ERJ open research
THE BEST IN OPEN ACCESS BASIC, TRANSLATIONAL & CLINICAL RESPIRATORY RESEARCH

Early View

Original research article

Maternal use of snus as smokeless tobacco in pregnancy and infant lung function

Karen Eline Stensby Bains, Ina Kreyberg, Martin Färdig, Berit Granum, Hrefna K. Gudmundsdóttir, Guttorm Haugen, Gunilla Hedlin, Katarina Hilde, Christine Monceyron Jonassen, Björn Nordlund, Eva M. Rehbinder, Corina Silvia Rueegg, Håvard O. Skjerven, Anne Cathrine Staff, Cilla Söderhäll, Riyas Vettukattil, Karin C. Lødrup Carlsen

Please cite this article as: Bains KES, Kreyberg I, Färdig M, *et al*. Maternal use of snus as smokeless tobacco in pregnancy and infant lung function. *ERJ Open Res* 2024; in press (https://doi.org/10.1183/23120541.00958-2023).

Maternal use of snus as smokeless tobacco in pregnancy and infant lung function

Karen Eline Stensby Bains, MD[1,2], Ina Kreyberg*, MD, PhD[3], Martin Färdig*, MSc[4,5], Berit Granum, PhD[6], Hrefna K. Gudmundsdóttir, MD[1,2], Guttorm Haugen, MD, PhD[2,7], Gunilla Hedlin, MD, PhD[4,5], Katarina Hilde, MD[2,7], Christine Monceyron Jonassen, PhD[8,9], Björn Nordlund, RN, PhD[4,5], Eva M. Rehbinder, MD, PhD[2,3], Corina Silvia Rueegg, PhD[10], Håvard O. Skjerven, MD, PhD [1,2], Anne Cathrine Staff, MD, PhD[2,7], Cilla Söderhäll, MSc, PhD[4,5], Riyas Vettukattil, MBBS, PhD[1,2], Karin C. Lødrup Carlsen, MD, PhD[1,2].
*Shared second authorship.

Affiliations:
1. Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway.
2. University of Oslo, Faculty of Medicine, Institute of Clinical Medicine, Oslo, Norway.
3. Department of Dermatology and Venerology, Oslo University Hospital, Oslo, Norway.
4. Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden
5. Department of Women's and Children's Health, Karolinska Institute, Stockholm, Sweden
6. Department of Environmental Health, Norwegian Institute of Public Health, Oslo, Norway.
7. Division of Obstetrics and Gynaecology, Oslo University Hospital, Oslo, Norway.
8. Department of Virology, Norwegian Institute of Public Health, Oslo, Norway.
9. Center for Laboratory Medicine, Østfold Hospital Trust, Grålum, Norway.
10. Oslo Centre for Biostatistics and Epidemiology, Oslo University Hospital, Oslo, Norway.

The study was performed within ORAACLE (the Oslo Research Group of Asthma and Allergy in Childhood; the Lung and Environment).

Address of correspondence to:
Karen Eline Stensby Bains
Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway,
Postboks 4956 Nydalen, 0424 Oslo.
E-mail: karen.eline.sh@gmail.com
Mobile: +47 97745444.

Short title: Maternal use of snus and infant lung function

Word count abstract: 249.

Word count manuscript (excluding figures and references): 3502

No. of tables: 3
No. of figures: 2

Key words: Snus, smokeless tobacco, nicotine-containing products, infant lung function, tidal flow-volume loops, PreventADALL.

Funding Source: The PreventADALL study has been funded by the following funding bodies: The Regional Health Board South East, The Norwegian Research Council, Oslo University Hospital, the University of Oslo, Health and Rehabilitation Norway, the Foundation for Healthcare and Allergy Research in Sweden – Vårdalstiftelsen, Swedish Asthma- and Allergy Association's Research Foundation, Swedish Research Council – the Initiative for Clinical Therapy Research, The Swedish Heart-Lung Foundation, SFO-V Karolinska Institutet, Østfold Hospital Trust, the European Union (MeDALL project), by unrestricted grants from the Norwegian Association of Asthma and Allergy, the Kloster Foundation, Thermo-Fisher Uppsala, Sweden, by supplying allergen reagents, Norwegian society of Dermatology and Venerology, Arne Ingel's legat. Funding information for this article has been deposited with the Crossref Funder Registry.

Financial Disclosure: The authors have no financial relationships relevant to this article to disclose, however Eva M. Rehbinder has received honoraria for lectures on atopic dermatitis from Sanofi-Genzyme, Novartis, Leo-Pharma, Perrigo and The Norwegian Asthma and Allergy Association.

Conflict of Interest: The authors have no conflicts of interest to disclose.

Clinical Trial Registration: ClincialTrials.gov NCT02449850.

What's Known on This Subject: Smoking in pregnancy is widely acknowledged to negatively affect infant respiratory function and disease, but the effects of antenatal exposure to snus in pregnancy on human lung function is largely unknown.

What This Study Adds: Exposure to snus in utero seems harmful to the developing fetal lung, with a tendency for lower post-natal function in exposed infants.

Abbreviations:
CI: confidence interval
GW: gestational week
OR: odds ratio
PreventADALL: Preventing Atopic Dermatitis and ALLergies in Children
SGA: Small for gestational age
SD: standard deviation
TFV: tidal flow-volume
t_{PTEF}: time to peak tidal expiratory flow, in seconds
t_E: expiratory time, in seconds
t_{PTEF}/t_E: time to peak tidal expiratory flow to expiratory time
V_E: expiratory volume, in milliliters
V_{PTEF}: volume at peak tidal expiratory flow, in milliliters
V_{PTEF}/V_E: volume at peak tidal expiratory flow to expiratory volume
V_T: tidal volume, in milliliters
V_T/kg: tidal volume per kilo

Abstract

Background

Smoking in pregnancy has detrimental effects on infant respiratory health, while the effects of other nicotine-containing products on infant lung function are unclear. We aimed to explore if smokeless tobacco such as snus used in pregnancy increased the risk of lower lung function in infancy and if the associations differed by sex.

Methods

From the Scandinavian population-based Preventing Atopic Dermatitis and ALLergies in Children birth cohort, we included 1163 infants with available tidal flow-volume measurements at three months of age and maternal self-reported use of nicotine-containing products in pregnancy. The risk of a ratio of time to peak tidal expiratory flow to total expiratory time <25th percentile by any nicotine exposure, snus exclusively and cigarette smoking with or without other nicotine-containing products, were explored by regression analyses adjusting for maternal age, education and asthma.

Results

Overall 120/1163 (10.3%) infants were exposed to any nicotine in-utero, 71/120 by snus exclusively and 49/120 by smoking, with six also exposed to snus. By pregnancy week six 85.8% of mothers reported stopping nicotine use. The risk of lower lung function was higher in children exposed in-utero to nicotine-containing products with an odds ratio (OR) of 1.63 (95% confidence interval 1.02,2.59) with a similar tendency for snus exclusively OR 1.55 (0.88,2.71) and smoking OR 1.79 (0.84,3.84). Effect estimates were similar after adjusting for covariates. No differences of the effect by sex was observed.

Conclusion

Our study suggests that in-utero exposure to not only cigarettes, but also snus, may negatively affect infant lung function.

Introduction

Reduced lung function in early life is associated with an increased risk of subsequent wheeze and asthma in childhood [1-3] and tracks through childhood and adolescence into adulthood, with lower initial values conferring greater risk of asthma or chronic obstructive pulmonary disease [4-6]. Observations of diminished lung function a few days after birth in neonates born to smoking mothers [7-9], point to origins of respiratory disease being established during pregnancy. Human lung development encompass lung budding in the embryonic stage, airway branching from around gestational week (GW) 7-17 and saccular development from around 24 GWs with alveolarization that continue through childhood [10]. Any of these stages may be affected by exposures [11, 12] with in-utero smoking shown to increase the risk of impaired infant lung function and increase risk for wheeze and asthma later in life [13]. Emerging evidence indicates that the negative effects of in-utero smoking exposure is largely mediated through nicotine [14], with fetal levels shown to be equal to, or even exceeding those of the mother [15].

