabolites.[20] The NMR metabolomics platform is widely used in various epidemiological studies.[19,26]

Additional biochemical, dietary, pubertal, and anthropometric measures

Measures of lipoprotein (a) [Lp(a)], C-reactive protein (CRP), and insulin concentrations were estimated from fasting venous blood samples. Lipoprotein (a) concentration was measured with a solid-phase immunoradiometric assay with a direct sandwich technique (Pharmacia/Mercordia, Uppsala, Sweden)[27] and was available for analyses at other age points except at age 19 years. High-sensitivity CRP was assayed by a turbidimetric immunoassay with a sensitivity of 0.06 mg/L and was available for analyses at other age points except at age 9 years. Serum insulin was measured with a micro-particle enzyme immunoassay (insulin IMX system reagent; Abbott, Chicago, IL, USA) or chemiluminescent micro-particle immunoassay (ARCHITECT insulin assay; Abbott) between age 7 and 13 years. From age 15 years onwards, serum insulin was measured by radioimmunoassay (Pharmacia Diagnostics, Uppsala, Sweden).[28] As an estimation of insulin resistance, HOMA-IR {fasting insulin mU/mL × [fasting glucose (mmol/L)/22.5]} measures were used.[29]

Food consumption was recorded by using a 4-day food record with parents or other caregivers completing them when participants were in early childhood.[8,9] A dietitian checked the records for accuracy during the study visit, and the food and nutrient intakes were analysed with a Micro-Nutrica programme (Research Center of the Social Insurance Institution, Turku, Finland).[30] The dietary target score was created to reflect achievement of key STRIP dietary intervention goals[31] with each participant given 1 point at every age for meeting each of the four targets: (i) the ratio of saturated fatty acid (SAFA) to monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) [SAFA/(MUFA + PUFA)] < 1:2, (ii) SAFA < 10E%, (iii) dietary fibre ≥80th age-specific percentile, and (iv) sucrose ≤20th age-specific percentile. The score ranged from 0 to 4 points.

Pubertal status was recorded beginning at the age of 9 years. Breast tissue diameter and testicular length were measured with a ruler, and the respective pubertal stages were then recorded according to the established criteria.[32] Pubertal status ranged from 1 to 5. Height was measured by a Harpenden Stadiometer (Holtain, Crymych, UK) and mass with an electronic scale (Soehnle S10; Soehnle, Murrhardt, Germany). Body mass index (BMI) was calculated as mass in kilograms divided by height in metres squared. Two 3-level categorical variables were used to represent parental

socio-economic status that was based on the highest education and occupation level obtained when study participants were aged 9 years.

Statistical analyses

Metabolic measures with skewed distributions were log(x + 1)-transformed prior to analyses. In analyses for passive tobacco smoke exposure, those reporting smoking at least once a week were excluded from the analyses, and an 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 at least 10 ng/mL). For each measure of metabolite concentration, a linear mixed-effects model for repeated measures was fitted with the exposure variable, sex, and age as fixed effects and subject as a random effect (Model 1). Analyses stratified by sex were also performed. In further multivariable analyses, a similar statistical model with the above-mentioned variables was fitted with additional adjustment for BMI, and categorical covariates for 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 subject as a random effect. Similar multivariable analyses with additional covariates as those mentioned for passive tobacco smoke exposure were performed.

To facilitate the comparison of effect sizes, all metabolic measures were scaled to SD units. The effect sizes reported thus correspond to the average difference in SD-scaled metabolic concentration between the participants with passive tobacco smoke exposure and participants not exposed to tobacco smoke or between the participants with daily tobacco smoking and participants not exposed to tobacco smoke. Multiple testing correction with Bonferroni adjustment for 75 independent tests provided a P-value threshold of 0.0007. Since the metabolic measures in part correlate, a principal component analysis was performed showing 23 metabolic measures explaining 95% of the observed variance in the dataset, and thus a P-value threshold of <0.002 was used as an indication in favour of association. Statistical analyses were conducted using R 3.6.1 software.[33]

Sensitivity analyses

The proportion of children experimenting with smoking, the number of active smokers among adolescents, and parental smoking has declined in Finland from the turn of the century.[34] For instance, in the 12–18 years age group, those exposed to tobacco smoke for at least an hour daily declined from 10% in 2001 to 5% in 2009. Similar trends have been observed in other countries, partly explaining the decrease in cotinine values, used to differentiate between active and passive tobacco smoke exposure.[25] To reflect this change in tobacco smoke exposure during our study period from 1998 to 2010, in addition to the cut-off point of 10 ng/mL, we applied the cut-offs of 3 and 15 ng/mL for serum cotinine to indicate passive tobacco smoke exposure. Linear mixed-effects models were then fitted for both exposure variables with age and sex included as fixed effects and subject as random effect. Similar multivariable analyses with additional covariates described above were performed. The results are shown in Supplementary material online, *Figure S1*, depicting also possible dose–response associations of passive tobacco smoke exposure with different metabolic measures.

To further analyse the associations between tobacco smoke exposure and the metabolic profile irrespective of self-reported smoking status, we conducted similar linear mixed-effects model analyses as described above by using continuous cotinine as an exposure variable while adjusting for age and sex. Similar multivariable analyses were performed, and the results are shown in Supplementary material online, *Figure S2*.

To investigate if parental smoking was confounding the associations between passive tobacco smoke exposure and metabolic profile, we created an additional categorical variable based on parent smoking status. We divided smoking status of the parents into three categories: neither parent smoked and the serum cotinine of the child was <1 ng/mL (n = 1474, for all applied age points), at least one parent smoked but the serum cotinine of the child was <1 ng/mL (n = 466), and at least one parent smoked and the serum cotinine 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 included as fixed effects and subject as random effect. The results are shown in Supplementary material online, *Figure S3*.

