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Childhood and adulthood passive smoking and non-alcoholic fatty liver in midlife: a 31-year cohort study

Short title: passive smoking and fatty liver

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WHAT IS KNOWN

- Non-alcoholic fatty liver is the most common cause of chronic liver disease in developed countries.
- Identifying early life risk factors remains key to the prevention of non-alcoholic fatty liver.
- Longitudinal influence of childhood passive smoking on adulthood risk of fatty liver is unknown.

WHAT IS NEW HERE

- Childhood passive smoking appears to be associated with an increased risk of fatty liver in adulthood.
- Individuals with persistent exposure to passive smoking between childhood and adulthood had the highest risk.
- The prevention of passive smoking should start as early as possible and maintain throughout lifetime.

ABSTRACT

Objectives Identifying early life risk factors remains key to the prevention of non-alcoholic fatty liver (hereinafter 'fatty liver') in adulthood. However, the longitudinal association of childhood passive smoking with adult fatty liver is not studied. We examined the association of childhood and adulthood passive smoking with fatty liver in midlife.

Methods This was a 31-year prospective cohort study of 1,315 participants. Information on childhood passive smoking (parental smoking) was collected in 1980 (aged 3-18 years) and 1983 and adulthood passive smoking in 2001, 2007 and 2011. Fatty liver was determined by ultrasound in 2011 (aged 34-49 years).

Results The prevalence of fatty liver was 16.3%. Both childhood and adulthood passive smoking were associated with higher risk of fatty liver, adjusting for potential confounders such as age, sex, childhood socioeconomic status, and adulthood physical activity and alcohol consumption (RR=1.41, 95%CI: 1.01-1.97 for childhood; 1.35, 1.01-1.82 for adulthood). Individuals with persistent exposure to passive smoking between childhood and adulthood had the highest risk (RR=1.99, 95%CI:1.14-3.45) compared to those without passive smoking in either childhood or adulthood.

Conclusions Passive smoking in both child and adult life are associated with increased risk of adult fatty liver, suggesting that the prevention of passive smoking should start as early as possible and maintain throughout lifetime.

Keywords: passive smoking, fatty liver, cohort

Introduction

Non-alcoholic fatty liver disease (hereinafter 'fatty liver') is a major public health issue due to its high prevalence and increased risk of complications (such as cirrhosis, liver cancer and coronary heart disease) and mortality(1, 2). As fatty liver is often asymptomatic and fatty liver with advanced fibrosis/cirrhosis is not curable, early life prevention is critical. In an effort to understand the antecedents of fatty liver that might help prevent the disease, our previous work has identified several early-life risk factors for adult fatty liver, including low birth weight, and child high insulin, high body mass index and socioeconomic disadvantage(3, 4).

Smoking is a major public health problem worldwide. Active smoking has been associated with an increased risk of fatty liver in adults (5). In contrast, little is known about the long-term influence of passive smoking on fatty liver(5, 6), particularly when the exposure occurs at an early stage of life(7). Only one study assessed the association of child and adult passive smoking with adult fatty liver, showing that only exposure to passive smoking in both child and adult life was associated with an increased risk of adult fatty liver(6). However, this study is limited by its cross-sectional design and likelihood of recall bias for the measurement of childhood passive smoking. Therefore, using data from a 31-year prospective population-based cohort study, we aimed to examine the association between passive smoking in child and adult life with adult risk of fatty liver.

Methods

Participants

The Cardiovascular Risk in Young Finns Study is an ongoing multi-center population-based cohort study to assess risk factors underlying cardiovascular diseases(8). In 1980, 3,596 individuals aged 3, 6, 9, 12, 15 and 18 years randomly selected from the national register of

the study areas in different parts of Finland participated in the baseline survey. The latest adult follow-up was conducted in 2011, when 2,063 participants were retained. This study comprised 1,315 participants who did not have active smoking in childhood or adulthood, had complete data on passive smoking in childhood and completed liver assessment in 2011 (aged 34-49 years). A flowchart of participation is given in Supplemental Figure 1. All participants provided written informed consent, and the study was approved by local ethics committees.

Fatty liver

In 2011, fatty liver was assessed by ultrasound imaging of the liver using a validated protocol and Sequoia 512 ultrasound mainframes (Acuson, Mountain View, CA, USA) with 4.0 MHz adult abdominal transducers(9). The presence of hepatic steatosis was visually assessed by one trained ultrasonographer blinded to participant's characteristics according to four criteria: liver-to-kidney contrast, parenchymal brightness, deep beam attenuation and bright vessel walls(9). By using data from the national hospital discharge registries, we were able to verify that none of the participants had viral or autoimmune causes of fatty liver(10). Potential advanced fibrosis among fatty liver patients was defined by the BARD score, composed of 3 variables: AST/ALT ratio ≥0.8 – 2 points; a BMI ≥28 – 1 point; and the presence of diabetes – 1 point. The possible score ranged from 0 to 4 points and ≥ 2 points was defined as having potential advanced fibrosis(11).