Maternal smoking during pregnancy has decreased over the last decades worldwide [16, 17], while alternative nicotine-containing products, including smokeless tobacco, are gaining popularity in several parts of the world [18, 19]. Snus, having long traditions for use in Norway and Sweden and exempted from EU legislation on sales ban, is currently more commonly used on daily basis in young men and women compared to cigarettes [20]. Among women aged 25-49, 5% reported daily use of snus in 2015 and 11% in 2022 [21-23] and a Norwegian register study found decreasing smoking rates during pregnancy in the years 2012-2017, while snus use remained constant [24]. Snus is a moist ground tobacco product placed under the lip either as a pinched portion or a premade portion in a sachet, containing nicotine and other chemicals which are absorbed mainly through the oral mucosa. Compared to cigarettes, snus gives a slower rise of plasma nicotine concentration, that stays elevated for a longer time period after removal, resulting in increased levels of absorbed nicotine after snus [25]. Sales of e-cigarettes containing nicotine is prohibited in Norway and only legally sold in Sweden after inclusion of mothers in this study, from July 2017 [26].

Use of snus in pregnancy is associated with increased risk of adverse outcomes in the off-spring including preterm delivery [27-29], small for gestational age (SGA) [30] and stillbirth [31], mainly observed in large register studies. Studies on human infant lung function after intrauterine exposure to nicotine-containing smokeless tobacco are lacking. Animal studies on

the effect of antenatal nicotine exposure have shown deleterious effects on structural changes in lung development, postnatal lung function and respiratory health, with striking similarities to the known hazardous effects of maternal smoking [14, 17].

Lung function measurement with tidal flow-volume (TFV) loops is feasible in both healthy and sick infants and children, either awake or asleep. Reduced ratio of time to peak tidal expiratory flow to expiratory time (t_{PTEF}/t_E) in infancy is associated with later obstructive disease, wheeze and asthma [2, 32, 33]. Lower t_{PTEF}/t_E ranging from <0.254 [3, 34] in sleeping infants to <0.20 [33] and <0.30 [2] in awake infants has previously demonstrated to be associated with later respiratory disease. The t_{PTEF}/t_E has been proven sensitive to detect impaired lung function at birth in infants of smoking mothers [7], as well as improved lung function in infants of smoking mothers receiving vitamin C supplementation during pregnancy compared to placebo [35].

Previous studies have demonstrated a possible sex-dependent response to early life exposures [36]. A reduced t_{PTEF}/t_E was observed in male but not female infants born to mothers with asthma [37], to mothers who smoked during pregnancy [9, 38], and in infants with subsequent lower respiratory tract infections with wheeze [34].

We hypothesize that exposure to snus in pregnancy increases the risk of lower lung function in infancy. The primary aim of the present study was therefore to explore if snus exposure in pregnancy is associated with lower lung function in early infancy. The secondary aim was to explore if the effect of snus exposure in pregnancy on infant lung function differed by fetal sex.

Study design and methods

Study design

This study is embedded in the prospective general population-based birth cohort Preventing Atopic Dermatitis and ALLergies in Children (PreventADALL) study [39]. Briefly, 2697 non-selected women were recruited mid-pregnancy in relation to national ultrasound examination at GW 18, between December 2014 and October 2016 in Oslo and Østfold (Norway) and Stockholm (Sweden). At birth, 2397 infants born after GW 35.0 without serious neonatal disease were included. This sub-study entails 1163 infants with lung function measurement at three months of age and information on antenatal exposure to nicotine-containing products (Figure 1).

Through electronic questionnaires completed by the participating mother, information on general demographics, maternal lifestyle and exposures was collected around GWs 18 and 34, and breastfeeding around three-months post-partum. Birth mode and concurrent anthropometric measurements were recorded from electronic hospital charts. Infant lung function was measured in study sites Oslo and Stockholm from July 2015 to July 2017 as part of the scheduled three-month follow-up visits.

Written informed consent was obtained from the women at primary enrolment in pregnancy and by both parents at newborn inclusion. The study was approved by the regional committees for medical and health research ethics in South-Eastern Norway (2014/518) and in Sweden (2014/2242-31/4). The study was registered at ClinicalTrials.gov (number NCT02449850).

Data collection

Detailed information on use of snus, cigarette smoking, electronic cigarettes, nicotine replacement therapies or other nicotine-containing products was obtained from electronic self-reported questionnaires; at enrolment around GW 18 for the period prior to pregnancy and by two-week intervals during pregnancy, and at GW 34 for the period since completing the previous. Questions regarding snus and cigarettes were mutually exclusive regarding time of use prior to and/or current use in pregnancy, including details quantifying products used and week of cessation when applicable, as described in detail elsewhere [40].

As tidal breathing parameters differ in awake versus sleeping tests [41], lung function was primarily measured in the awake state at three months of age with the infant in a supine

position with head and neck in neutral position, using the Exhalyzer ® D (EcoMedics, Duernten, Switzerland) equipment [42]. Measurement of tidal breathing was attempted in all children at the three-month follow-up in the study sites Oslo and Stockholm. When not feasible in the awake state, due to an uneasy or crying child or failure to obtain stable tidal breathing, TFV loops were obtained in supine naturally sleeping infants, and for some when possible in both arousal states. Evaluation of TFV loops and post-processing analyses were performed according to standardized criteria as described elsewhere [42].

Outcomes, explanatory variables and confounders

The primary outcome was low lung function defined as t_{PTEF}/t_E below the 25th percentile in awake infants and the secondary outcome was low lung function in sleeping infants. Further lung function outcome was tidal volume per kilo (V_T/kg) as a continuous variable. As there is no consensus on a cut-off limit differentiating between lower lung function by tidal breathing and association with respiratory disease, we used t_{PTEF}/t_E <0.25 in awake infants and <0.20 in sleeping infants for sensitivity analyses.

We generated two exposure variables to nicotine containing products: First no nicotine in pregnancy ("never") versus any use of nicotine ("any nicotine"), including cigarettes, snus and other nicotine-containing products. Second, nicotine exposure was categorized into "never", snus use exclusively ("snus"), and smoking including those using snus in addition to cigarettes or other nicotine-containing products ("smoke/dual") as we explored the effect of snus alone on infant lung function. Household smoking during pregnancy was not included due to minimally reported exposure (three mothers only, see online supplement for details).

We identified the following confounders to be included in the multivariable models, based on a directed acyclic graph (Supplementary Figure 1): maternal age, maternal asthma (yes, no) and maternal education (<4 years, ≥4 years). Only factors arising before pregnancy affecting both the exposure and the outcome were considered.

Statistical analysis

Categorical variables are presented with numbers and percentages; continuous variables with means, standard deviation (SD) or range. We assessed associations between nicotine exposure in utero and low lung function with univariable and multivariable regression models, and report adjusted and unadjusted odds ratios (ORs) with 95% confidence intervals (CI) and p-

values from logistic regression models for t_{PTEF}/t_E, and beta-coefficients with 95% CI and p-values for V_T/kg from linear regression models. Missing data on maternal education and maternal asthma were defined as separate categories in the multivariable models. For the sensitivity analyses, we performed exact logistic regression due to small samples in the exposed groups. All statistical analyses were conducted separately according to arousal state, and lung function tests for infants with measurements in both arousal states were included in the analysis of both awake and sleeping tests. To explore potential differential associations by fetal sex, an interaction between fetal sex and nicotine exposure was included in the multivariable logistic and linear regression models. The significance level was set to 5%. Analyses were performed using SPSS Statistics (version 29; IBM, Chicago, IL, USA) for the logistic regression, and Stata/SE (version 16.1; StataCorp LLC, College station, TX) for the linear regression and sensitivity analyses.

Results

Among the 1163 infants included in this study, 49% were girls, born at mean±SD GW 40.0±1.4 with mean±SD birth weight of 3.55±0.48 kg. At the time of TFV measurement their mean±SD age was 13.2±1.0 weeks and mean±SD weight 6.23±0.78 kg. The included infants (Figure 1) were largely comparable to not included infants except a higher proportion of girls, mothers of higher age and education level, lower birth weight and lower age at the three-month follow-up visit (Supplementary table 1). Background characteristics among infants of mothers who used nicotine-containing products in pregnancy were similar to non-exposed infants (Table 1). Lung function measurements were available in 881 awake and 371 sleeping infants; 89 infants had measurements in both arousal states.