Results

This study investigated the association of passive tobacco smoke exposure on the circulating metabolic profile of 360–539 participants at 6 age points between 9 and 19 years of age. Additionally, we investigated how daily smoking is reflected in the metabolic profile of participants at 15 (n = 13), 17 (n = 36), and 19 (n = 49) years of age. Supplementary material online, *Table S1* presents the number of participants in the two study settings and in different serum cotinine categories.

The associations of passive tobacco smoke exposure on metabolic profile from childhood to early adulthood are illustrated in *Figures 1–3*. Sensitivity, continuous cotinine, parental smoking, and sex-stratified analyses are shown in Supplementary material online, *Figures S1–S4*. For ease of comparison, results from the multivariable analysis are shown in the same Figures when applicable. The most prominent associations between passive tobacco smoke exposure and metabolic measures were observed with fatty acid measures and sizes of very-low-density lipoprotein (VLDL) and HDL. *Figures 4–6* depict the associations between daily smoking and metabolic profile in adolescents, with the most prominent associations observed with fatty acid measures and triglyceride-rich lipoprotein measures.

Fatty acid measures

Passive tobacco smoke exposure showed some robust associations with circulating fatty acids (*Figure 1*). Passive tobacco smoke exposure was strongly and directly associated with the ratio of MUFAs to total fatty acids {β = 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 serum concentration of MUFAs. The most prominent inverse associations were observed between passive tobacco smoke exposure and the ratios of omega-6 and PUFAs to total fatty acids. Other PUFA-related measures and the number of double bonds per fatty acid followed a similar pattern, except that no association was observed between passive tobacco smoke exposure and omega-3 fatty acids. Associations were also not observed between passive tobacco smoke exposure and SAFAs or total concentration of fatty acids. All the above-mentioned associations remained similar after adjustment for potential confounding factors.

Sex-stratified results for the fatty acid metabolic measures are shown in Supplementary material online, *Figure S4*. The results for males mostly coincided with the sex-combined results, whereas only a weak direct association between passive tobacco smoke exposure and MUFAs was observed for females. These associations remained similar after adjustment for potential confounders.

Fatty acid measures for adolescents who smoked daily are shown in *Figure 4*. Daily smoking was inversely associated with the ratios of omega-3 and omega-6 fatty acids, linoleic acid, 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 omega-3 fatty acids. Similar to passive tobacco smoke exposure, the most prominent direct association was observed between daily smoking and the ratio of MUFAs to total fatty acids {β = 0.57 SD [95% 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 adjustment for confounding factors. No significant associations were observed between daily smoking and SAFAs, or total fatty acid concentration, with

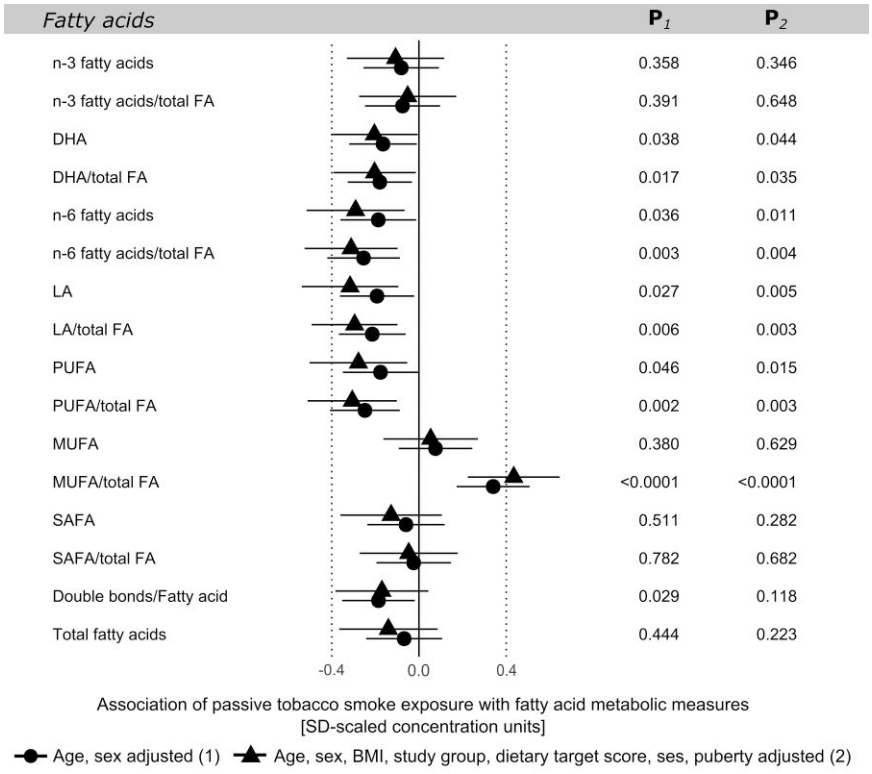


Figure 1 Differences in circulating fatty acid metabolic measures between study participants with passive tobacco smoke exposure (serum cotinine concentration ≥10 ng/mL) and participants with serum cotinine concentration <1 ng/mL. Effect estimates are standard deviation-scaled differences in metabolic measures between the exposed and non-exposed. Statistical Model 1, adjusted for age and sex, is denoted by a dot. Statistical Model 2, adjusted also with body mass index, study group, dietary target score, pubertal, and socio-economic status, is denoted by a triangle. The error bars indicate 95% confidence intervals. Metabolic measures are from pooled analyses across the six time points. n-3 fatty acids/total FA, the ratio of omega-3 fatty acids to total fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SAFA, saturated fatty acids; DHA, docosahexaenoic acid; LA, linoleic acid.

the associations further attenuated after adjusting for potential confounders.