Childhood passive smoking and serum cotinine concentrations

Childhood passive smoking was measured by parental smoking status. In 1980 and 1983, one parent responding on behalf of both parents self-reported whether the mother and/or father had ever smoked daily for at least 1 year and those who responded 'yes' were designated as 'regular smokers'. A two-level variable was generated to indicate child passive smoking

status(12): 0 = not exposed to passive smoking if neither mother nor father was a regular smoker at both time points, and 1 = exposed to passive smoking if either mother or father was a regular smoker at either time point. This variable has been previously shown to associate with serum cotinine(13).

Child serum cotinine concentrations were measured in 2014 from frozen blood samples taken in 1980 using standardized methods(14). Cotinine values between 3 and 20 ng/mL in nonsmokers were considered as indicative of positive exposure to passive smoking (the concentration that could be detected reproducibly in the assay)(15). Participants aged 12 years or older self-reported their own smoking in 1980 and those aged 3 to 9 years were presumed to be nonsmokers. Childhood parental smoking hygiene was defined as: 0 = no parental smoking and nondetectable cotinine level, 1 = parental smoking and nondetectable cotinine level (hygienic parental smoking), and 2 = parental smoking and detectable serum cotinine (nonhygienic parental smoking). Those who did not have parental smoking but had detectable cotinine level were excluded from analysis (n=2).

Adult passive smoking

Self-administrated questionnaires were used to collect information about participants' daily exposure to cigarette smoke at home, at work or other places (in hours) in 2001, 2007 and 2011. In 2007 and 2011, participants were also asked a separate question if they were exposed to cigarette smoke. In 2001, we also asked participants if their spouse or other people smoke in the participant's residence (responses 'yes' or 'no'). Participants were considered as having passive smoking exposure in 2001, 2007 and 2011 if their daily exposure to cigarette smoke was at least one hour and in 2001 also if spouse or other people smoked in the participant's residence. Participants were not considered as having passive smoking exposure if participants' daily exposure were 0 hours and in 2001 also if spouse or other people did not smoke in the participant's residence and in 2007 and 2011 if the

participant answered that they were not exposed to cigarette smoke. A two-level variable was generated to indicate adulthood passive smoking: 0 = no passive smoking at any time points; 1 = passive smoking in either 2001, 2007 or 2011.

Long-term passive smoking status between child and adult life was defined as: 0 = did not have child or adult passive smoking, 1= child passive smoking only, 2 = adult passive smoking only, and 3 = persistent passive smoking between child and adult life.

Possible child and adult confounders

Childhood BMI, serum insulin levels and parental school years (as indicator of socioeconomic status) were measured in 1980. Height and weight were measured and BMI calculated (weight /height^2, kg/m^2). Serum insulin levels were measured using a modification of the immunoassay method of Herbert et al.(16). Parental school years were measured by questionnaire (the average length of parents' school years was used for analyses). Data on birth weight was verified by well-baby clinic records. Childhood diet habits (by the parents of children 3 or 6 years of age) were collected using a 15-item nonquantitative questionnaire that asked the frequency of consuming fruit, vegetable, meat, and fish consumption, among others during the past month (grouped as daily/almost daily vs. all others)(17). Information on adult physical activity, smoking and alcohol consumption was obtained with questionnaires in 2001, 2007 and 2011. The mean value of physical activity and alcohol consumption from the three time points and daily smoking in any of the adult follow-ups were used in analyses.

Possible adult mediators

On the basis of previous studies(9, 18), BMI, waist circumference, systolic blood pressure, and serum levels of insulin and triglycerides, all well-established risk factors for fatty liver(9) and previously shown to associate with passive smoking(19, 20), were considered as possible

mediators between the association of child and adult passive smoking with adult fatty liver. These variables were measured in adult follow-ups (2001, 2007, 2011), and the mean value from these follow-ups were used for data analyses. Height and weight were measured, and BMI calculated as indicated above. Waist circumference was measured midway between the iliac crest and the lowest rib at the midaxillary line using a non-stretch plastic covered cloth measuring tape to the nearest 0.1 cm. Blood pressure was measured using a random zero sphygmomanometer in adulthood with the average of three measurements calculated. Venous blood samples were drawn after an overnight fast to measure serum triglycerides and insulin using standard enzymatic methods as previously described(21).

Statistical analysis

Mean (standard deviation), median (interquartile range) or number (%) were used to describe characteristics of participants with or without fatty liver in adult life. Log-binomial regression was used to examine unadjusted and adjusted associations of child, adult, and long-term passive smoking with adult risk of fatty liver and advanced fibrosis among fatty liver patients. We selected potential confounders from the risk factors of fatty liver identified in previous studies(4, 9) (age, sex, childhood BMI, insulin levels, parental school years, birthweight, adulthood physical activity, alcohol consumption and smoking) based on the biological plausibility and statistical significance of an association of a factor with both the exposure of interest and the outcome. Dietary intakes of fruit, vegetables, meat and fish products were not adjusted as they were not associated with passive smoking or fatty liver. To assess the influence of potential mediators, we further adjusted for adult BMI and other potential adult mediators (waist circumference, systolic blood pressure, triglycerides, and insulin levels) in above-mentioned adjusted log-binomial models.

