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

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Shareable abstract (@ERSpublications)
Smoking in pregnancy is known to affect infant respiratory health negatively, while effects of *in utero* exposure to smokeless tobacco are unknown. This study suggests that maternal use of snus in pregnancy may be harmful to the developing fetal lung. https://bit.ly/3v7lQdI

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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 3 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 was explored by regression analyses adjusting for maternal age, education and asthma.

Results Overall 120 out of 1163 (10.3%) infants were exposed to any nicotine *in utero*, 71 out of 120 by snus exclusively and 49 out of 120 by smoking, with six also exposed to snus. By pregnancy week 6, 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 (CI) 1.02–2.59) with a similar tendency for snus exclusively (OR 1.55, 95% CI 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 were 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 COPD [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 encompasses lung budding in the embryonic stage, airway branching from around gestational week (GW) 7–17 and saccular development from around 24 GWs with alveolarisation 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 a daily basis in young men and women compared to cigarettes [20]. Among women aged 25–49 years, 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 [25]. Sale of e-cigarettes containing nicotine is prohibited in Norway, but they were sold legally in Sweden from July 2017, *i.e.* after the inclusion of mothers in this study [26].

According to some large register studies, use of snus in pregnancy is associated with increased risk of adverse outcomes in the offspring including preterm delivery [27–29], small for gestational age (SGA) [30] and stillbirth [31]. 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 been 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 hypothesise 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 at or after GW 35.0 without serious neonatal disease were included. In this sub-study, 1163 infants underwent lung function measurement at 3 months of age and information on antenatal exposure to nicotine-containing products was obtained (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 at around 3 months post-partum. Birth mode and concurrent anthropometric measurements were recorded

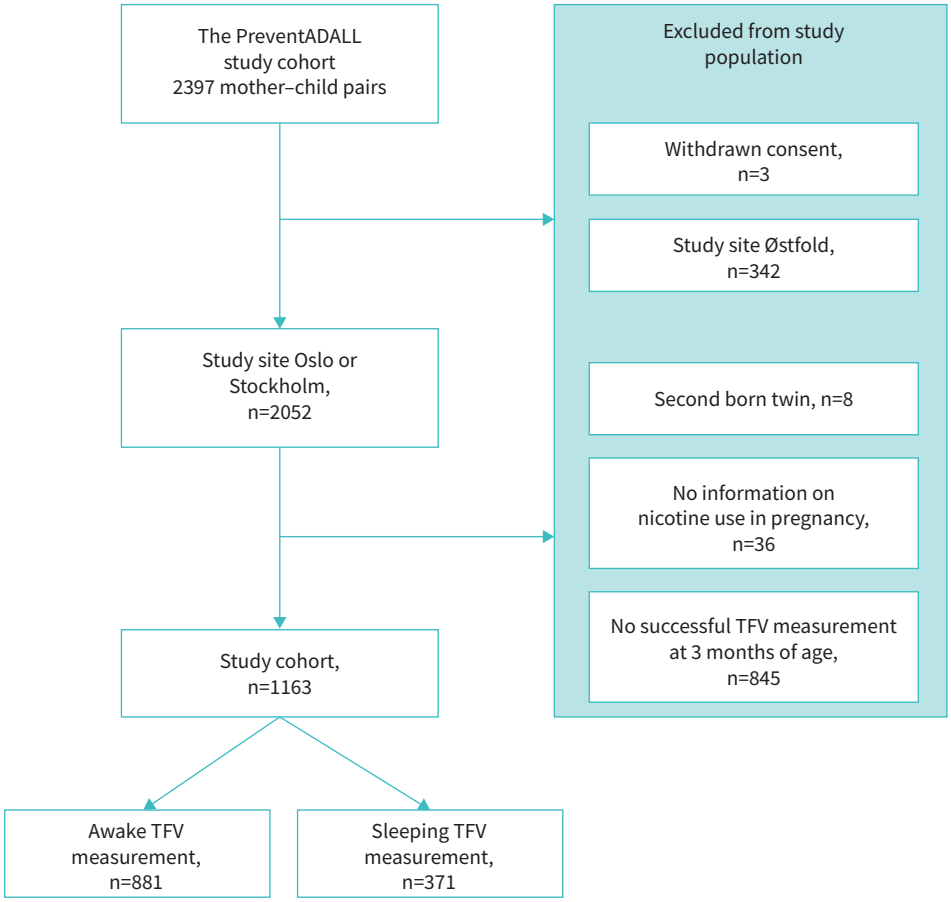

FIGURE 1 Flow chart of the study cohort. Of 1163 infants with lung function measurements, 89 infants had measurements in both the awake and sleeping state. TFV: tidal flow volume.

from electronic hospital charts. Infant lung function was measured at the Oslo and Stockholm study sites from July 2015 to July 2017 as part of the scheduled 3-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 at 2-week intervals during pregnancy, and at GW 34 for the period since completing the previous questionnaire. 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 3 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 3-month follow-up at the study sites in 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 standardised 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 per 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 categorised 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 supplementary material for details).

We identified the following confounders to be included in the multivariable models, based on a directed acyclic graph (supplementary figure S1): 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 as n (%) and continuous variables as mean±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 β-coefficients with 95% CI and p-values for V_T per 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, USA) 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 birthweight 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 the excluded infants except for a higher proportion of girls, mothers of higher age and education level, lower birthweight and lower age at the 3-month follow-up visit (supplementary table S1). 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 out of 1163 women (10.3%), of whom 71 (59.2%) reported use of snus exclusively. Among the 49 (40.8%) women categorised 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 6, 103 out of 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 was 0.39±0.08 (0.19–0.63) in awake infants 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

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*[#]		Infants not exposed to nicotine *in utero*[¶]	
	n	Mean±SD or %	n	Mean±SD or %
Mothers				
Age of 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)				
Female	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
Birthweight 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. [#]: n=120; [¶]: n=1043.