Lipoprotein measures

Figure 2 displays the associations between passive tobacco smoke exposure and lipoprotein measures. In more conventional lipoprotein measures, exposure to passive tobacco smoke was inversely associated with serum concentrations of total cholesterol, non–HDL-cholesterol, LDL cholesterol, HDL-cholesterol, and apolipoprotein A1 (apo A1), with the concentration of apolipoprotein B (apo B) following a similar pattern, but these did not reach statistical significance. There was a tendency for a direct association between passive tobacco smoke and serum concentration of triglycerides.

An inverse association was observed between passive tobacco smoke exposure and the number of LDL particles, while no association was found with the number of VLDL or HDL particles. In contrast, there was a direct association between passive tobacco smoke exposure and VLDL particle size [$\beta = 0.28$ SD, (0.11–0.45), $P = 0.001$] and an inverse association with HDL particle size [$\beta = -0.21$ SD, (−0.34 to −0.07), $P = 0.003$], whereas no association was observed between passive tobacco smoke exposure and the size of LDL particles.

In line with the 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.

Passive tobacco smoke exposure was directly associated with the concentration of triglycerides in VLDL particles and inversely associated with cholesterol concentration in large HDL particles.

Sex-stratified analyses showed a mostly similar pattern for males compared with the sex-combined analyses (see Supplementary material online, *Figure S4*). A direct association between passive tobacco smoke exposure and HDL particle concentration and the total lipid concentrations of medium and small HDL subclasses was observed in females but not males, with adjustment for confounding factors diluting these associations.

Daily smoking generally showed similar differences in the lipoprotein measures with passive tobacco smoke exposure (*Figure 5*) 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.

Other metabolic measures

The associations of passive tobacco smoke exposure with serum glycolysis-related metabolic measures, amino acids, ketone bodies, metabolic waste products, and other metabolic measures are shown

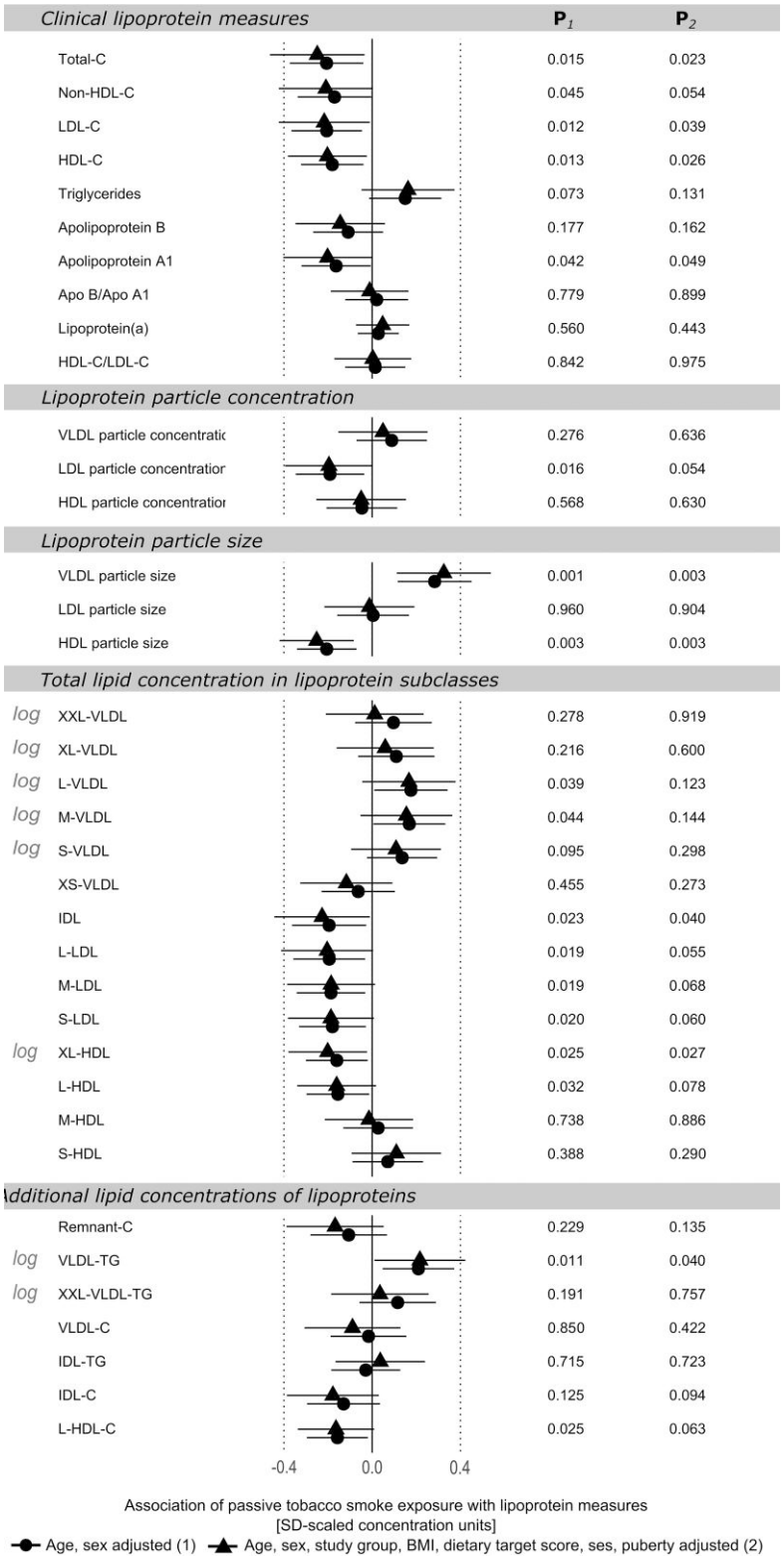


Figure 2 Differences in serum lipid and lipoprotein measures between participants with passive tobacco smoke exposure (serum cotinine concentration ≥10 ng/mL) and participants with cotinine concentration <1 ng/mL. Effect estimates are standard deviation-scaled differences between the exposed and non-exposed. Statistical Model 1, adjusted for age and sex, is denoted by a dot. Statistical Model 2, adjusted also with body mass index, dietary target score, study group, pubertal, and socio-economic status, is denoted by a triangle. Error bars indicate 95% confidence intervals. Metabolic measures are from pooled analyses across the six time points, and those with skewed distributions were log(x + 1)-transformed prior to analyses. C, cholesterol; TG, triglycerides; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein.