We performed mediation analysis for the association of child and adult passive smoking with fatty liver using age, sex, adult physical activity, alcohol consumption and smoking as

confounders, and the following adult variables as potential mediators in separate models: BMI, waist circumference, systolic blood pressure, triglycerides, and insulin levels (complete case analysis)(22). In addition to a direct causal relationship between the exposure and the outcome, the mediation analysis also hypothesizes an indirect causal relationship where the exposure causes the mediator, which in turn causes the outcome(23). The results of the mediation analysis are shown as natural direct effect, natural indirect effect (through mediator), total effect, and the proportion mediated of the total effect (%).

Missing data for child insulin levels (n=29), BMI (n=10), physical activity (n=4), parental school years (n=29) and adult alcohol consumption (n=3) were imputed using age, sex, child passive smoking, and adult fatty liver as predictors by multivariate imputation using chained equations (linear regression imputation method) and 20 datasets were imputed. Rubin's combination rules were used to consolidate the individual estimates from complete data analyses using imputed data. We assumed all values were missing at random.

Secondary analysis was performed for the association between parental smoking hygiene and adult risk of fatty liver. We tested for interactions between child and adult passive smoking with fatty liver, and between each passive smoking variable and age or sex with fatty liver by including the product term (e.g., child × adult passive smoking) in the fully adjusted model. Sensitivity analyses included: 1) To minimise the possibility of having fatty liver at baseline for estimating the association between passive smoking and fatty liver, we excluded participants with potential fatty liver identified by fatty liver index in 2001(24); 2) we examined the associations between each passive smoking variable and fatty liver by excluding those with excessive alcohol consumption in adulthood (>20 g/day for women and >30 g/day for men). All analyses were conducted in Stata 14.0 (Stata Corporation, Texas, USA) and a two-tailed p value <0.05 was considered statistically significant.

Results

Of the 2,063 participants, 748 were excluded because they had active smoking in childhood or adulthood, had missing data on childhood passive smoking or adult fatty liver. On average, participants were followed-up for 31 years. The proportion of fatty liver was 16.3% (n=215). Compared with participants who did not have fatty liver, those with fatty liver were more often males, had lower parental education level, were older, had higher BMI and insulin at both time-points, and higher adult systolic blood pressure, triglycerides, alcohol consumption but had a lower level of physical activity (**Table 1**).

Passive smoking with adulthood fatty liver and advanced fibrosis

Both child and adult passive smoking were associated with increased risk of fatty liver after adjustment for age, sex, child BMI and serum insulin levels, parental school years, and adult physical activity and alcohol consumption (risk ratio (RR)=1.41, 95% confidence interval (CI): 1.01 to 1.97 for child and RR=1.35, 95%CI: 1.01 to 1.82 for adult) (**Table 2**). Individuals with persistent exposure to passive smoking between childhood and adulthood had the highest risk (relative risk=1.99, 95% confidence interval: 1.14 to 3.45) compared to those without passive smoking in either childhood or adulthood (**Table 3**). Change in BMI from childhood to adulthood was associated with adult fatty liver (RR= 1.14, 95%CI: 1.12 to 1.16). After further adjusting for adult BMI and other metabolic factors, the associations of passive smoking with fatty liver were largely reduced (Model 3 and 4, **Table 2 and 3**). Neither child not adult passive smoking was associated with advanced fibrosis, with or without adjusting for confounders (**Supplemental Table 1**).

Adult pathways (mediators) linking passive smoking with fatty liver

Adult risk factors linking child passive smoking and adult fatty liver included BMI (proportion of the association mediated=20.2%) and serum triglycerides levels (15.1%).

Adult risk factors linking adult passive smoking with fatty liver included BMI (51.2%), waist circumference (44.1%), and insulin levels (7.6%) (**Table 4**).

Parental smoking hygiene and fatty liver

Child exposure to nonhygienic parental smoking (exposed to parental smoking with detectable cotinine levels) was associated with an increased risk of adult fatty liver, but this association was no longer significant after adjustment for confounders (RR=1.66, 95%CI: 0.94 to 2.92; Model 2, **Supplemental Table 2**). In comparison, child exposure to hygienic parental smoking (exposed to parental smoking with nondetectable cotinine levels) had a smaller magnitude of association with fatty liver, which was also not significant after adjustment for confounders.

Interaction and sensitivity analyses

There were no significant interactions between child and adult passive smoking, or between passive smoking in child or adult life, or the change in passive smoking status with age or sex for fatty liver ($p>0.05$ for all). Sensitivity analyses excluding those who had a possibility of fatty liver in 2001 showed slight increase in the association between child and adult passive smoking and fatty liver (**Supplemental Table 3**). Sensitivity analyses excluding those with excessive alcohol consumption in adulthood showed similar results for the association between each passive smoking variable with fatty liver (data not shown).

Discussion

This 31-year population-based cohort study showed that both child and adult passive smoking were associated with an increased risk of fatty liver in adulthood. These associations were partly mediated through adult body mass index, waist circumference and serum triglyceride levels. Moreover, individuals with persistent exposure to passive smoking between child and adult life had the highest risk of adult fatty liver. These findings suggest that effective

strategies for preventing passive smoking in both child and adult life may substantially reduce adult risk of fatty liver.

The association between passive smoking and fatty liver is biologically plausible. Our findings suggested that the associations were largely mediated through BMI and waist circumference. Although the mechanism for the association of passive smoking and obesity is unclear and likely complicated, one hypothesis is that passive smoking causes dysregulation of adipose tissue-derived endocrine hormones (e.g., adipokines(25)) that favour fat accumulation(26). In addition, previous studies also suggested that individuals exposed to passive smoking might have unhealthier behaviours (e.g., physical inactivity(27)) that increase the risk of obesity. However, more studies are needed to confirm these hypotheses. Moreover, Yuan et al. showed that mice exposed to side-stream whole smoke for 19 weeks had significantly increased levels of triglycerides in the liver tissue compared to controls(28). Their further analysis suggested that this effect might be mediated through the inactivation of 5′-AMP-activated protein kinase and increased activation of sterol response element binding protein-1, two molecules that play important roles in lipid synthesis(28). In line, our study in humans showed that the association of child passive smoking with fatty liver as adults was partly mediated through increased serum triglyceride levels (proportion of association mediated = 15.1%).

Our findings that both child and adult passive smoking were associated with higher risk of adulthood fatty liver, and that individuals with persistent exposure to passive smoking between child and adult life had the highest risk, have important implications. They suggest that effective strategies should be developed to prevent passive smoking throughout lifetime to achieve maximum benefits to reduce the risk of fatty liver. Only two cross-sectional studies assessed the association of child and/or adult passive smoking with fatty liver(6, 7), showing conflicting results. A small cross-sectional study showed that children who had any

exposure to passive smoking at home had about 4 times higher risk of fatty liver(7), but this association may be overestimated as only age was adjusted. Moreover, Liu et al. found that exposure to passive smoking in childhood or adulthood only was not associated with fatty liver and only exposure to passive smoking in both child and adult life was associated with higher risk of fatty liver in women but not men(6). The discrepancy may be partly explained by that child passive smoking being retrospectively measured after at least 22 years(6), introducing potentially major recall bias. In contrast, our study assessed passive smoking prospectively and both child and adult passive smoking were determined by multiple surveys, minimising the potential recall bias and misclassification.

We found no association between passive smoking and advanced fibrosis among fatty liver patients. This may be partly explained by the low positive predictive value of the fibrosis score (only 43%(11)). Of note, BARD score, as other non-invasive fibrosis scores, was primarily developed for identifying fatty liver patients without advanced fibrosis, that is, high negative predictive value(11). Thus, future research using more accurate measurement of fibrosis among fatty liver patients is needed to confirm our findings.

Although the association was not statistically significant, children who were exposed to nonhygienic parental smoking had a 66% higher risk of adult risk of fatty liver compared with those who were not exposed to passive smoking, which is about 70% higher than those who had hygienic parental smoking. Although these findings need to be confirmed by studies of larger sample size, similar results in our cohort were previously observed for the association of child parental smoking hygiene with adult carotid atherosclerotic plaque(13). Thus, parents who smoke should be encouraged to quit smoking, but among those who are either unable or unwilling to quit smoking, they are able to limit the effects of their smoking on their child offspring by maintaining good smoking hygiene.

The main strength of this study is the long-term prospective follow-up of a large population-based cohort, allowing us to examine the early-life exposure to passive smoking with adult risk of fatty liver. In addition, passive smoking in both child and adult life was prospectively assessed by multiple surveys and child parental smoking hygiene assessed by serum cotinine, minimising the potential for recall bias and misclassification. This study has limitations. First, fatty liver in childhood was not measured. Although we were not able to rule out fatty liver patients in childhood, the associations of passive smoking with fatty liver were only slightly increased after excluding potential fatty liver patients identified by fatty liver index score 10 years prior to the last follow-up, suggesting minimum influence on our conclusions. Second, fatty liver was determined by ultrasound imaging, while liver biopsy is the standard method to measure fatty liver. However, liver biopsy is invasive and may lead to severe complications, limiting its efficacy in large epidemiological studies of the general population(29). In contrast, ultrasound imaging is cost-effective and has high specificity, which has been widely used in epidemiological studies of the general population. Although it has low sensitivity, it has been shown to have reliable and accurate detection of moderate-severe fatty liver(30). Moreover, the prevalence of fatty liver in the current study was largely comparable with previous data in Finland and other countries(31, 32). Third, although none of the participants had diagnosed viral or autoimmune hepatitis based on national hospital discharge registries data, there might be participants who had viral or autoimmune causes of chronic hepatitis but did not see a doctor and thus had been undiagnosed. It has been shown that the estimated incidence of acute hepatitis B infection in Finland is 4.2 times higher than the register-based data(33). Nonetheless, the lifetime risk of acute and chronic hepatitis B in Finland is low, estimated to be 0.13% and 0.01%, respectively(33). Thus, this limitation is unlikely to affect our results. Fourth, as with all single country studies, the findings may not be generalisable to other areas of the world. For example, the Global Adult Tobacco Survey

showed a high exposure to passive smoking in the home among children in most low- and middle-income countries, such as Indonesia (79%)(34). Thus, there is an urgent need to assess the influence of passive smoking on health outcomes in those countries to provide evidence to inform preventive interventions and health policy. Lastly, we had missing data due to loss to follow-up after an extensive study period of 31 years. However, the current study sample is likely to be representative of the original cohort given that the study sample has been largely dynamic and we have previously shown no differences in baseline risk factor between participants who were lost to follow-up and those who were not at the latest follow-ups(35).

In conclusion, both child and adult passive smoking were associated with increased risk of fatty liver in adulthood. Individuals with persistent exposure to passive smoking between child and adult life had the highest risk of adult fatty liver. These findings suggest that effective strategies for preventing passive smoking in both child and adult life may substantially reduce adult risk of fatty liver.

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Table 1. Child and adult characteristics of participants in the Cardiovascular Risk in Young Finns Study according to presence of fatty liver in adulthood (n=1,315)

	Presence of Fatty liver		P-value
	No	Yes	
N	1100	215	
Male sex, n (%)	409 (37)	140 (65)	<0.001
Child factors			
Age (years)	10.2 (4.9)	12.0 (4.6)	<0.001
Parental school years [a]	10.3 (3.2)	9.4 (3.0)	<0.001
Body mass index (kg/m^2)	17.6 (2.9)	18.8 (3.7)	<0.001
Insulin (mU/L) (Median, interquartile range)	9.28 (5 to 12.5)	11.59 (6 to 16)	<0.001
Adult factors			
Age (years)	41.2 (4.9)	42.9 (4.6)	<0.001
Body mass index (kg/m^2)	25.0 (4.1)	30.0 (5.3)	<0.001
Insulin (mU/L)	7.98 (10.26)	15.09 (11.18)	<0.001
Systolic blood pressure (mmHg)	117 (12)	126 (12)	<0.001
Triglycerides (mmol/L)	1.17 (0.68)	1.94 (1.10)	<0.001
Physical activity	9.2 (1.6)	8.4 (1.5)	<0.001
Alcohol consumption (units/day) [b]	0.62 (0.74)	1.12 (1.51)	<0.001
Waist circumference (cm)	85.3 (11.4)	100.9 (13.1)	<0.001

Values are mean (standard deviation) unless otherwise stated.

[a] Indicates average length of parents' school years. [b] One unit = 14 g of alcohol.

Table 2. Relative risk (RR) and 95% confidence interval (CI) of child and adult passive smoking with adulthood fatty liver

Passive smoking		Model 1	Model 2	Model 3	Model 4
Child [a]	Cases of fatty liver, n/N (%)	RR (95%CI)	RR (95%CI)	RR (95%CI)	RR (95%CI)
No	34/316 (10.8)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Yes	181/999 (18.2)	**1.68 (1.19 to 2.38)**	**1.41 (1.01 to 1.97)**	1.25 (0.91 to 1.72)	1.23 (0.89 to 1.69)
Adult [b]					
No	102/774 (13.2)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Yes	49/214 (22.9)	**1.74 (1.28 to 2.36)**	**1.35 (1.01 to 1.82)**	1.12 (0.85 to 1.48)	1.20 (0.91 to 1.58)

Bold denotes statistical significance, $p<0.05$.

Model 1, unadjusted.

Model 2, adjusted for age, sex, child serum insulin (log-transformed), body mass index (age-specific standardised) and parental school years, and adult physical activity and alcohol consumption.

Model 3, model 2 + adult mean BMI.

Model 4, model 3 + adult waist circumference, systolic blood pressure, triglycerides, and insulin levels.

[a] No, both mother and father did not have ever smoked daily for at least 1 year in 1980 (baseline) and 1983; Yes, either mother or father had ever smoked daily for at least 1 year in either 1980 or 1983.

[b] No, did not have passive smoking at any adult follow-ups in 2001, 2007 and 2011; Yes, had passive smoking at any adult follow-ups in 2001, 2007 and 2011.

Table 3. Relative risk (RR) and 95% confidence interval (CI) of the association between change in passive smoking status between childhood and adulthood with adult fatty liver (n=988)

		Model 1	Model 2	Model 3	Model 4
Passive smoking	Cases of fatty liver, n/N (%)	RR (95%CI)	RR (95%CI)	RR (95%CI)	RR (95%CI)
Persistently no	15/194 (7.7)	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
Child only	87/580 (15.0)	1.94 (1.15 to 3.27)	1.58 (0.95 to 2.63)	1.39 (0.84 to 2.30)	1.26 (0.76 to 2.09)
Adult only	7/39 (18.0)	**2.32 (1.01 to 5.32)**	1.96 (0.90 to 4.26)	1.52 (0.70 to 3.32)	1.29 (0.58 to 2.88)
Persistently yes	42/175 (24.0)	**3.10 (1.78 to 5.40)**	**1.99 (1.14 to 3.45)**	1.45 (0.85 to 2.49)	1.47 (0.85 to 2.52)

Bold denotes statistical significance, $p<0.05$.

Model 1, unadjusted.

Model 2, adjusted for age, sex, child serum insulin (log-transformed), body mass index (age-specific standardised) and parental school years, and adult physical activity and alcohol consumption.

Model 3, model 2 + adult mean BMI.

Model 4, model 3 + adult waist circumference, systolic blood pressure, triglycerides, and insulin levels.

Table 4. Pathways (mediators) linking the association of child and adult passive smoking with adulthood fatty liver.

	Natural direct effect	Natural indirect effect through mediator	Total effect	Proportion mediated of the Total effect
Child passive smoking	RR (95%CI)	RR (95%CI)	RR (95%CI)	%
Adult mediators [a]				
Body mass index	1.32 (0.93 to 1.82)	**1.06 (1.00 to 1.13)**	1.40 (1.00 to 1.96)	20.2
Waist circumference	1.37 (1.95 to 1.85)	1.06 (0.99 to 1.13)	1.45 (1.03 to 2.00)	19.1
Triglycerides	1.31 (0.95 to 1.83)	**1.04 (1.02 to 1.07)**	1.36 (0.98 to 1.92)	15.1
Insulin	1.41 (1.00 to 1.96)	1.01 (0.99 to 1.04)	1.42 (1.01 to 2.00)	3.1
Systolic blood pressure	1.49 (1.07 to 2.11)	1.01 (0.96 to 1.06)	1.50 (1.08 to 2.13)	1.7
Adult passive smoking				
Adult mediators [a]				
Body mass index	1.16 (0.84 to 1.54)	**1.14 (1.05 to 1.25)**	1.32 (0.96 to 1.78)	51.2
Waist circumference	1.26 (0.94 to 1.69)	**1.16 (1.06 to 1.28)**	1.46 (1.07 to 1.97)	44.1
Systolic blood pressure	1.34 (0.96 to 1.78)	1.04 (0.99 to 1.10)	1.39 (1.00 to 1.87)	14.1
Insulin	1.38 (0.98 to 1.88)	**1.02 (1.01 to 1.09)**	1.41 (0.99 to 1.88)	7.6
Triglycerides	1.49 (1.07 to 1.99)	1.00 (0.97 to 1.05)	1.50 (1.08 to 1.99)	1.2

Bold denotes statistical significance, $p<0.05$.

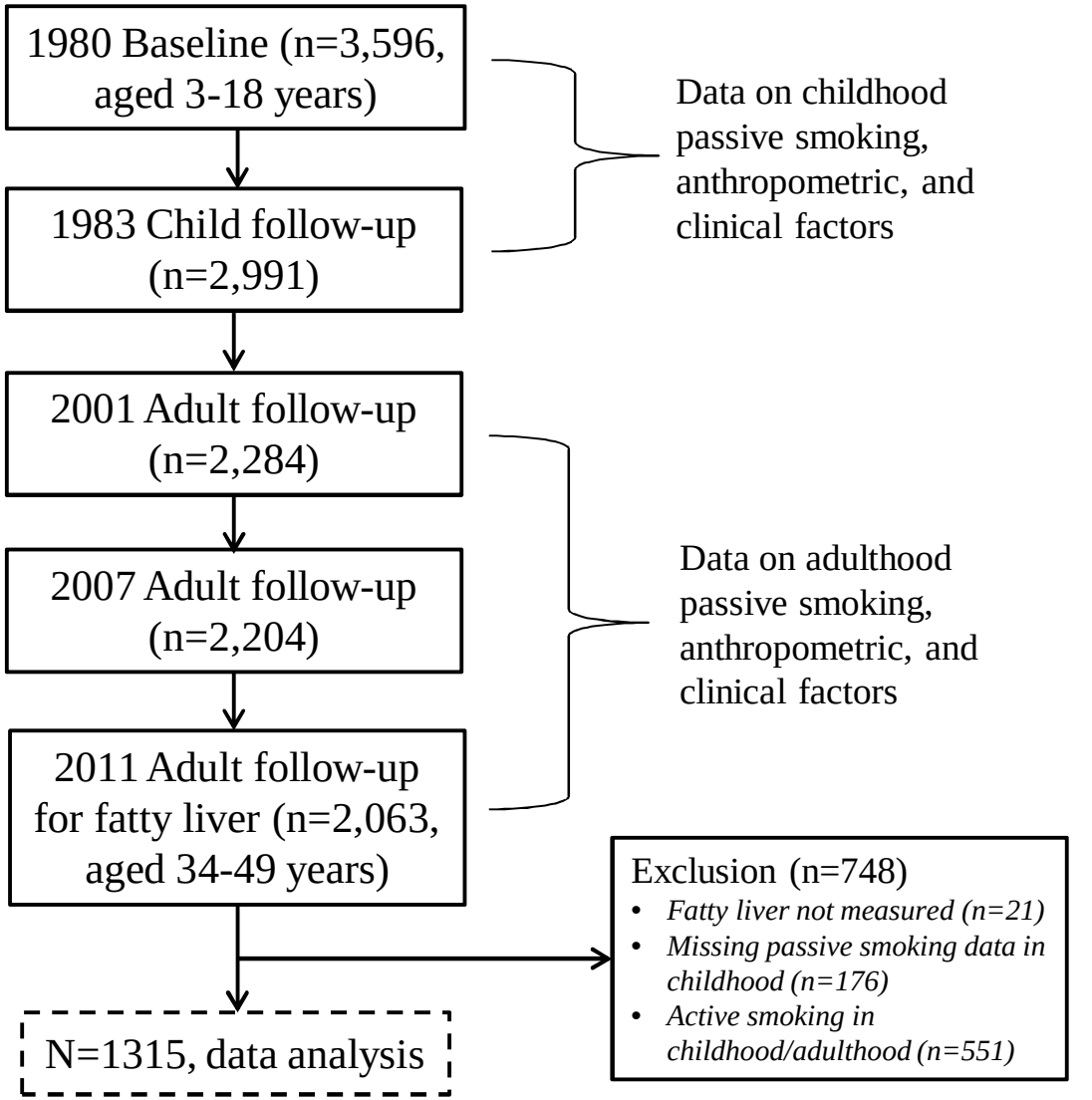
[a] mean value of adult follow-ups from 2001, 2007 and 2011.

The analyses were adjusted for age, sex, and adult physical activity and alcohol consumption.

RR=relative risk; CI= confidence interval.

Figure legend

Supplemental Figure 1 Flowchart of study participants.


1980 Baseline (n=3,596, aged 3-18 years)
1983 Child follow-up (n=2,991)
Data on childhood passive smoking, anthropometric, and clinical factors
2001 Adult follow-up (n=2,284)
2007 Adult follow-up (n=2,204)
2011 Adult follow-up for fatty liver (n=2,063, aged 34-49 years)
Data on adulthood passive smoking, anthropometric, and clinical factors
Exclusion (n=748)
Fatty liver not measured (n=21)
Missing passive smoking data in childhood (n=176)
Active smoking in childhood/adulthood (n=551)
N=1315, data analysis

Supplemental Table 1. Relative risk (RR) and 95% confidence interval (CI) of the association between passive smoking and advanced fibrosis defined by BARD score in fatty liver patients.

Passive smoking		**Model 1**	**Model 2**
Child [a]	**Cases of advanced fibrosis, n/N (%)**	**RR (95%CI)**	**RR (95%CI)**
No	26/29 (89.7)	1.00 (reference)	1.00 (reference)
Yes	139/160 (86.9)	0.97 (0.84 to 1.11)	0.87 (0.73 to 1.03)
Adult [b]			
No	89/99 (89.9)	1.00 (reference)	1.00 (reference)
Yes	33/43 (76.7)	0.85 (0.71 to 1.02)	0.92 (0.80 to 1.06)

Bold denotes statistical significance.

Model 1, unadjusted.

Model 2, adjusted for age, sex, child serum insulin (log-transformed), body mass index (age-specific standardised) and parental school years, and adult physical activity and alcohol consumption.

[a] No, both mother and father did not have ever smoked daily for at least 1 year in 1980 (baseline) and 1983; Yes, neither one of the parents had ever smoked daily for at least 1 year in 1980 (baseline) or 1983.

[b] No, did not have passive smoking at any adult follow-ups in 2001, 2007 and 2011; Yes, had passive smoking at any adult follow-ups in 2001, 2007 and 2011.

Supplemental Table ~~1~~2. Relative Risk (RR) and 95% confidence interval (CI) of childhood parental smoking hygiene with adulthood fatty liver

		Model 1	Model 2
Parental smoking hygiene	**Cases of fatty liver, n/N (%)**	**RR (95%CI)**	**RR (95%CI)**
No parental smoking [a]	21/215 (9.8)	1.0	1.0
Hygienic parental smoking [b]	103/618 (16.7)	**1.71 (1.10 to 2.66)**	1.38 (0.89 to 2.12)
Nonhygienic parental smoking [c]	18/71 (25.4)	**2.60 (1.47 to 4.58)**	1.66 (0.94 to 2.92)

Bold denotes statistical significance.

Model 1, unadjusted.

Model 2, adjusted for age, sex, child serum insulin (log-transformed), body mass index (age-specific standardised) and parental school years, and adult physical activity and alcohol consumption.

[a] both mother and father did not have ever smoked daily for at least 1 year in 1980 (baseline) and 1983.

[b] either mother or father had ever smoked daily for at least 1 year in either 1980 or 1983, but child had a nondetectable serum cotinine level.

[c] either mother or father had ever smoked daily for at least 1 year in either 1980 or 1983, and child had a detectable serum cotinine level.