Any use of nicotine-containing products during pregnancy was reported by 120/1163 women (10.3%), of whom 71 (59.2%) reported use of snus exclusively. Among the 49 (40.8%) women categorized to "smoke/dual", 42 mothers reported use of cigarettes only, six were dual users of cigarettes and snus, and one reported use of other nicotine-containing products. None of the participants reported use of e-cigarettes or nicotine replacement therapy. By GW six, 103/120 (85.8%) reported quitting use of any nicotine-containing products, whereas eight women (6.7%) continued use of nicotine-containing products after pregnancy week 30.

The mean±SD (range) t_{PTEF}/t_E in awake infants was 0.39±0.08 (0.19-0.63) and 0.27±0.07 (0.13-0.47) in sleeping infants. The 25th percentile cut off value for t_{PTEF}/t_E was 0.33 and 0.23 in awake and sleeping infants, respectively.

The risk for having a t_{PTEF}/t_E below the 25th percentile among awake infants was significantly increased by in-utero exposure to any type of nicotine-containing products during pregnancy (OR 1.63 (1.02,2.59), p=0.040; Figure 2 and Table 2 in the univariable analysis. After adjusting for maternal age, maternal education and maternal asthma, the estimate was similar but no longer statistically significant (OR 1.51 (0.94,2.43), p=0.086; Figure 2 and Table 2. When looking at exclusive snus use during pregnancy, effect estimates were similar and pointed towards an increased risk for reduced lung function (OR 1.44 (0.82, 2.54) in the multivariable model, Table 2). The risk was similar for children exposed to smoking (with or without snus) (OR 1.67, (0.77, 3.60)) in the multivariable model. Both estimates did not reach statistical significance. Effect estimates were smaller and not significant in sleeping infants (Table 2). In the sensitivity analyses, the risk of t_{PTEF}/t_E <0.25, as observed in 4/881 awake

infants whom were exposed to nicotine-containing products in-utero, and <0.20, as observed in 4/371 sleeping infants, was not statistically significant with OR 0.79 (0.20, 2.25) and OR 0.81 (0.20, 2.43), respectively.

Sleeping infants exposed to any nicotine in-utero had a significantly higher means of V_T/kg compared to non-exposed infants (beta-coefficient 0.70 (0.06, 1.34)) in the multivariable model. There were no significant differences observed among infants measured in the awake state or stratified to separate nicotine-containing products (Table 3).

The associations between in-utero nicotine exposure and infant lung function did not differ by infant sex, all $P_{interaction}$>0.065 (Table 2 and Table 3).

Discussion

Infants in this non-selected population-based birth-cohort study born to the 10.3% of women who used snus, cigarettes or other nicotine-containing products during pregnancy, had an increased risk of low lung function at three months of age compared to non-exposed infants. In general, two thirds of the total nicotine exposure was through exclusive snus-use and nicotine exposure ceased around GW six in 85.8%. Exclusive snus use, as well as smoke/dual use, tended to increase the risk of lower lung function in the three-month old infants, although not reaching statistical significance. A small increase in difference in means for V_T/kg was observed in sleeping infants exposed to any nicotine, while the other analyses on V_T/kg as well as the sensitivity analyses for t_{PTEF}/t_E <0.25 in awake and <0.20 in sleeping infants did not reveal significant associations. No differential effect on the association between nicotine exposure and lung function was detected for male and female infants.

To our knowledge, this is the first prospective mother-child study exploring the effects of in-utero snus exposure on human infant lung function. The increased risk of low lung function at three months of age by in-utero exposure to nicotine dominated by snus use is partly novel and is supported by previous studies demonstrating reduced lung function in infants of smoking mothers [7-9]. We are not aware of previous human studies exploring the potential effects of in-utero smokeless tobacco exposure on infant lung function, however one registry study found increased risk of asthma/wheeze in children of mothers using snus before and in early pregnancy [43], and the findings in this study are in line with the suggested aberrant effects on lung function and development being largely attributed to nicotine per se [14]. The risk of low lung function was only statistically significant among awake infants exposed to any nicotine. However, effect estimates in infants exposed to maternal snus exclusively, were similar to that of any nicotine exposure, although somewhat lower than for the one third exposed to smoking including dual use with snus, neither reaching statistical significance, probably due to small numbers.

In contrast to a previous birth cohort study from the 1990's where reduced lung function was associated with in-utero maternal cigarette smoking reported by 27% of mothers [7], any nicotine exposure was reported by 10.3% of mothers in the present study, with two thirds being exposed to snus only, in line with the shift from use of cigarettes to snus in recent years in the Scandinavian populations [21, 22]. Previously, a dose-dependent increased risk of extreme premature (GW <28) has been associated with continued use of snus and cigarettes

after GW 8-12 [27]. Further, women who ceased the use of snus or cigarettes early in pregnancy had no increased risk of premature birth compared to non-users of tobacco, while mothers using snus or cigarettes throughout pregnancy had an increased risk of both very and moderately premature birth [28] as well as both preterm and term offspring being born SGA, but not if the use of snus ceased before pregnancy week 8-12. In contrast, for smoking the association with term SGA was evident even for early-pregnancy use of cigarettes [30]. While Hoo et al. [9] examined premature infants where smoking in pregnancy was defined as smoking after pregnancy week four and found a significantly lower t_{PTEF}/t_E ratio in exposed infants, most women in our study stopped the use of nicotine-containing products before pregnancy week six, and yet we found an increased risk for reduced t_{PTEF}/t_E in infants exposed to any nicotine-containing products in-utero and similar effect estimates when stratifying for exclusive snus use. Overall, the in-utero snus and smoking exposure in the PreventADALL study appears relatively limited and of shorter duration compared to previous studies demonstrating aberrant effect of smoking on off-spring birth outcomes and lung function [7-9]. Collectively therefore it is likely that our study was underpowered to conclude on the risk of lower lung function by exclusive in-utero snus exposure.

The ratio of t_{PTEF}/t_E reflects the degree to which expiratory flow and timing are modulated, however the exact effect of nicotine on infant lung function is not fully known. As summarized by Spindel et al., antenatal nicotine exposure in different animal models resulted in reduced lung function, increased hyperreactivity and morphological changes in the lung, consistent with alterations in infants of smoking mothers [14]. Although the critical period for exposure to nicotine in pregnancy is unclear, studies in mice suggest a primary effect of nicotine on airway growth on conducting airways rather than the alveolarization period, with increased number of airways of small diameter with nicotine exposure [44]. Lødrup Carlsen et al. found declining ratios of t_{PTEF}/t_E with increasing exposure to cigarettes in pregnancy [7], although the study did not explore potential critical exposure periods for infant lung function impairment. Supporting the findings in the present study, where 85.8% of the women stopped snus use or smoking by pregnancy week six, in a pooled analysis of eight birth cohorts in Europe, maternal smoking only in the first trimester, but not later in pregnancy or in the first year postnatally, was associated with an increased risk of wheeze and asthma at 4-6 years of age [13]. Collectively, one may therefore speculate that exposure to nicotine including smokeless tobacco may be particularly critical in early organogenesis. Our results suggest that exposure to nicotine-containing products early in pregnancy may impact lung budding starting

in the fourth week of fetal life, preceding airway branching which completes during the second trimester, with potential life-long effects demonstrated by tracking of lung function through life [17].

We did not observe significant differences for t_{PTEF}/t_E in nicotine-exposed versus non-exposed sleeping infants. We are not aware of other studies exploring the effect of exposure to nicotine-containing products in-utero comparing arousal states, as previous studies have been conducted in either awake or sleeping infants [7-9, 35]. The significant increase in mean difference in V_T/kg in sleeping infants exposed to any nicotine in the multivariable model only, is in contrast to a previous study where no difference in V_T/kg was detected between infants exposed to smoking in utero or not [9], however statistical methods differed. No significant findings in the sensitivity analyses may be due to small sample size in single strata.