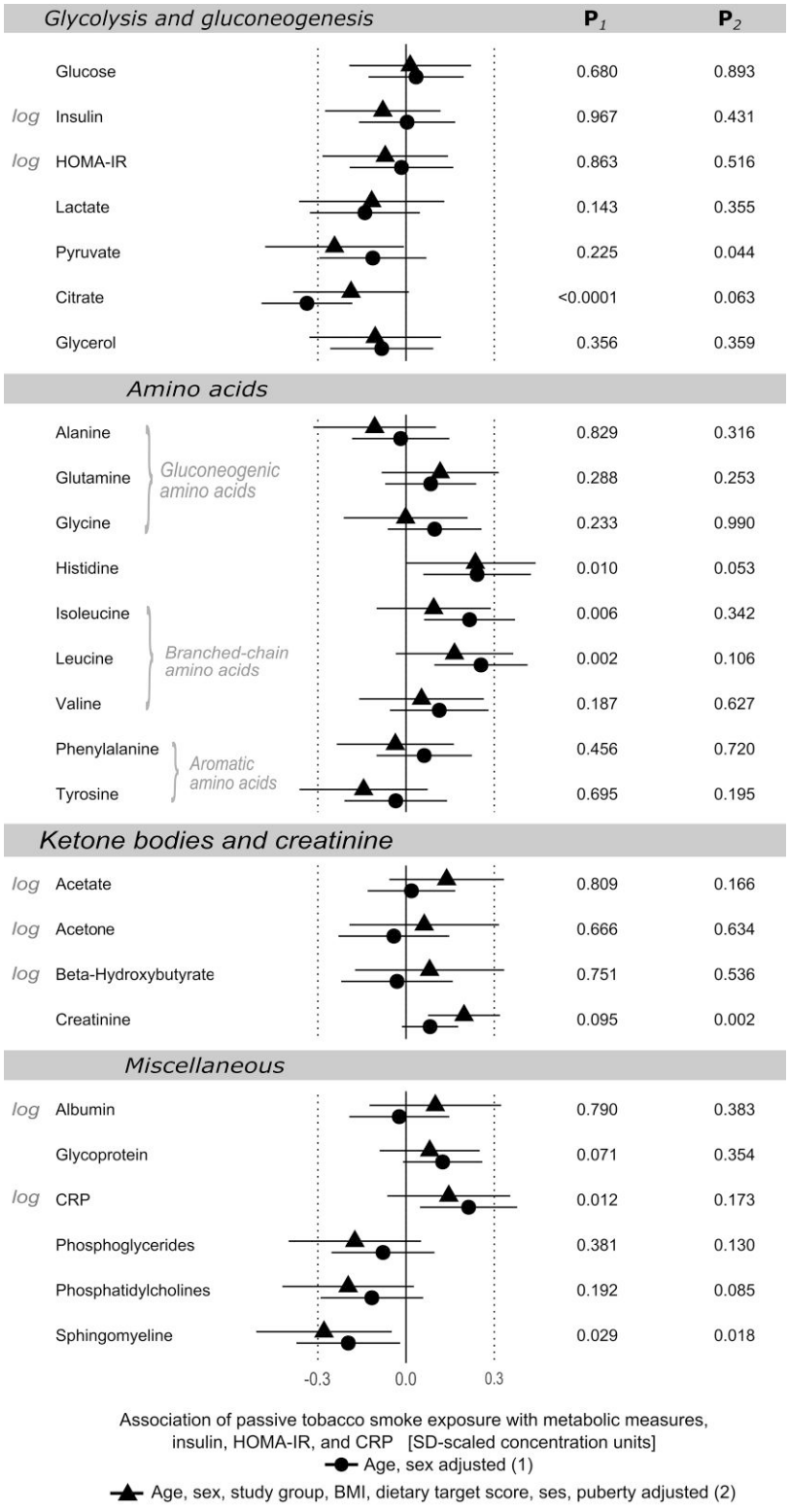


Figure 3 Differences in circulating metabolic measures, insulin, HOMA-IR, and C-reactive protein, between study participants with passive tobacco smoke exposure (serum cotinine concentration ≥10 ng/mL) and participants with serum cotinine concentration <1 ng/mL. Effect estimates are standard deviation-scaled differences between the exposed and non-exposed. Statistical Model 1, adjusted for age and sex, is denoted by a dot. Statistical Model 2, adjusted also with body mass index, dietary target score, study group, pubertal, and socio-economic status, is denoted by a triangle. The error bars indicate 95% confidence intervals. Metabolic measures are from pooled analyses across the six time points. HOMA-IR, homeostatic model assessment for insulin resistance; CRP, C-reactive protein.

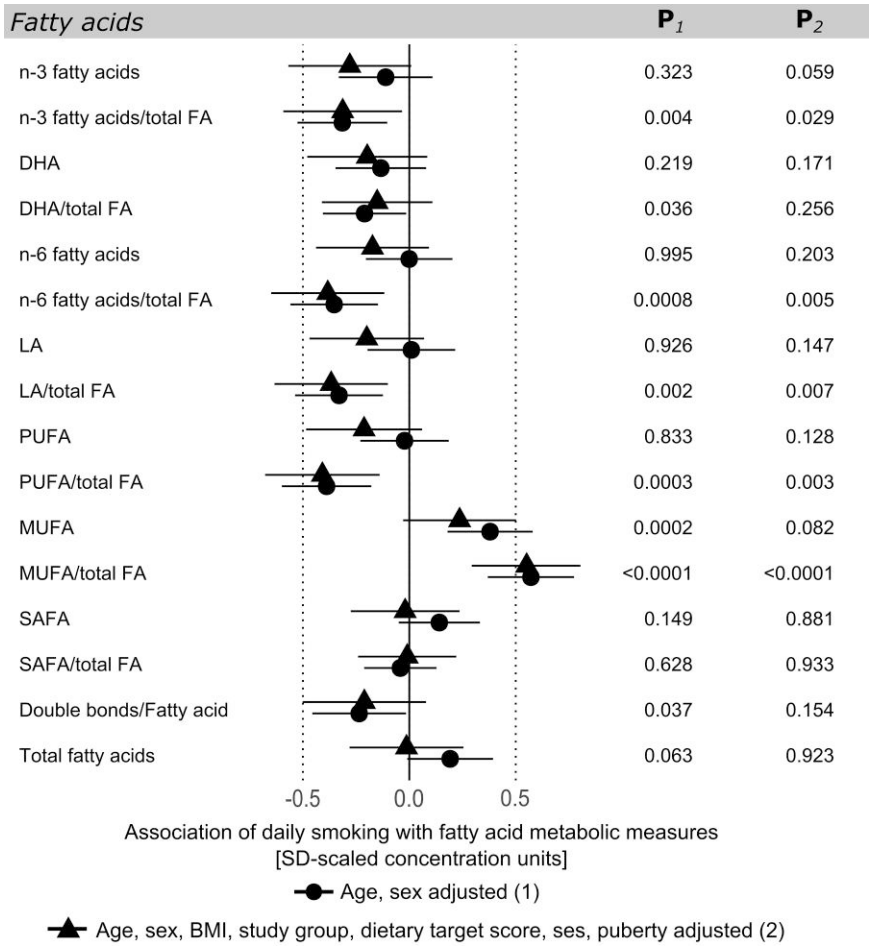


Figure 4 Differences in circulating fatty acid metabolic measures between study participants reporting daily smoking and participants with no reported smoking and serum cotinine concentration <1 ng/mL. Effect estimates are standard deviation-scaled differences in metabolic measures between smokers and non-smokers. Statistical Model 1, adjusted for age and sex, is denoted by a dot. Statistical Model 2, adjusted also with body mass index, study group, dietary target score, pubertal, and socio-economic status, is denoted by a triangle. The error bars indicate 95% confidence intervals. Metabolic measures are from pooled analyses across the three time points. n-3 fatty acids/total FA, the ratio of omega-3 fatty acids to total fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SAFA, saturated fatty acids; DHA, docosahexaenoic acid; LA, linoleic acid.

in *Figure 3*. No significant association was observed between passive tobacco smoke exposure and serum concentrations of glucose or insulin. 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 for a stronger direct association seen between passive tobacco smoke exposure and serum concentration of creatinine.

Sex-stratified results are shown in Supplementary material online, *Figure S4*. Males had a stronger inverse association between passive tobacco smoke and serum concentration of citrate than females. Direct associations between passive tobacco smoke exposure and the concentrations of histidine, glycoprotein acetylation, and CRP were observed for females, but only for CRP in males. Adjusting for potential confounders mostly diluted the observed associations while slightly strengthening the inverse association between passive tobacco smoke and phospholipids in males and tyrosine in females.

The associations of daily smoking with the metabolic measures are shown in *Figure 6*. Unlike exposure to passive tobacco smoke, daily smoking was weakly associated with serum concentrations of glucose, insulin, and HOMA-IR. Similar to 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. Adjusting for confounders diluted most of these associations.

Sensitivity analyses and parental smoking

Use of different cotinine concentration cut-offs of 3 and 15 ng/mL to indicate passive tobacco smoke exposure showed mostly similar metabolic profiles as presented in the main analysis for cotinine concentrations ≥10 ng/mL (see Supplementary material online, *Figure S1*). The associations between passive tobacco smoke exposure and metabolic measures were generally weaker for cotinine concentrations ≥3 ng/mL and stronger for cotinine concentrations ≥15 ng/mL, an indication of a possible dose–response relationship.

