THE USE OF NICOTINE PRODUCTS AND EFFECTS OF SNUS IN PREGNANCY

Ina Kreyberg

University of Oslo, Institute of Clinical Medicine

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Oslo University Hospital, Division of Paediatric and Adolescent Medicine

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ORAACLE

Oslo Research group of Asthma and Allergy in Childhood; the Lung and Environment

Oslo 2020

Series of dissertations submitted to the
Faculty of Medicine, University of Oslo

ISBN 978-82-8377-802-1

Cover: Hanne Baadsgaard Utigard.
Print production: Reprosentralen, University of Oslo.

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1 Preface

1.1 Acknowledgments

First, I would like to thank all the PreventADALL participants – the parents and their children for contributing to this study by answering questionnaires and coming to the follow-up visits in a time that is busy enough with parenting.

Second, I am very grateful to my main supervisor Professor Karin C Lødrup Carlsen and my co-supervisor Professor emeritus Kai-Håkon Carlsen for giving me the opportunity to be a part of this wonderful project under the excellent ORAACLE group (Oslo Research group of Asthma and Allergy in Childhood; the Lung and Environment), to explore the world of research and take on the role of being a Ph.D. student.

I have been very lucky to have Karin as my main supervisor with her experience and high-quality achievements and merits within her field of paediatric research. She has been an excellent supervisor in every step of this project as Project leader, always interested and asking the right questions, quick to respond and ensuring the best possible results through thorough planning, guidance and advice. Thank you!

Further I would like to thank my co-supervisors Professor emeritus Kai-Håkon Carlsen for always sharing his extensive knowledge of the harm of tobacco as well as Professor Guttorm Haugen for excellent advice in the PhD-work and providing insight and perspectives from the field of obstetrics and fetal medicine.

The local principal investigators from the different study sites: Eva Maria Rehbinder, now currently local PI in Oslo, following Håvard O. Skjerven and Linn A. Landrø. In Stockholm local PI Björn Nordlund and at Østfold Hospital Trust local PI Christine M. Jonassen with their respective study teams.

Special thanks to the wonderful PreventADALL Oslo team:

My fellow Ph.D. students: Eva Maria Rehbinder, Hrefna Katrín Gudmundsdottír, Karen Eline Stensby Bains, Live S. Nordhagen (extra thanks for the collaboration with our published mini review highly relevant for the background section of this thesis),

Katarina Hilde, Kim M. Advocaat S. Endre, Carina M. Saunders, Anine Lie, Åshild Wik Despriée, and Marius Kurås Skram.

My colleagues: Liv Julie Sørdal, Oda Cecilie Lødrup Carlsen, Thea A. Fatnes, Mari Rønning Kjendsli, Malén Gudbrandsgard, Angelica J. Winger, Vibeke Dyrseth, Kristine Wedum Davanger, Ingvild Essén, Peder A. Granlund, Sofie Rabo Carlsen, Hilde Aaneland, Elke Maes, Johanne Uthus Hermansen and Andrea Dystvold Hansen.

For eminent advice in statistical methods I thank statistician Corina S. Rueegg, and Riyas Vettukatil for managing the PreventADALL database.

Thank you Birgitte Kordt Sundet for the cooperation with the statistical analyses for paper 2, Meryam Sugulle for being an excellent main writer and master of coordinating, and of course Professor Anne Cathrine Staff for all valuable advices and revisions.

I would like to thank my family and friends for cheering me on – you know who you are. And last, but not least, thank you Kjetil for always being there and for holding the fort at home with our children Olav (3) and Liv (10 months) while I finalized the PhD work at our home office.

1.2 Summary of the thesis

1.2.1 Background

Today snus is the most commonly used nicotine product among young women in Scandinavia. The extent of maternal use of snus during pregnancy is however less investigated as well as risk factors associated with such use. It is well known that smoking during pregnancy is harmful for the mother and the unborn child increasing the risk of several adverse health effects, and evidence suggest that nicotine is one of the main mediators behind these effects. Studies on the effect of in-utero exposure to snus have found increased risk of effects that at are similar to those of cigarettes smoke such as premature delivery, stillbirth, congenital malformations, neonatal apnoea and altered heart rate variability. There are however conflicting results when it comes to the effect of snus on infant birth weight.