The lack of differential association by sex of exposure to nicotine-containing products antenatally and lung function is in contrast to other studies where boys exposed to maternal smoking in utero were found to have lower lung function compared to girls [9, 38]. However, the non-significant findings could also be a result of low power in our dataset, different statistical methods and lung function measurement techniques.

Strengths and limitations

A strength of this study is the prospective design of a large population of generally healthy infants with detailed questionnaires on use of nicotine-containing products reported during pregnancy to avoid recall bias. The lung function measurements were conducted by trained study personnel and standardized according to international guidelines and a standard operating procedure [42]. The maternal population is representative for the general female Scandinavian population with low smoking rates and higher prevalence of snus use.

Due to the relatively infrequent nicotine use during pregnancy in this non-selected population of pregnant women, we did not quantify the amount of tobacco use. As none reported use of e-cigarettes and household smoking was rare, these were not included in further analyses. Largely healthy term infants were included in this study, and we cannot rule out a possible under-observation of adverse outcomes related to snus exposure in preterm infants [28]. We did not asses and could thus not control for exposure to nicotine through breastfeeding. Lung function measurements were only obtained in two out of three study cites due to

organizational considerations, limiting our group of participants. We were unable to quantify a potential contribution of nicotine or other hazardous constitutes in snus such as tobacco specific nitrosamines (TSNAs) that may also adversely impact the fetus [17, 20], although snus lacks combustion products such as carbon monoxide.

Clinical implications

At present, it is not clear if the effects of snus use in pregnancy are similar to, or less detrimental on lung function and development than is smoking, and our study could not confirm or reject this. This study adds to the hypothesis that nicotine-related alterations in lung development can occur in the first weeks of fetal life, even before the woman knows she is pregnant. Advances in recent years regarding reduced smoking rates in pregnancy could be lost if knowledge on the likely detrimental effects of nicotine from other nicotine-containing products are not adequately addressed.

Conclusion

Our study suggests that not only exposure to cigarette smoking but also other nicotine-containing products such as snus during pregnancy may negatively affect early life infant lung function. Our study supports the advice against any use of nicotine-containing products in pregnancy.

Acknowledgements

We sincerely thank all families participating in the PreventADALL (Preventing Atopic Dermatitis and ALLergies in children) study as well as study personnel who have contributed to recruit participants, perform lung function measurements and other relevant examinations and in general managing the study:

Live S. Nordhagen, RN[1], Carina Saunders, MD,[2,3], Kim A. M. S. Endre, MD, PhD[4], Oda C. Lødrup Carlsen, MD[5], Runa H. Kaldestad[2], Sandra Götberg[6,7], Caroline-Aleksi Olsson Mägi, PhD, [6,7], Sandra G. Tedner, MD, PhD[6,7], Ellen Tegnerud[6,7]. In memoriam Kai-Håkon Carlsen.

Affiliations:

1. VID Specialized University, Oslo, Norway.
2. Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway.
3. University of Oslo, Faculty of Medicine, Institute of Clinical Medicine, Oslo, Norway.
4. Department of Dermatology and Venerology, Oslo University Hospital, Oslo, Norway.
5. Department of Acute Psychiatry, Akershus University Hospital, Oslo, Norway.
6. Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden.
7. Department of Women´s and Children´s Health, Karolinska Institute, Stockholm, Sweden.

Tables

Table 1. Background characteristics of 1163 mother-child pairs included in the present study from the PreventADALL cohort.

Characteristics	Infants exposed to nicotine in utero n=120		Infants not exposed to nicotine in utero n=1043	
	n	**Mean (SD) or %**	**n**	**Mean (SD) or %**
Mothers				
Age mother (years)	120	32.0 (3.8)	1043	33.0 (4.0)
Maternal education ≥4 years	54/120	45%	631/956	66%
Married/cohabitant mother	115/120	96%	943/963	98%
Parity <1 (nullipara)	82/120	68%	619/1043	59%
Maternal asthma	24/120	20%	156/959	16%
Infants (birth)				
Girl	66/120	55%	506/1043	49%
Gestational age at birth (weeks)	119	40.2 (1.4)	1039	40.0 (1.4)
Caesarian section	20/120	17%	170/1041	16%
Birth weight (kg)	120	3.56 (0.4)	1037	3.55 (0.5)
Infants (3 months)				
Age (weeks)	119	13.3 (1.1)	1042	13.2 (1.0)
Weight (kg)	119	6.26 (0.8)	1040	6.23 (0.8)
Length (cm)	118	61.8 (2.4)	1030	61.8 (2.4)
Breastfed exclusively at 3 months	63/103	61%	615/900	68%

Data are presented as n, mean (SD) or %. The group "Infants exposed to nicotine in utero" entails all infants of mothers reporting any use of nicotine-containing products in pregnancy, including snus, cigarettes, dual use of snus and cigarettes or other nicotine-containing products.
Abbreviations: SD – standard deviation, n – number of subjects.

Table 2. Association between in-utero exposure to nicotine-containing products and infant t_{PTEF}/t_E below the 25th percentile at three months of age from logistic regression models, in awake (n=224) and sleeping (n=94) infants.

	Awake t_{PTEF}/t_E <25th percentile							**Sleeping t_{PTEF}/t_E <25th percentile**						
	n	**Univariable OR (95% CI)**	**p-value**	**n**	**Multivariable OR (95% CI)**	**p-value**	**$p_{interaction}$**	**n**	**Univariable OR (95% CI)**	**p-value**	**n**	**Multivariable OR (95% CI)**	**p-value**	**$p_{interaction}$**
Nicotine exposure			*0.040*			0.086	0.876			0.540			0.757	0.940
Never (ref.)	791	1.0		791	1.0			330	1.0		330	1.0		
Any nicotine	90	*1.63 (1.02-2.59)*		90	1.51 (0.94-2.43)			41	1.25 (0.61-2.57)		41	1.12 (0.54-2.34)		
Nicotine exposure			0.114			0.217	0.987			0.824			0.951	0.918
Never (ref.)	791	1.0		791	1.0			330	1.0		330	1.0		
Snus only	60	1.55 (0.88-2.71)		60	1.44 (0.82-2.54)			21	1.21 (0.45-3.22)		21	1.09 (0.41-2.94)		
Smoke/dual	30	1.79 (0.84-3.84)		30	1.67 (0.77-3.60)			20	1.30 (0.48-3.48)		20	1.15 (0.42-3.17)		

The reference group "never" includes all females who did not report use of tobacco or nicotine in pregnancy. The "any nicotine" group includes all women who reported use of any nicotine containing products at any time during pregnancy. The "snus" group includes women who reported use of snus exclusively during pregnancy. The "smoke/dual" group include smokers and dual smokers and snus users during pregnancy, including one woman reporting use of other nicotine-containing products than cigarettes and snus.
Covariates used in the multivariable analysis: maternal age, maternal asthma, maternal education level. The interaction term included infant sex by nicotine exposure.
Abbreviations: n – number of subjects, CI – confidence interval, ref. – reference.

Table 3. Association between in-utero exposure to nicotine-containing products and infant V_T/kg at three months of age from linear regression models, in awake and sleeping infants.