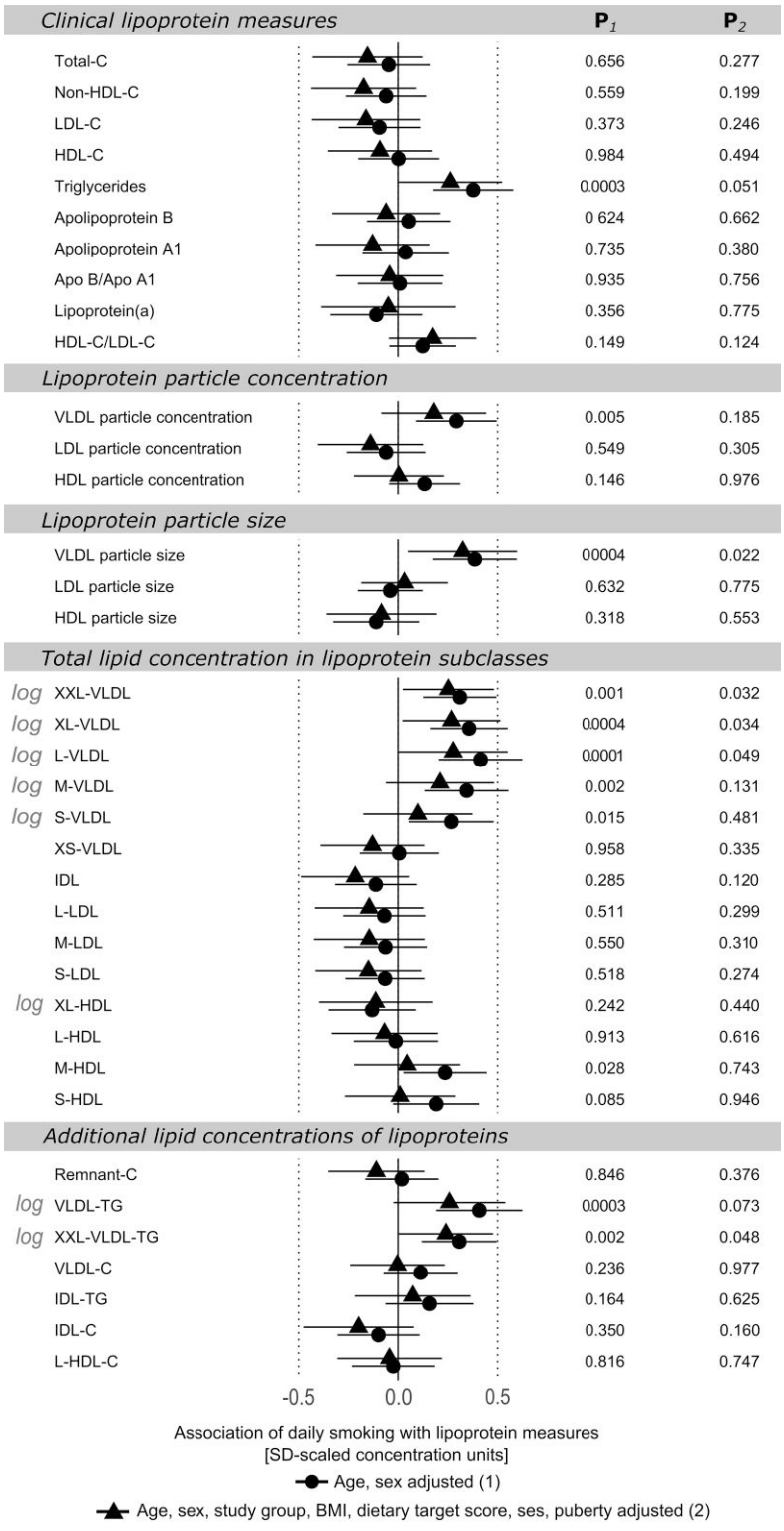


Figure 5 Differences in serum lipoprotein measures between participants reporting daily smoking and participants with no reported smoking and serum cotinine concentration <1 ng/mL. Effect estimates are standard deviation-scaled differences between smokers and non-smokers. Statistical Model 1, adjusted for age and sex, is denoted by a dot. Statistical Model 2, adjusted also with body mass index, dietary target score, study group, pubertal, and socio-economic status, is denoted by a triangle. The error bars indicate 95% confidence intervals. Metabolic measures are from pooled analyses across the three time points, and those with skewed distributions were log(x + 1)-transformed prior to analyses. C, cholesterol; TG, triglyceride; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein.