Normal fetal growth and development is dependent on a healthy placenta throughout pregnancy. The normal placenta growth is relying on well-functioning vasculogenesis and angiogenesis, processes regulated by pro- and anti-angiogenic factors. Two of these factors, called placental growth factor (PlGF) and soluble fms-like tyrosine kinase receptor 1 (sFlt-1), represent potential useful biomarkers for identifying placental dysfunction. The level of biomarkers has been shown to differ between male and female pregnancies. To our knowledge neither the potential effect of snus on these biomarkers are not previously been investigated, nor the effect of snus exposure in a sexdimorphic way. The specific aims of this thesis were therefore:

1. To determine the maternal prevalence, pattern of use and cessation rate of snus and other nicotine products during pregnancy
2. To identify women at risk using snus in pregnancy
3. To determine if maternal snus use pre- and perinatally affect placental biomarkers in midpregnancy and/or neonatal anthropometric measures at birth
4. To explore if the effects of in-utero snus exposure differ by fetal sex, focusing on midterm angiogenetic placenta biomarkers

1.2.2 Methods

The study participants were included in the prospective mother-child birth cohort PreventADALL (Preventing Atopic Dermatitis and Allergies). A population-based, multicenter study with a two-by-two factorial randomized controlled design. The pregnant women were included at 18 weeks gestational age (GA) at the routine ultrasound examination at three study sites in Oslo and Østfold, Norway and Stockholm, Sweden. The pregnant women completed electronic questionnaires at 18- and 34-week pregnancy, and blood samples for placenta biomarkers were drawn at the inclusion visit. The healthy infants born at GA 35 weeks or later were included with the first 1-2 days of life, and anthropometric measurements were conducted. Information about previous and current tobacco use including snus, conventional cigarettes and other products such as e-cigarettes (electronic cigarettes) and nicotine replacement therapies (NRT) were included. Logistic regression analyses were conducted to identify risk factors of snus use during pregnancy, and linear regression models were used to investigate the effect of snus use during pregnancy on placenta biomarkers (PlGF, sFlt-1 and PlGF/sFlt-1 ratio) as well as anthropometric measurements for infant birth size. The biomarkers were analysed at Oslo University Hospital, Dept. of Medical Biochemistry.

1.2.3 Results

In total 2697 women with 2701 pregnancies constituting of 2397 mother-child pairs were included. Mean maternal age was 32 years, 57.1% had more than 4 years of education, the women were mostly urban living (76.8%) either married (41.2%) or in a cohabitant relationship (55.9%). Mean estimated infant gestational age was 39.2 (35.2-42.9) weeks and 52.7% were boys.

Overall 11.3% reported use of any type nicotine product during pregnancy, with snus alone being the most commonly used product reported by 6.5%, followed by 4.1% cigarettes smoke and 0.2% use of NRT/e-cigarettes. There was a significant increased use of snus among Norwegian compared to Swedish women (8.2% versus 5.5%, p-value 0.035). However, more than 90% reported cessation of snus or cigarettes when recognizing pregnancy, in total 87.2% of the snus using women within

pregnancy week 6. Risk factors for cigarette smoking were younger age, urban living, being cohabitant and native born in Norway or Sweden as well a personal and in-utero history of smoking.

The early snus exposure during pregnancy was significantly associated with lower PlGF (p-value 0.020) compared to never users in multivariable regression analyses. There was no significantly change in level of sFlt-1 and sFlt-1/PlGF ratio in women who reported use of snus compared to never users.

Snus exposure during pregnancy did not affect anthropometric or proportional neonatal size measures in multivariable regression analyses when adjusting for relevant covariates including maternal weight gain from pre-pregnancy to gestational week 18.

When exploring whether the effect of snus exposure differ between fetal sex, we tested the interaction between fetal sex and snus exposure in relation to PlGF. As this was found positive, further analyses were conducted stratified by fetal sex. Women carrying a male fetus and with in-utero snus exposure had significantly lower pro-angiogenic PlGF levels compared to never users (0-value 0.002). This was not the case for female fetuses (p-value=0.194).

1.2.4 Conclusions

Snus is the most commonly used nicotine product during pregnancy reported by 7% of the women, but with 87% stopping when knowing about their pregnancy within pregnancy week 6. Young, urban, cohabitant women, with previous smoking history and being exposed to snus in-utero themselves were at risk of using snus during pregnancy. Early snus exposure was associated with lower midpregnancy PlGF levels, but it did not affect infant birth size. When stratifying for fetal sex women carrying a male fetus had lower PlGF levels compared to never users. The same effect was not reproduced in women carrying a female fetus, supporting the perception that male fetuses are more susceptible to environmental factors in-utero.

1.3 Abbreviations

BPD	biparietal diameter
BMI	body mass index
CI	confidence intervals
E-cigarettes	electronic cigarettes
GA	gestational age
IQR	interquartile range
NCDs	non-communicable diseases
NRT	nicotine replacement therapy
MBR	Medical Birth Register
MBRN	Medical Birth Register of Norway
OR ()	Odds Ratio with 95% confidence interval
PlGF	placental growth factor
PreventADALL	Preventing Atopic Dermatitis and ALLergies
SD	standard deviation
SGA	small for gestational age
sFlt-1	soluble fms-like tyrosine kinase receptor 1
SOP	standard operating procedures
VEGF	vascular endothelial growth factor

1.4 List of papers

I Stopping when knowing: use of snus and nicotine during pregnancy in Scandinavia.

Ina Kreyberg, Karen Eline S. Bains, Kai-Håkon Carlsen, Berit Granum, Hrefna Katrín Gudmundsdóttir, