

## **Online Supplementary Data**

### **Tobacco smoke exposure in early life and adolescence in relation to lung function**

Jesse D. Thacher,<sup>1\*</sup> Erica S. Schultz,<sup>1</sup> Jenny Hallberg,<sup>1,3,4</sup> Ulrika Hellberg,<sup>2</sup> Inger Kull,<sup>1,3,4</sup> Per Thunqvist,<sup>3,4</sup> Göran Pershagen,<sup>1,2</sup> Per M. Gustafsson,<sup>5,6</sup> Erik Melén,<sup>1,3, †</sup> and Anna Bergström,<sup>1,2, †</sup>

\*Corresponding author. Jesse Thacher, MPH, Institute of Environmental Medicine, Karolinska Institutet, Box 210, SE-171 77 Stockholm, Sweden. [Jesse.Thacher@ki.se](mailto:Jesse.Thacher@ki.se), +46 704 220 115.

†Both authors contributed equally to this work.

#### **Affiliations:**

<sup>1</sup> Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

<sup>2</sup> Centre for Occupational and Environmental Medicine, Stockholm County Council, Stockholm, Sweden

<sup>3</sup> Sachs' Children's Hospital, Södersjukhuset, Stockholm, Sweden

<sup>4</sup> Department of Clinical Science and Education, Södersjukhuset, Karolinska Institutet, Stockholm, Sweden

<sup>5</sup> The Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

<sup>6</sup> Department of Pediatrics, Central Hospital, Skövde, Sweden

## Methods

### Lung function assessment

For spirometry indices, multiple maximal expiratory flow volume measurements were performed by each participant and the highest values of forced expiratory volume in one second (FEV<sub>1</sub>), and forced vital capacity (FVC) were used in analyses [1]. The FEV<sub>1</sub>/FVC ratios were expressed as percentages. Standard deviation scores for FEV<sub>1</sub>, FVC, and FEV<sub>1</sub>/FVC were computed taking age, sex, height, and ethnicity into account [2]. The spirometer was calibrated daily using a 3-L precision syringe.

Impulse oscillometry (IOS) indices were assessed by having participants perform tidal breathing through a mouthpiece while pressure impulses were delivered from a loud speaker throughout the respiratory tract [1]. Each participant performed the manoeuvre at least twice and quality control checks were conducted by visual examination of waveforms at the time of the manoeuvre [3]. Daily accuracy checks for the IOS system were conducted using a reference resistance ( $0.20 \text{ kPa} \cdot \text{L}^{-1} \cdot \text{s}^{-1}$ ) [3].

IOS is a form of FOT (forced oscillation technique) which means that pressure oscillations are forced upon the respiratory system and that information about the mechanical properties of the respiratory system can be derived from the "opposing forces" that the respiratory system exerts. These are resistance (R) and reactance (X). By separating data with respect to frequency of signals more detailed information about the properties of the respiratory system can be further derived. For instance, frequency dependence of resistance (FDR; i.e. R5-R20, resistance at 5 Hz-resistance at 20 Hz) reflects the heterogenous distribution of peripheral resistance. Furthermore, capacitive reactance which is reflected in reactance between 5 Hz and resonant frequency reflects reduced compliance of the respiratory system and small airways in particular. The AX (area of reactance) is the integrated value of reactance with respect to frequency from 5 Hz to resonant

frequency. As such it multiplies to variables that both increase in absolute magnitude with increasing peripheral airway obstruction. However, taking the square root of AX will linearize this variable and create a more robust reactance index than any reactance value at a particular frequency. In most cases there are very good correlations between FDR and square root of AX, even in a single subject. So despite that fact that FDR and AX are different physical / mechanical properties of the respiratory system they cross-confirm each other.

### **Saliva cotinine**

Saliva collection kits were sent to participants along with detailed instructions. Saliva was collected in the morning and evening time (prior to oral hygiene) using sterile dental rolls (braided cotton dental rolls; Salivetter®, SARSTEDT AG & Co., D-51582 Nümbrecht).

Participants were instructed to keep the roll in their mouths until it was saturated with saliva and then replace it in the pre-labelled sample tube and sent directly to the laboratory via post.

Samples were centrifuged and stored at -80 degrees Celsius. A total of 20 evening samples had too little saliva and were excluded from analysis.

### **Confounder selection**

Various covariates were considered in the model using stepwise linear regression and included age, sex, height, birth weight, gestational age, weight (at age 16 years), socioeconomic status (categorized on the basis of parents' occupation as manual and non-manual workers), municipality, breast feeding (<4 months, ≥4 months), air pollution from local road traffic (using NO<sub>x</sub> as a continuous indicator[4]), puberty status, and parental history of allergic disease (asthma

or hay fever ever). Variables that changed the  $\beta$  coefficient by 10% and likelihood ratio test was different (p-value <0.05) from a more simple model were included in the final models.

The BAMSE study and all subsequent follow-ups were approved by the Regional Ethical Review Board, Karolinska Institutet, Stockholm, Sweden, and parents gave informed consent for data collection and analysis.

**Table E1.** Distribution of selected exposure characteristics among included (N = 2295) and excluded (N = 1794) study participants.

	Included participants (N = 2295)		Excluded participants (N = 1794)		<i>P-value</i> <sup>**</sup>
	n	%	n	%	
<b>Male sex</b>	1092	47.6	973	54.2	<0.001
<b>Maternal smoking during pregnancy</b> <sup>*</sup>	254	11.1	273	15.2	<0.001
<b>SHS during infancy</b>	447	19.6	408	22.8	0.01
<b>SHS exposure at 16 years</b>	271	12.4	142	16.7	<0.01
<b>Parental allergic disease</b> <sup>¶</sup>	729	32.1	471	26.6	<0.001
<b>Maternal age &lt; 26 years</b> <sup>†</sup>	160	7.0	159	8.9	0.02
<b>Socioeconomic status</b> <sup>‡</sup>					
Manual workers	320	14.2	375	21.4	<0.001
Non-manual workers	1942	85.9	1381	78.6	<0.001
<b>Breastfeeding ≥ 4 months</b>	1814	80.6	1302	78.0	0.05
<b>Furred pets at home</b> <sup>‡</sup>	323	14.1	306	17.1	0.01
<b>Type of home</b> <sup>‡</sup>					
Single family home	412	18.0	278	15.5	0.04
Multifamily building	1882	82.0	1515	84.5	0.04
<b>Adolescent smoking</b> <sup>  </sup>	280	12.2	93	11.4	0.57
	<b>Mean</b>	<b>SD</b>	<b>Mean</b>	<b>SD</b>	
<b>Birth weight (grams)</b>	3518.7	552.3	3544.4	565.2	0.15
<b>Birth length (cm)</b>	50.1	2.5	50.2	2.8	0.27
<b>Gestational age (weeks)</b>	39.8	1.8	39.8	2.1	0.99

<sup>\*</sup> Mother smoked at least one cigarette per day at any point in time during pregnancy.

<sup>†</sup> At birth of the child.