Supplemental Table ~~2~~3. Relative risk (RR) and 95% confidence interval (CI) of the association between ~~adult~~ passive smoking and fatty liver after excluding participants with a possible fatty liver in 2001.

Passive smoking[a]		**Model 1**	**Model 2**
Child[a]	**Cases of fatty liver, n/N (%)**	**RR (95%CI)**	**RR (95%CI)**
No	14/218 (6.4)	1.00 (reference)	1.00 (reference)
Yes	90/686 (13.1)	**2.04 (1.19 to 3.51)**	**1.78 (1.04 to 3.05)**
Adult[b]			
No	~~90~~56/~~712~~616 (~~12.6~~9.1)	1.00 (reference)	1.00 (reference)
Yes	~~41~~25/~~177~~ 149 (~~23~~16.~~28~~)	**1.8~~5~~3 (1.~~32~~ 19 to 2.~~55~~86)**	1.4~~5~~3 (~~1.~~0.9~~0~~4 to ~~1.98~~2.24)

Bold denotes statistical significance.

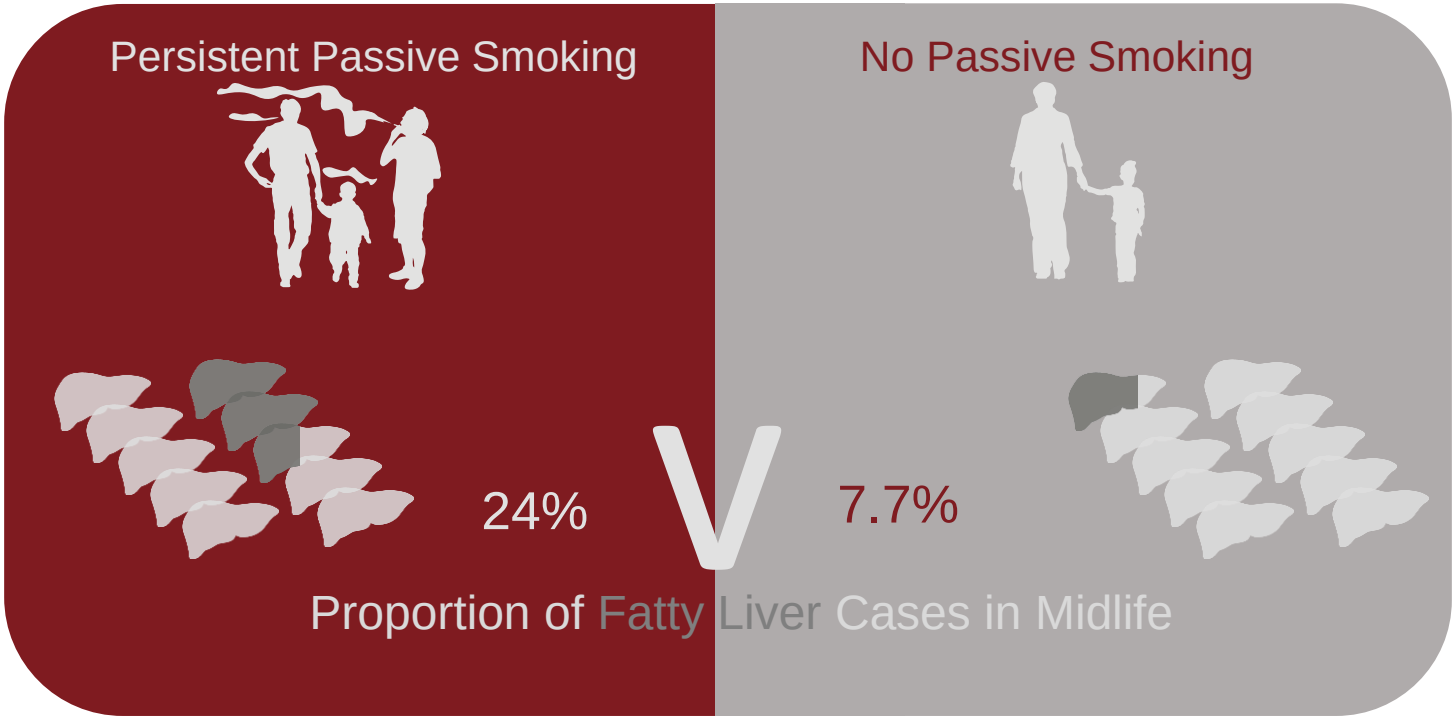
Model 1, unadjusted.

Model 2, adjusted for age, sex, child serum insulin (log-transformed), body mass index (age-specific standardised) and parental school years, and adult physical activity and alcohol consumption.

[a] No, both mother and father did not have ever smoked daily for at least 1 year in 1980 (baseline) and 1983; Yes, neither one of the parents had ever smoked daily for at least 1 year in 1980 (baseline) or 1983.

[b] No, did not have passive smoking at any adult follow-ups in 2001, 2007 and 2011; Yes, had passive smoking at any adult follow-ups in 2001, 2007 and 2011.

CHILDHOOD AND ADULTHOOD PASSIVE SMOKING AND MIDLIFE FATTY LIVER



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