Awake V_t/kg								
	n	**Univariable difference in means* (95% CI)**	**p-value**	**Marginal means* (95%CI)**	**Multivariable difference in means* (95% CI)**	**p-value**	**Marginal means* (95% CI)**	**p_{int}**
Nicotine exposure			0.132			0.079		0.067
Never	784	Ref.		7.03 (6.88,7.18)	Ref.		7.02 (6.87,7.17)	
Any nicotine	89	0.36 (-0.11,0.83)		7.39 (6.94,7.83)	0.43 (-0.05,0.90)		7.45 (7.00,7.90)	
Nicotine exposure			0.267				0.170	0.065
Never	785	Ref.		7.03 (6.88,7.18)	Ref.		7.02 (6.87,7.17)	
Snus	59	0.46 (-0.11,1.03)		7.49 (6.94,8.03)	0.53 (-0.04,1.11)		7.55 (7.00,8.11)	
Smoke/dual	30	0.17 (-0.61,0.95)		7.19 (6.43,7.96)	0.22 (-0.57,1.01)		7.24 (6.47,8.01)	
Sleeping V_t/kg								
	n	**Univariable difference in means* (95% CI)**	**p-value**	**Marginal means* (95% CI)**	**Multivariable difference in means* (95% CI)**	**p-value**	**Marginal means* (95% CI)**	**p_{int}**
Nicotine exposure			0.058			*0.032*		0.971
Never	329	Ref.		7.78 (7.57,7.99)	Ref.		7.77 (7.56,7.98)	
Any nicotine	41	0.61 (-0.22,1.24)		8.39 (7.79,8.98)	0.70 (0.06,1.34)		8.47 (7.87,9.07)	
Nicotine exposure			0.156			0.093		0.999
Never	329	Ref.		7.78 (7.57,7.99)	Ref.		7.77 (7.56,7.98)	
Snus	21	0.70 (-0.15,1.56)		8.48 (7.65,9.31)	0.76 (-0.10,1.62)		8.53 (7.69,9.36)	
Smoke/dual	20	0.51 (-0.37,1.38)		8.29 (7.43,9.14)	0.64 (-0.25,1.53)		8.41 (7.55,9.27)	

The reference group "never" includes all females who did not report use of tobacco or nicotine in pregnancy. The "any nicotine" group includes all women who reported use of any nicotine containing products at any time during pregnancy. The "snus" group includes women who reported use of snus exclusively during pregnancy. The "smoke/dual" group includes smokers and dual smokers and snus users during pregnancy, including one woman reporting use of other nicotine-containing products than cigarettes and snus.

Covariates used in the multivariable analysis: maternal age, maternal asthma, maternal education level. The interaction term included infant sex by nicotine exposure.

Abbreviations: n – number of subjects, CI – confidence interval, ref. – reference value, p_{int} – $p_{interaction}$

* Given in milliliters per kilo

References

1. Yuksel B, Greenough A, Giffin F, et al. Tidal breathing parameters in the first week of life and subsequent cough and wheeze. *Thorax* 1996: 51(8): 815-818.
2. Haland G, Carlsen KC, Sandvik L, et al. Reduced lung function at birth and the risk of asthma at 10 years of age. *N Engl J Med* 2006: 355(16): 1682-1689.
3. Young S, Arnott J, Le Souef PN, et al. Flow limitation during tidal expiration in symptom-free infants and the subsequent development of asthma. *J Pediatr* 1994: 124(5 Pt 1): 681-688.
4. Stern DA, Morgan WJ, Wright AL, et al. Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study. *Lancet* 2007: 370(9589): 758-764.
5. Guerra S, Lombardi E, Stern DA, et al. Fetal Origins of Asthma: A Longitudinal Study from Birth to Age 36 Years. *Am J Respir Crit Care Med* 2020: 202(12): 1646-1655.
6. Bui DS, Lodge CJ, Burgess JA, et al. Childhood predictors of lung function trajectories and future COPD risk: a prospective cohort study from the first to the sixth decade of life. *Lancet Respir Med* 2018: 6(7): 535-544.
7. Lodrup Carlsen KC, Jaakkola JJ, Nafstad P, et al. In utero exposure to cigarette smoking influences lung function at birth. *Eur Respir J* 1997: 10(8): 1774-1779.
8. Stick SM, Burton PR, Gurrin L, et al. Effects of maternal smoking during pregnancy and a family history of asthma on respiratory function in newborn infants. *Lancet* 1996: 348(9034): 1060-1064.
9. Hoo AF, Henschen M, Dezateux C, et al. Respiratory function among preterm infants whose mothers smoked during pregnancy. *Am J Respir Crit Care Med* 1998: 158(3): 700-705.
10. Kurihara C, Kuniyoshi KM, Rehan VK. Preterm Birth, Developmental Smoke/Nicotine Exposure, and Life-Long Pulmonary Sequelae. *Children (Basel)* 2023: 10(4).
11. Stocks J, Hislop A, Sonnappa S. Early lung development: lifelong effect on respiratory health and disease. *Lancet Respir Med* 2013: 1(9): 728-742.
12. Martinez FD. Early-Life Origins of Chronic Obstructive Pulmonary Disease. *N Engl J Med* 2016: 375(9): 871-878.
13. Neuman A, Hohmann C, Orsini N, et al. Maternal smoking in pregnancy and asthma in preschool children: a pooled analysis of eight birth cohorts. *Am J Respir Crit Care Med* 2012: 186(10): 1037-1043.
14. Spindel ER, McEvoy CT. The Role of Nicotine in the Effects of Maternal Smoking during Pregnancy on Lung Development and Childhood Respiratory Disease. Implications for Dangers of E-Cigarettes. *Am J Respir Crit Care Med* 2016: 193(5): 486-494.
15. Luck W, Nau H, Hansen R, et al. Extent of nicotine and cotinine transfer to the human fetus, placenta and amniotic fluid of smoking mothers. *Dev Pharmacol Ther* 1985: 8(6): 384-395.
16. Lange S, Probst C, Rehm J, et al. National, regional, and global prevalence of smoking during pregnancy in the general population: a systematic review and meta-analysis. *Lancet Glob Health* 2018: 6(7): e769-e776.
17. McEvoy CT, Spindel ER. Pulmonary Effects of Maternal Smoking on the Fetus and Child: Effects on Lung Development, Respiratory Morbidities, and Life Long Lung Health. *Paediatr Respir Rev* 2017: 21: 27-33.
18. O'Connor R, Schneller LM, Felicione NJ, et al. Evolution of tobacco products: recent history and future directions. *Tob Control* 2022: 31(2): 175-182.

19. Sieminska A, Jassem E. The many faces of tobacco use among women. *Med Sci Monit* 2014: 20: 153-162.
20. Folkehelseinstituttet. Helserisiko ved snusbruk. https://www.fhi.no/globalassets/bilder/rapporter-og-trykksaker/2019/helserisiko-ved-snusbruk-rapport-2019-v2.pdf, 2019.
21. Folkehelseinstituttet. Folkehelserapporten - Røyking og snusbruk i Noreg. 2022 [cited 2023 22.05.23]; Available from: https://www.fhi.no/nettpub/hin/levevaner/royking-og-snusbruk-i-noreg/
22. Folkhälsomyndigheten. Vuxnas bruk av tobaks- och nicotinprodukter. [cited 2023 22.05.23]; Available from: https://www.folkhalsomyndigheten.se/livsvillkor-levnadsvanor/andts/utveckling-inom-andts-anvandning-och-ohalsa/anvandning/anvandning-av-tobaks-och-nikotinprodukter/vuxnas-bruk-av-tobaks--och-nikotinprodukter/
23. Folkehelseinstituttet. Utbredelse av snusbruk i Norge. https://www.fhi.no/le/royking/tobakkinorge/bruk-av-tobakk/utbredelse-av-snusbruk-i-norge/?term=, 2023.
24. Rygh E, Gallefoss F, Grotvedt L. Trends in maternal use of snus and smoking tobacco in pregnancy. A register study in southern Norway. *BMC Pregnancy Childbirth* 2019: 19(1): 500.
25. (SCENIHR). SCoEaNIHR. Health effects of smokeless tobacco products. . Brussels, European Comission 2008 [cited 03.11.23]; Available from: https://ec.europa.eu/health/ph_risk/committees/04_scenihr/docs/scenihr_o_013.pdf
26. Reduction GSoTH. Smoking, vaping, HTP, NRT and snus in Sweden. 2024 [cited; Available from: https://gsthr.org/countries/profile/swe/
27. Dahlin S, Gunnerbeck A, Wikstrom AK, et al. Maternal tobacco use and extremely premature birth - a population-based cohort study. *BJOG* 2016: 123(12): 1938-1946.
28. Baba S, Wikstrom AK, Stephansson O, et al. Influence of smoking and snuff cessation on risk of preterm birth. *Eur J Epidemiol* 2012: 27(4): 297-304.
29. Wikstrom AK, Cnattingius S, Galanti MR, et al. Effect of Swedish snuff (snus) on preterm birth. *BJOG* 2010: 117(8): 1005-1010.
30. Baba S, Wikstrom AK, Stephansson O, et al. Changes in snuff and smoking habits in Swedish pregnant women and risk for small for gestational age births. *BJOG* 2013: 120(4): 456-462.
31. Wikstrom AK, Cnattingius S, Stephansson O. Maternal use of Swedish snuff (snus) and risk of stillbirth. *Epidemiology* 2010: 21(6): 772-778.
32. Hevroni A, Goldman A, Blank-Brachfeld M, et al. Use of tidal breathing curves for evaluating expiratory airway obstruction in infants. *J Asthma* 2018: 55(12): 1331-1337.
33. Lodrup Carlsen KC, Carlsen KH, Nafstad P, et al. Perinatal risk factors for recurrent wheeze in early life. *Pediatr Allergy Immunol* 1999: 10(2): 89-95.
34. Martinez FD, Morgan WJ, Wright AL, et al. Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. *N Engl J Med* 1988: 319(17): 1112-1117.
35. McEvoy CT, Schilling D, Clay N, et al. Vitamin C supplementation for pregnant smoking women and pulmonary function in their newborn infants: a randomized clinical trial. *JAMA* 2014: 311(20): 2074-2082.
36. Liptzin DR, Landau LI, Taussig LM. Sex and the lung: Observations, hypotheses, and future directions. *Pediatr Pulmonol* 2015: 50(12): 1159-1169.