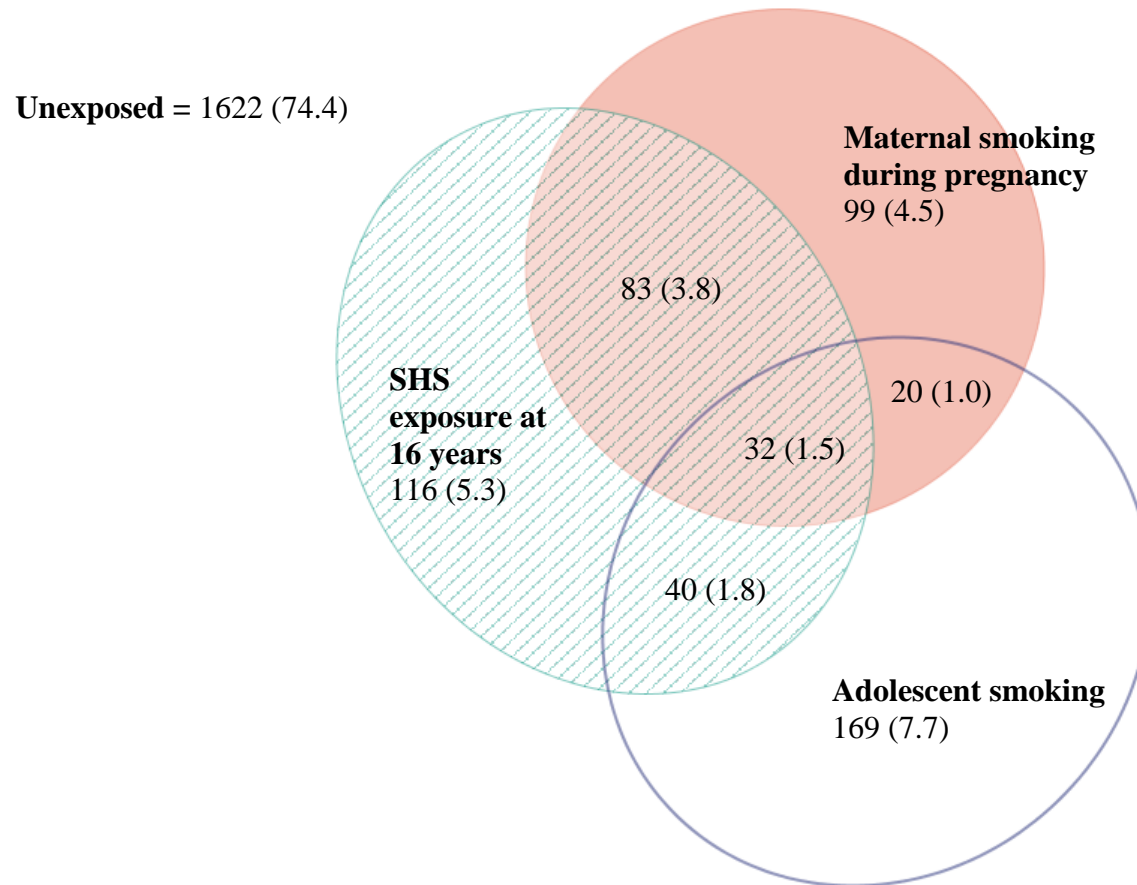
<sup>‡</sup> At baseline.

<sup>¶</sup> Mother or father with asthma or hay fever.

<sup>||</sup> Daily or occasionally.

<sup>\*\*</sup> P-values obtained from two-sample test of proportions.

**Figure E1.** Distribution of SHS smoke exposure at 16 years, adolescent smoking, and maternal smoking during pregnancy (N=2181).



**Table E2.** Differences in lung function between exposed and unexposed children to tobacco smoke and lung function at age 16 years mutually adjusted model (n=2112).

	FEV <sub>1</sub> (ml)		FVC (ml)		FEV <sub>1</sub> /FVC (%)	
	Diff.	95% CI	Diff.	95% CI	Diff.	95% CI
<b>Maternal smoking during pregnancy*</b>						
No	Reference		Reference		Reference	
Yes	-51.6	-121.9,18.7	-6.4	-89.6,76.8	-1.0	-2.1,0.02
<b>SHS exposure during infancy†</b>						
No	Reference		Reference		Reference	
Yes	-1.1	-59.4,57.2	8.2	-59.7,76.2	-0.2	-1.1,0.6
<b>SHS exposure at 16 years‡</b>						
No	Reference		Reference		Reference	
Yes	55.5	-12.7,123.6	60.7	-20.2;141.7	-0.04	-1.1;1.0
<b>Participants smoking¶</b>						
Non-smokers	Reference		Reference		Reference	
Adolescent smoking	-12.4	-72.1;47.3	6.2	-64.5;77.0	-0.8	-1.7;0.1
Occasional smokers	-32.8	-104.1;38.5	9.3	-74.6;93.3	-0.9	-1.9;0.2
Daily smokers	25.9	-72.7;124.5	-0.2	-117.3;116.9	-0.6	-2.1;0.9

\* Calculated by linear regression adjusted for SHS exposure during infancy, adolescent smoking at 16, SHS exposure at 16 years, sex, age, and height.

† Adjusted for maternal smoking during pregnancy, adolescent smoking at 16, SHS exposure at 16 years, sex, age, and height.

‡ Adjusted for maternal smoking during pregnancy, SHS exposure during infancy, adolescent smoking at 16, sex, age, and height.

¶ Adjusted for maternal smoking during pregnancy, SHS exposure during infancy, SHS exposure at 16 years, sex, age, and height.