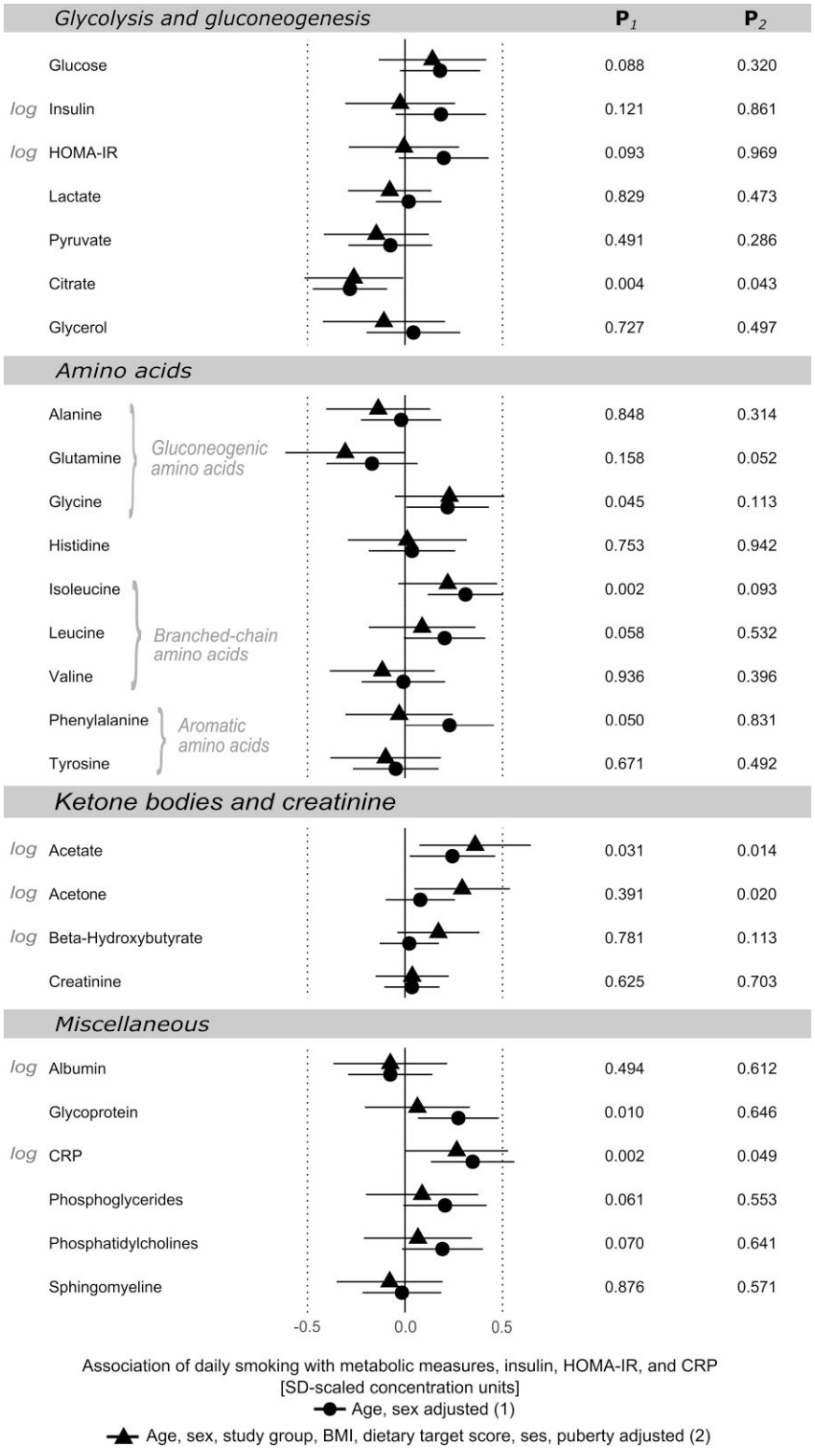


Figure 6 Differences in circulating metabolic measures, insulin, HOMA-IR, and C-reactive protein between study participants reporting daily smoking and participants with no reported smoking and serum cotinine concentration <1 ng/mL. Effect estimates are standard deviation-scaled differences between smokers and non-smokers. Statistical Model 1, adjusted for age and sex, is denoted by a dot. Statistical Model 2, adjusted also with body mass index, diet score, study group, pubertal, and socio-economic status, is denoted by a triangle. The error bars indicate 95% confidence intervals. Metabolic measures are from pooled analyses across the three time points. HOMA-IR, homeostatic model assessment for insulin resistance; CRP, C-reactive protein.

When examining the associations of tobacco smoke exposure and metabolic profiles regardless of the self-reported smoking status, the results further confirmed the associations observed among both actively and passively exposed individuals (see Supplementary material online, *Figure S2*).

When further analysing the observed associations between passive tobacco smoke exposure and metabolic profiles with respect to parents' smoking status and children's cotinine concentration (<1 vs. ≥ 1 mg/mL), we found that the associations were mostly similar, regardless of the child's cotinine concentration when having a smoking parent (see Supplementary material online, *Figure S3*).

Discussion

We found that exposure to passive tobacco smoke from childhood to early adulthood is associated with numerous serum metabolic measures, with these associations largely persisting after adjustment for potential confounders. Notably, associations tended to be stronger when using a higher cotinine cut-off, indicative of greater passive tobacco smoke exposure. Passive tobacco smoke exposure was directly associated with serum ratio of MUFAs to total fatty acids and inversely related to serum PUFAs, particularly omega-6 fatty acids, including linoleic acid. Higher serum MUFA concentrations and lower omega-6 PUFA concentrations have been associated with increased cardiovascular disease (CVD) risk,[35] while the concentration of linoleic acid has been inversely linked to total mortality in older adults.[36] Recent study found that the ratio of MUFA to total fatty acids was the biomarker associated across the highest number of endpoints in the UK Biobank data.[37] Therefore, the authors suggested that it may be considered as a marker of systemic inflammation more so than of recent diet. Additionally, we found a direct association between passive tobacco smoke exposure and VLDL particle size and an inverse association with HDL particle size. Decreased HDL particle size has been associated with an unfavourable cardio-metabolic risk profile,[38] while larger VLDL size has been linked with insulin resistance,[39] though conflicting evidence exists.[40] Overall, the role of lipoprotein subclasses and particle size in atherosclerosis is debated.[41] We also observed weak associations between passive tobacco smoke exposure and concentrations of isoleucine and leucine—branched chain amino acids linked with higher risk for Type 2 diabetes.[42] Passive tobacco smoke exposure was weakly associated with CRP, a biomarker for inflammation shown to reflect insulin resistance in smokers[43] and a known non-causal biomarker for CVD.[44] In general, both passive smoking and daily smoking exposures were associated with similar deviations in the metabolic profile when compared with individuals who were not exposed.

Fatty acid measures

Only a few metabolomics studies with small sample sizes or cross-sectional designs have been conducted on children exposed to tobacco smoke,[45,46] observing changes in pathways related to steroid biosynthesis and fatty acid metabolism.[46] Our study is the first to report longitudinal data on these associations during childhood and adolescence. Previous research on fatty acid profiles has primarily focused on active smoking in adults,[47,48] with most studies being cross-sectional, having small sample sizes, and relying on self-reported smoking data. A study comparing the metabolic profiles of 25 male smokers and non-smokers found a direct association between smoking and plasma MUFA concentrations,[49] similar to our findings. Another study profiling the lipidomes of 40 smokers, smokers with chronic obstructive pulmonary disease, and never smokers found direct associations between smoking and concentrations of phosphoglycerides and MUFAs, as well as an inverse association with omega-3 fatty acids.[50] A recent study on adult humans and mice found an inverse association between passive smoking and docosahexaenoic acid;[51] however, the mechanisms underlying the associations observed with PUFAs are not yet understood. One possible mechanism suggested for the association of MUFA% in smokers is altered endogenous fatty acid desaturation.[49,52] Collectively, our results regarding fatty acids among adolescents who smoked daily show similarities to those observed in adult smokers and indicate slight differences compared with passive tobacco smoke exposure during childhood and adolescence.