37. de Gouveia Belinelo P, Collison AM, Murphy VE, et al. Maternal asthma is associated with reduced lung function in male infants in a combined analysis of the BLT and BILD cohorts. *Thorax* 2021: 76(10): 996-1001.
38. Li YF, Gilliland FD, Berhane K, et al. Effects of in utero and environmental tobacco smoke exposure on lung function in boys and girls with and without asthma. *Am J Respir Crit Care Med* 2000: 162(6): 2097-2104.
39. Lodrup Carlsen KC, Rehbinder EM, Skjerven HO, et al. Preventing Atopic Dermatitis and ALLergies in Children-the PreventADALL study. *Allergy* 2018: 73(10): 2063-2070.
40. Kreyberg I, Bains KES, Carlsen KH, et al. Stopping when knowing: use of snus and nicotine during pregnancy in Scandinavia. *ERJ Open Res* 2019: 5(2).
41. Bains KES, Fardig M, Gudmundsdottir HK, et al. Infant tidal flow-volume parameters and arousal state. *ERJ Open Res* 2022: 8(4).
42. Bains KES, Gudmundsdottir HK, Fardig M, et al. Infant lung function: criteria for selecting tidal flow-volume loops. *ERJ Open Res* 2022: 8(4).
43. Lundholm C, Gunnerbeck A, D'Onofrio BM, et al. Smoking and snuff use in pregnancy and the risk of asthma and wheeze in pre-schoolchildren-A population-based register study. *Clin Exp Allergy* 2020: 50(5): 597-608.
44. Wongtrakool C, Wang N, Hyde DM, et al. Prenatal nicotine exposure alters lung function and airway geometry through alpha7 nicotinic receptors. *Am J Respir Cell Mol Biol* 2012: 46(5): 695-702.

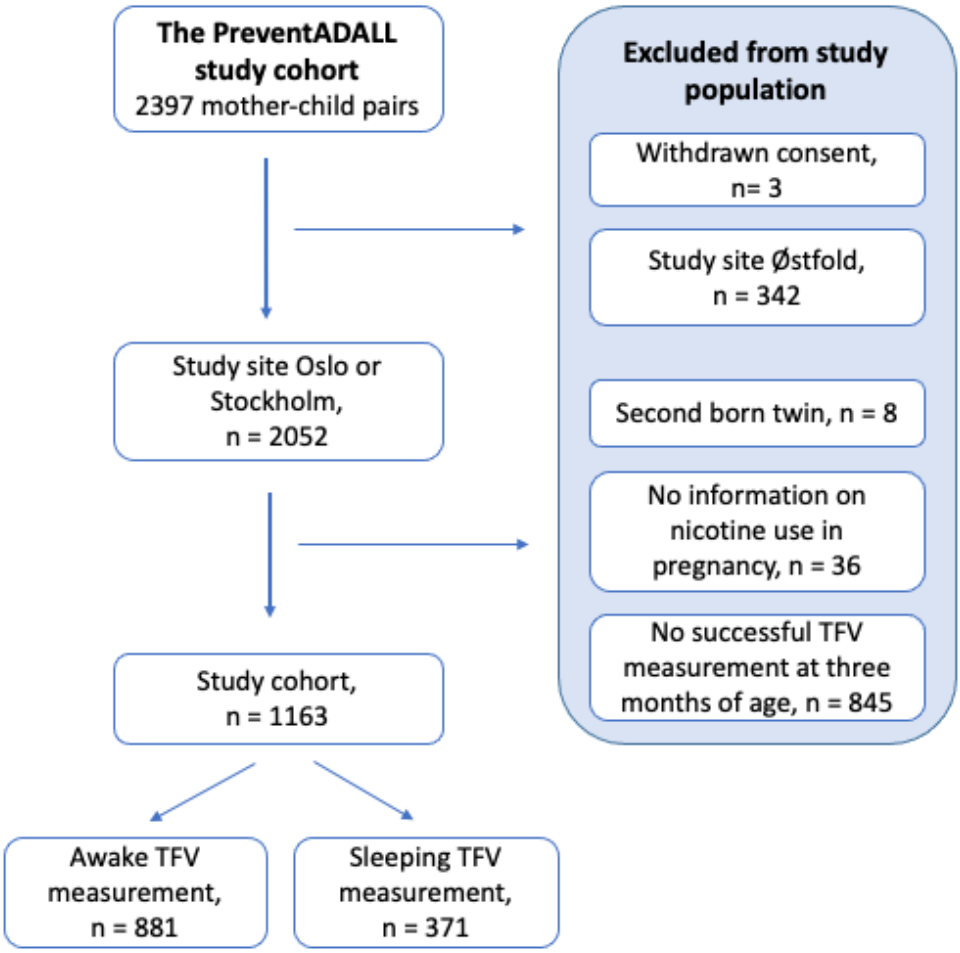
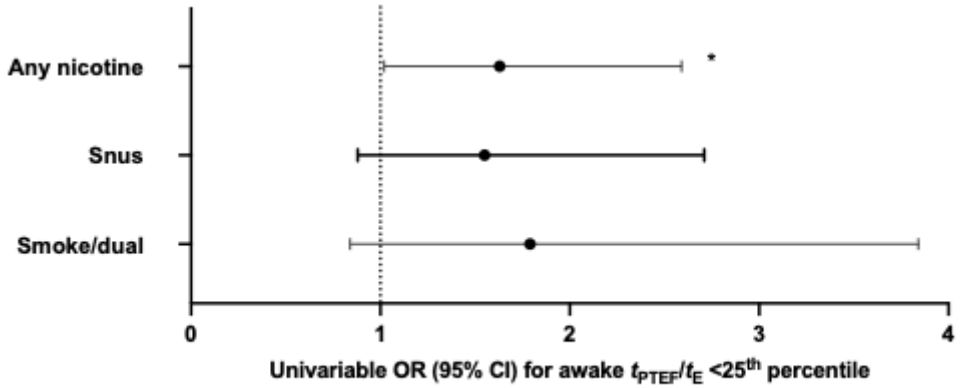

The PreventADALL study cohort
2397 mother-child pairs
Excluded from study population
Withdrawn consent, n= 3
Study site Østfold, n = 342
Study site Oslo or Stockholm, n = 2052
Second born twin, n = 8
No information on nicotine use in pregnancy, n = 36
No successful TFV measurement at three months of age, n = 845
Study cohort, n = 1163
Awake TFV measurement, n = 881
Sleeping TFV measurement, n = 371

Figure 1


Any nicotine
Snus
Smoke/dual
*
0
1
2
3
4
Univariable OR (95% CI) for awake t_{PTEF}/t_E <25th percentile

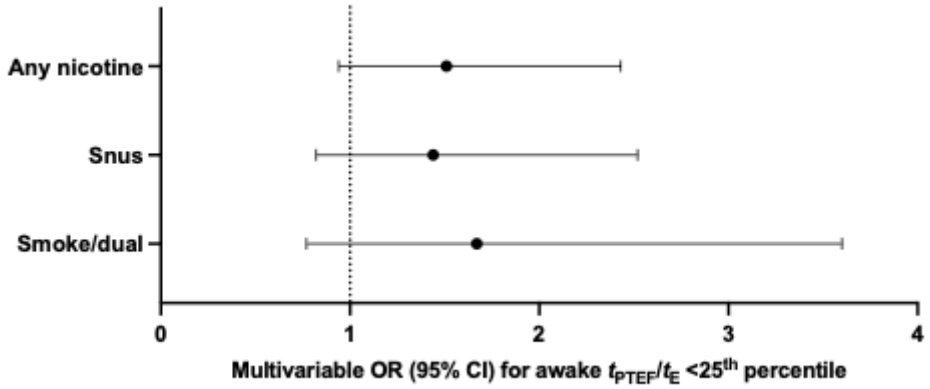

Any nicotine
Snus
Smoke/dual
0
1
2
3
4
Multivariable OR (95% CI) for awake t_{PTEF}/t_E <25th percentile

Figure 2

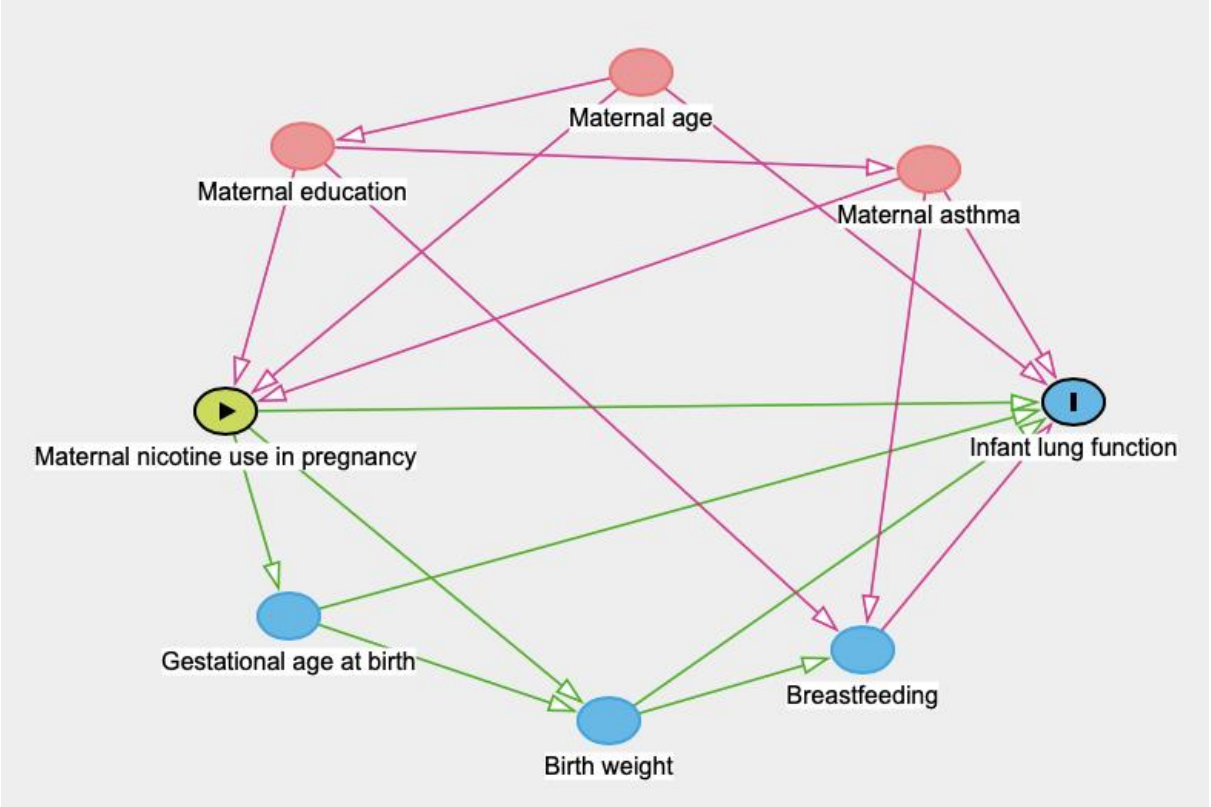

Maternal age
Maternal education
Maternal asthma
Maternal nicotine use in pregnancy
Infant lung function
Gestational age at birth
Breastfeeding
Birth weight

Figure 3

Supplementary Table 1. Baseline characteristi`cs of the 1163 included infants in the present PreventADALL sub-study with lung function measurements and of the 881 remaining infants, all from the study locations in Oslo, Norway, and Stockholm, Sweden.

Characteristics	Included infants, n=1163		Remaining cohort, n=881		
	N	Mean (SD) or %	n	Mean (SD) or %	P-value*
Mothers					
Age mother	1163	32.9 (4.0)	881	32.4 (3.9)	**0.006**
Maternal education ≥4 years	685/1076	64%	458/789	58%	**0.014**
Urban living environment pregnancy	1045/1079	97%	767792	97%	0.995
Married/cohabitant mother	10581083	98%	776/795	98%	0.908
Parity <1 (nullipara)	701/1163	60%	552/880	63%	0.260
Maternal asthma	180/1079	17%	132/792	17%	0.993
Nicotine exposure in pregnancy					
Any nicotine exposure in pregnancy	120/1163	10.3%	100/845	11.8%	0.283
Snus use in pregnancy	71/1163	6.1%	58/845	6.9%	0.493
Smoke/dual use in pregnancy	49/1163	4.2%	42/845	5.0%	0.421
Infants (birth)					
Girl	572/1163	49%	393/881	45%	**0.040**
GA at birth in weeks	1158	40.0 (1.4)	880	39.9 (1.3)	0.920
Caesarian section	190/1161	16%	140/880	16%	0.782
Birth weight in kg	1157	3.55 (0.48)	878	3.60 (0.48)	**0.037**
Infants (3 months)					
Age in weeks	1161	13.2 (1.0)	673	13.3 (1.3)	**0.025**
Weight in kg	1156	6.23 (0.78)	673	6.26 (0.76)	0.442
Length in cm	1148	61.8 (2.4)	672	62.0 (2.4)	0.092
Breastmilk exclusively at 3 months	678/1003	68%	410/602	68%	0.833

Data are presented as n, mean (SD) or %. The "any nicotine" group includes all women who reported use of any nicotine containing products at any time during pregnancy. The "snus" group includes women who reported use of snus exclusively during pregnancy. The "smoke/dual" group include smokers and dual smokers and snus users during pregnancy, including one woman reporting use of other nicotine-containing products than cigarettes and snus.
* Independent t-test or Chi^2 test.
Abbreviations: SD – standard deviation, n – number of subjects.

Online supplement:

Maternal use of snus in pregnancy and infant lung function

Karen Eline Stensby Bains, MD[1,2], Ina Kreyberg*, MD, PhD[3], Martin Färdig*, MSc[4,5], Berit Granum, PhD[6], Hrefna K. Gudmundsdóttir, MD[1,2], Guttorm Haugen, MD, PhD[2,7], Gunilla Hedlin, MD, PhD[4,5], Katarina Hilde, MD[2,7], Christine Monceyron Jonassen, PhD[8,9], Björn Nordlund, RN, PhD[4,5], Eva M. Rehbinder, MD, PhD[2,3], Corina Silvia Rueegg, PhD[10], Håvard O. Skjerven, MD, PhD [1,2], Anne Cathrine Staff, MD, PhD[2,7], Cilla Söderhäll, MSc, PhD[4,5], Riyas Vettukattil, MBBS, PhD[1,2], Karin C. Lødrup Carlsen, MD, PhD[1,2].

*Shared second authorship.