**Table E3.** Differences in lung function between exposed and unexposed participants to tobacco smoke and lung function at age 16 years (n=2295).

	n	FEV <sub>1</sub> (ml)		FVC (ml)		FEV <sub>1</sub> /FVC (%)	
		Diff.*	95% CI	Diff.*	95% CI	Diff.*	95% CI
No MSP no adolescent smoking	1818	Reference		Reference		Reference	
Yes MSP no adolescent smoking	196	-18.9	-85.8,48.1	18.3	-60.9,97.5	-0.8	-1.8,0.1
No MSP yes adolescent smoking	222	2.1	-61.2,65.3	18.0	-56.0,92.1	-0.6	-1.6,0.2
Yes MSP yes adolescent smoking	58	-78.1	-196.1,39.9	17.6	-123.5,158.7	-2.5	-4.3,-0.7

\* Calculated by linear regression adjusted for sex, age, and height.

† Daily or occasional smoking.

MSP – maternal smoking during pregnancy.



**Table E4.** Participant's reported smoking habits by categorical cotinine levels (excluding smokeless tobacco users) (n=1468).

	<b>Cotinine level &lt;12 ng/ml</b>	<b>Cotinine level ≥12 ng/ml</b>
	<b><u>n (%)</u></b>	<b><u>n (%)</u></b>
Non-smokers	1251 (92.5)	101 (7.5)
Occasional smokers	34 (45.3)	41 (54.7)
Daily smokers	1 (2.4)	40 (97.6)

**Table E5.** Saliva cotinine levels and lung function at 16 years (restricted to children who did not use smokeless tobacco) (n=1431).

	Salivary cotinine <12 ng/ml	Salivary cotinine ≥12 ng/ml	
		Diff.*	95% CI
<b>Spirometry</b>			
FEV <sub>1</sub>	Reference	-5.8	-76.7,65.1
FVC	Reference	81.7	2.0,161.4
FEV <sub>1</sub> /FVC (%)	Reference	-1.5	-2.5,-0.4
<b>IOS</b>			
R <sub>5</sub> (Pa*L <sup>-1</sup> )*s <sup>†</sup>	Reference	-8.3	-22.3,5.7
R <sub>20</sub> (Pa*L <sup>-1</sup> )*s <sup>†</sup>	Reference	-7.1	-18.9,4.7
R <sub>5-20</sub> (Pa*L <sup>-1</sup> )*s <sup>†</sup>	Reference	3.2	-4.4,10.8
AX <sup>0.5</sup> (Pa*L <sup>-1</sup> ) <sup>0.5†</sup>	Reference	0.003	-0.78,0.79
FeNO (ppb) <sup>†</sup>	Reference	-0.4	-0.9,0.1

\* Calculated by linear regression, adjusted for sex, age, and height.

**Table E6. Association between children's smoking habits and spirometry at age 16 years stratified by current wheeze.**

	No wheeze		Any wheeze (last 12 months)	
	$\beta^\dagger$	95% CI	$\beta^\dagger$	95% CI
<b>FEV<sub>1</sub></b>				
Non-smokers		Reference		Reference
Any smoking	-22.8	-83.0, 37.4	97.7	-113.8, 309.1
<b>FVC</b>				
Non-smokers		Reference		Reference
Any smoking	24.6	-47.2, 96.5	81.9	-150.2, 314.0
<b>FEV<sub>1</sub>/FVC</b>				
Non-smokers		Reference		Reference
Any smoking	-1.1	-2.0, -0.2	0.5	-3.2, 4.1

<sup>†</sup> Calculated by linear regression on the mean adjusted for sex, age, and height.

**Table E7. Associations between children's smoking habits and lung function at 16 years stratified by current asthma.**

	No asthma			Asthma at age 16 years		
	N	$\beta^*$	95% CI	N	$\beta^*$	95% CI
<b>FEV<sub>1</sub> <i>p-value for interaction</i>= 0.04</b>						
Non-smokers	1776		Reference	141		Reference
Any smoking	249	-28.7	-87.9, 30.5	11	213.5	-60.8, 487.9
<b>FVC <i>p-value for interaction</i>= 0.41</b>						
Non-smokers	1671		Reference	143		Reference
Any smoking	233	22.2	-48.5, 92.8	11	135.1	-161.2, 431.5
<b>FEV<sub>1</sub>/FVC (%) <i>p-value for interaction</i>= 0.05</b>						
Non-smokers	1618		Reference	229		Reference
Any smoking	136	-1.2	-2.1, -0.3	11	2.1	-2.5, 6.7

\* Calculated by linear regression adjusted for sex, age, height.