Lipoprotein measures

Research examining the associations between passive tobacco smoke exposure and traditional lipid risk profiles in children and adolescents is limited and has produced mixed results. For example, one study on US adolescents ($n = 1822$) found that passive tobacco smoke exposure was directly associated with total and LDL cholesterol and inversely with HDL cholesterol.[17] However, another US study ($n = 2008$) found no differences in lipid profiles between those passively exposed to tobacco smoke and those not exposed.[11] A large study on Iranian children ($n = 14\,400$) found that passive tobacco smoke exposure was directly associated with triglycerides but not with LDL, HDL, or total cholesterol.[18] Although our study aligns with some of these findings, such as HDL cholesterol and triglycerides, it is important to note that these studies on children and adolescents are cross-sectional and used varying definitions for passive tobacco smoke exposure, limiting the potential for direct comparison.

In our previous studies using the same STRIP cohort ($n = 327$ at age 11 years, and $n = 494$ at age 13 years), we investigated the association between longitudinal tobacco smoke exposure [four cotinine measurements by age 11 years, divided into non-cotinine, low cotinine, and top decile (cotinine ≥1.7 ng/mL) group, and two to six cotinine measurements divided into tertiles at age 13 years] and lipid measures evaluated at a single age point. We found no significant associations for triglycerides, total, HDL, or LDL cholesterol.[13,14] At age 13 years, higher longitudinal tobacco smoke exposure was directly associated with apolipoprotein and the ratio of apolipoprotein B to apolipoprotein, while no association was observed with apolipoprotein A1.[14] The current study, including six age points for both cotinine and NMR measurements, found weak associations between passive tobacco smoke exposure and most conventional lipid measures, with no association observed between passive tobacco smoke exposure and the ratio of apolipoprotein B to apolipoprotein A1.

Few studies have investigated the associations between active smoking during adolescence and traditional lipid measures. One showed a direct association between active smoking and triglycerides and an inverse association with LDL cholesterol,[11] while no association was found for HDL cholesterol. Another study found direct associations between active smoking in adolescents and concentrations of total cholesterol and triglycerides and an inverse association with HDL cholesterol.[17] Adult smokers have been shown to have a direct association with triglycerides and an inverse association with HDL cholesterol.[53,54] One proposed mechanism for these lipid alterations is increased lipolysis in adipose tissue due to nicotine,[55–57] with a recent study showing these lipids to partly mediate the association between smoking and coronary artery disease.[58] Our data revealed direct associations between daily smoking and triglycerides, VLDL particle concentration, VLDL particle size, and the majority of VLDL subclasses. However, these associations were diluted after adjustment, suggesting that the observed associations may be confounded by differences in factors such as body size and diet between smokers and non-smokers.

Other metabolic measures

In terms of other metabolic measures, passive tobacco smoke exposure has been associated with inflammatory markers,[59,60] which is consistent with our observation related to CRP. Our study also shows that

passive tobacco smoke exposure is inversely associated with phospholipids. One possible explanation is that phospholipids are the main constituents of HDL particles, whose size was inversely associated with passive tobacco smoke exposure. Like passive tobacco smoke exposure, daily smoking during adolescence showed a direct association with CRP, as previously reported.[61] Moreover, active smoking is associated with an increased risk of Type 2 diabetes,[62] which aligns with the associations we observed for glucose, insulin, and HOMA-IR.

Strengths and limitations

The main strength of our study is the longitudinal design with a relatively large sample size, providing data on an extensive metabolic profile, cotinine concentrations, and self-reported smoking status—all assessed at multiple age points. To our knowledge, this is the first study to report the associations of passive tobacco smoke exposure obtained using serum cotinine concentrations and daily smoking, combined with a comprehensive serum metabolic profile assessed repeatedly with high-throughput NMR in healthy children and adolescents. Therefore, opportunities to compare with prior studies are limited. A possible limitation of the NMR methodology is the separation of lipoprotein subclasses in NMR, which is based merely on particle size, and further identification between, e.g. VLDL or a chylomicron remnant, is not possible.[20] We recognize that our study cannot draw conclusions about causality. We also acknowledge the potential for selection bias during the study's recruitment phase, as more health-conscious families might have been more eager to participate in the trial. This may have also been reflected in the lower prevalence of active smoking in the cohort than in the general population. Additionally, the use of a self-reported data on smoking status can be subject to recall or reporting bias, and as a single country study comprising White participants, the findings presented here might not generalize to other parts of the world. Lastly, bias due to differential loss to follow-up might have occurred. However, the current sample is likely representative of the original study cohort as we have previously shown no systematic differences in key characteristics between participants lost to follow-up and those who continued in the study.[8,63]

Conclusions

Passive tobacco smoke exposure during childhood and adolescence is associated with several circulating metabolic measures indicative of increased cardio-metabolic risk. These associations generally remain after adjusting for potential confounders. Additionally, passive tobacco smoke exposure during adolescence is associated with a similar metabolic profile as active daily smoking. Our findings suggest that reducing both active and passive tobacco smoke exposures during childhood and adolescence could reduce the risk of future cardio-metabolic disease.

Supplementary material

Supplementary material is available at *European Journal of Preventive Cardiology*.

Authors' contributions

M.L. designed the research, analysed the data, and wrote the first draft of the manuscript. C.G.M. participated in the design of the research, gave critical comments, and wrote the manuscript. H.N. and H.L. designed the research and participated in data acquisition. S.P.R. and T.T.L. helped to design the research and gave critical comments. J.S.A.V., T.R., and A.J. participated in data acquisition and gave critical comments. M.A.-K. designed and performed NMR metabolomics data acquisition and gave critical comments. K.P. designed the research, participated in data collections, wrote the manuscript, and gave critical comments. O.T.R. participated in data acquisition, designed the research, wrote the manuscript, and gave critical comments. All authors approved the final version of the manuscript as submitted and agree to be accountable for all aspects of the work. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Conflict of interest: None declared.

Data availability

Selected variables and their descriptions without personal identification codes are distributed to investigators and collaborators working on specific projects. The rights to the data belong to the STRIP research group. Data sharing outside the STRIP group requires a data sharing agreement. Investigators can submit an expression of interest to the STRIP Steering Committee.

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