Affiliations:

1. Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway.
2. University of Oslo, Faculty of Medicine, Institute of Clinical Medicine, Oslo, Norway.
3. Department of Dermatology and Venerology, Oslo University Hospital, Oslo, Norway.
4. Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden
5. Department of Women's and Children's Health, Karolinska Institute, Stockholm, Sweden
6. Department of Environmental Health, Norwegian Institute of Public Health, Oslo, Norway.
7. Division of Obstetrics and Gynaecology, Oslo University Hospital, Oslo, Norway.
8. Department of Virology, Norwegian Institute of Public Health, Oslo, Norway.
9. Center for Laboratory Medicine, Østfold Hospital Trust, Grålum, Norway.
10. Oslo Centre for Biostatistics and Epidemiology, Oslo University Hospital, Oslo, Norway.

The study was performed within ORAACLE (the Oslo Research Group of Asthma and Allergy in Childhood; the Lung and Environment).

Address of correspondence to:

Karen Eline Stensby Bains

Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway,

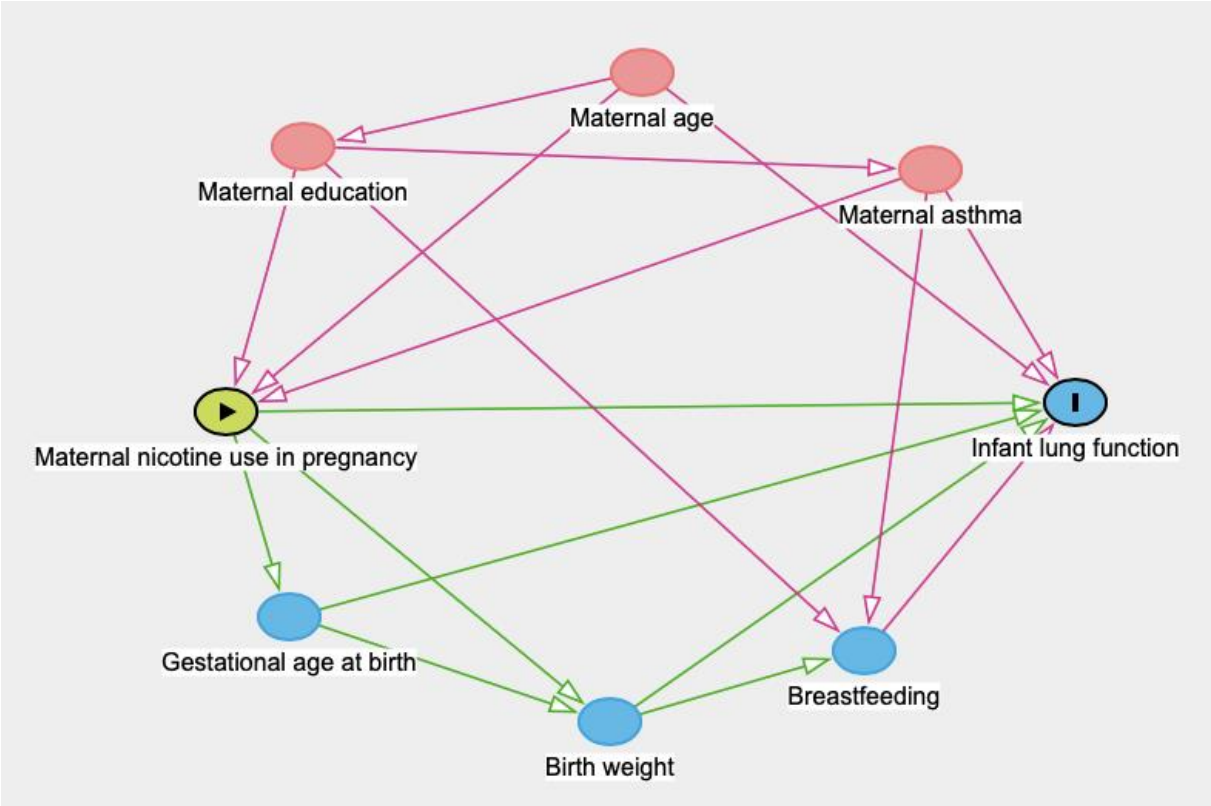
Postboks 4956 Nydalen, 0424 Oslo.

E-mail: karen.eline.sh@gmail.com

Mobile: +47 97745444.

Methods

Supplementary figure 1: We identified the confounders to be included in the multivariable models, based on a directed acyclic graph, for the exposure, maternal nicotine use in pregnancy, and the outcome, infant lung function. Red nodes: Variables associated with maternal use of nicotine in pregnancy. Blue nodes: Variables associated with infant lung function or offspring asthma.



Results

Supplementary Table 1. Baseline characteristics of the 1163 included infants in the present PreventADALL sub-study with lung function measurements and of the 881 remaining infants, all from the study locations in Oslo, Norway, and Stockholm, Sweden.

Characteristics	Included infants, n=1163		Remaining cohort, n=881		
	n	Mean (SD) or %	n	Mean (SD) or %	P-value*
Mothers					
Age mother	1163	32.9 (4.0)	881	32.4 (3.9)	**0.006**
Maternal education ≥4 years	685/1076	64%	458/789	58%	**0.014**
Urban living environment pregnancy	1045/1079	97%	767792	97%	0.995
Married/cohabitant mother	10581083	98%	776/795	98%	0.908
Parity <1 (nullipara)	701/1163	60%	552/880	63%	0.260
Maternal asthma	180/1079	17%	132/792	17%	0.993
Nicotine exposure in pregnancy					
Any nicotine exposure in pregnancy	120/1163	10.3%	100/845	11.8%	0.283
Snus use in pregnancy	71/1163	6.1%	58/845	6.9%	0.493
Smoke/dual use in pregnancy	49/1163	4.2%	42/845	5.0%	0.421
Infants (birth)					
Girl	572/1163	49%	393/881	45%	**0.040**
GA at birth in weeks	1158	40.0 (1.4)	880	39.9 (1.3)	0.920
Caesarian section	190/1161	16%	140/880	16%	0.782
Birth weight in kg	1157	3.55 (0.48)	878	3.60 (0.48)	**0.037**
Infants (3 months)					
Age in weeks	1161	13.2 (1.0)	673	13.3 (1.3)	**0.025**
Weight in kg	1156	6.23 (0.78)	673	6.26 (0.76)	0.442
Length in cm	1148	61.8 (2.4)	672	62.0 (2.4)	0.092
Breastmilk exclusively at 3 months	678/1003	68%	410/602	68%	0.833

Data are presented as n, mean (SD) or %. The "any nicotine" group includes all women who reported use of any nicotine containing products at any time during pregnancy. The "snus" group includes women who reported use of snus exclusively during pregnancy. The "smoke/dual" group include smokers and dual smokers and snus users during pregnancy, including one woman reporting use of other nicotine-containing products than cigarettes and snus.
* Independent t-test or Chi^2 test.
Abbreviations: SD – standard deviation, n – number of subjects.

Household smoking:

In the electronic questionnaire at GW 34, 40 mothers reported smoking by other persons in the household, and among these indoor household smoking was reported "rarely" by one, "sometimes, but not weekly" by one, and "weekly, but not daily" by one mother.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract (*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4 4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5-6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7-8
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up ~~(*b*) For matched studies, give matching criteria and number of exposed and unexposed~~	7-8
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	7-8
Bias	9	Describe any efforts to address potential sources of bias	The manuscript was screened using OHAT (Office of Health Assessment and Translation) handbook, with low risk of bias, and Supplementary figure 1.
Study size	10	Explain how the study size was arrived at	7 and figure 1

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	8-9
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8-9
		(b) Describe any methods used to examine subgroups and interactions	8-9
		(c) Explain how missing data were addressed	8
		(d) If applicable, explain how loss to follow-up was addressed	-
		(e) Describe any sensitivity analyses	8
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7 and 10 and figure 1
		(b) Give reasons for non-participation at each stage	7
		(c) Consider use of a flow diagram	Given in figure 1
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7-8, 10 and Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) Summarise follow-up time (eg, average and total amount)	-
Outcome data	15*	Report numbers of outcome events or summary measures over time	10-11 and Table 1-3

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8, 10-11, Table 2, Table 3, Supplementary figure 1
		(*b*) Report category boundaries when continuous variables were categorized	8, 10
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	-
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8, 10
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12-15
Generalisability	21	Discuss the generalisability (external validity) of the study results	12, 14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	2

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.