**Table E8.** Associations between tobacco smoke exposure and children's lung function at 16 years stratified by sex (n=2295).

Males							Females								
<u>FEV<sub>1</sub></u> n    Diff.*    95% CI				<u>FVC</u> Diff.*    95% CI		<u>FEV<sub>1</sub>/FVC (%)</u> Diff.*    95% CI		<u>FEV<sub>1</sub></u> n    Diff.*    95% CI				<u>FVC</u> Diff.*    95% CI		<u>FEV<sub>1</sub>/FVC (%)</u> Diff.*    95% CI	
<b>Maternal smoking during pregnancy</b>															
No	974		Reference		Reference		Reference	1066		Reference		Reference		Reference	
Yes	118	-75.9	-174.9;23.0	-28.7	-147.9;90.6	-0.8	-2.2;0.5	136	-6.46	-74.2;61.2	37.3	-42.1;116.8	-1.4	-2.5;-0.2	
<b>SHS exposure during infancy</b>															
No	882		Reference		Reference		Reference	951		Reference		Reference		Reference	
Yes	204	27.7	-52.3;107.6	30.3	-63.9;124.4	0.2	-0.9;1.3	243	-35.0	-88.3;18.3	6.0	-56.3;68.4	-1.3	-2.2;-0.4	
<b>SHS exposure at 16 years</b>															
No	912		Reference		Reference		Reference	999		Reference		Reference		Reference	
Yes	133	21.5	-73.1;116.1	33.6	-80.8;147.9	-0.4	-1.7;0.9	138	47.2	-19.7;114.1	92.0	12.8;171.1	-0.9	-2.0;0.2	
<b>Participants smoking</b>															
Non-smokers	963		Reference		Reference		Reference	1052		Reference		Reference		Reference	
Any smoking	129	40.1	-55.8;136.0	49.4	-65.9;164.8	-0.4	-1.7;1.0	151	-55.7	-119.9;8.5	-4.8	-79.3;69.7	-1.3	-2.4;-0.3	
Occasional smokers	83	27.7	-88.6;144.0	78.1	-61.3;217.6	-0.4	-2.0;1.2	95	-86.8	-165.5;-8.1	-26.4	-117.7;65.0	-1.6	-2.9;-0.3	
Daily smokers	46	63.1	-92.7;219.0	-5.7	-195.2;183.8	-0.4	-2.6;1.8	56	-2.4	-103.4;98.6	32.1	-84.9;149.1	-0.9	-2.5;0.8	

\* Calculated by linear regression on the mean adjusted for sex, age, and height.

† Mother only.

**Table E9.** Associations between tobacco smoke exposure and children's lung function at age 16 years (z-scores) (n=2295).

	FEV <sub>1</sub>		FVC		FEV <sub>1</sub> /FVC (%)	
	Diff.*	95% CI	Diff.*	95% CI	Diff.*	95% CI
<b>Maternal smoking during pregnancy</b>						
No		Reference		Reference		Reference
Yes	-0.08	-0.20;0.04	0.01	-0.11;0.14	-0.17	-0.30;-0.04
<b>SHS exposure during infancy</b>						
No		Reference		Reference		Reference
Yes	-0.20	-0.12;0.08	0.02	-0.08;0.12	-0.10	-0.20;0.01
<b>SHS exposure at 16 years</b>						
No		Reference		Reference		Reference
Yes	0.07	-0.05;0.19	0.12	-0.01;0.24	-0.11	-0.24;0.03
<b>Participants smoking</b>						
Non-smokers		Reference		Reference		Reference
Any smoking	-0.04	-0.16;0.08	0.03	-0.09;0.15	-0.15	-0.28;-0.02
Occasional smokers	-0.09	-0.23;0.06	0.03	-0.12;0.18	-0.17	-0.33;-0.02
Daily smokers	0.05	-0.14;0.24	0.04	-0.15;0.23	-0.11	-0.32;0.09

\* Calculated by linear regression on the mean.

† Mother only.

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