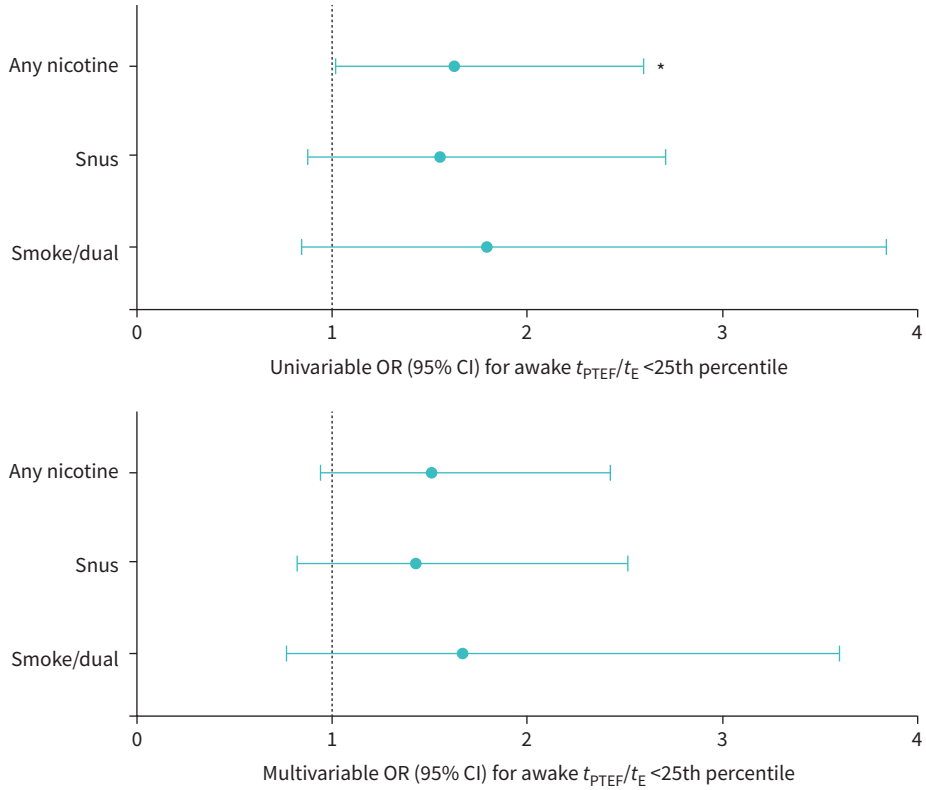

FIGURE 2 Forest plot showing the odds ratio with 95% confidence intervals for awake t_{PTEF}/t_E <25th percentile at 3 months of age and nicotine exposure *in utero* in the groups "any nicotine" (n=90), "snus" (n=60) and "smoke/dual" (n=30). The reference group is no nicotine exposure in pregnancy (n=791). *p-value=0.040. Multivariable analysis adjusting for maternal age, maternal education and maternal asthma. t_{PTEF}/t_E: ratio of time to peak tidal expiratory flow to expiratory time.

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TABLE 2 Association between *in utero* exposure to nicotine-containing products and infant t_{PTEF}/t_E below the 25th percentile at 3 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 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.

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 four out of 881 awake infants who were exposed to nicotine-containing products *in utero*, and <0.20, as observed in four out of 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 significantly higher mean values of V_T per kg compared to non-exposed infants (β-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 $p_{interaction} \geqslant 0.065$ (tables 2 and 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 3 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 6 in 85.8%. Exclusive snus use, as well as smoke/dual use, tended to increase the risk of lower lung function in the 3-month old infants, although not reaching statistical significance. A small increase in difference in mean values for V_T per kg was observed in sleeping infants exposed to any nicotine, while the other analyses on V_T per 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 3 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 the small numbers involved.

In contrast to a previous birth cohort study from the 1990s 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 population [21, 22]. Previously, a dose-dependent increased risk of extreme prematurity (GW <28) has been associated with continued use of snus and cigarettes after GW 8–12 [27]. Further, women who ceased 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 4 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 6, 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 offspring birth outcomes and lung function [7–9]. Collectively, therefore, it

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TABLE 3 Association between *in utero* exposure to nicotine-containing products and infant tidal volume (V_T) per kilogram at 3 months of age from linear regression models, in awake and sleeping infants

	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}
Awake V_T per kg								
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 per kg								
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. p_{int}: $p_{interaction}$. [#]: given in millilitres per kilogram.

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 summarised by Spindel *et al.* [14], 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. Although the critical period for exposure to nicotine in pregnancy is unclear, studies in mice suggest a primary effect of nicotine on airway growth of conducting airways rather than the alveolarisation period, with an increased number of airways of small diameter with nicotine exposure [44]. Carlsen *et al.* [7] found declining ratios of t_{PTEF}/t_E with increasing exposure to cigarettes in pregnancy, 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 6, 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 per 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 per kg was detected between infants exposed to smoking *in utero* or not [9]; however, statistical methods used 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, and 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 standardised 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.

Owing 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 assess and could thus not control for exposure to nicotine through breastfeeding. Lung function measurements were only obtained in two out of three study sites due to organisational 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 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 those of smoking or less detrimental on lung function and development, 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.

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This study is registered at www.clinicaltrials.gov with identifier number NCT02449850.

Ethics statement: 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).

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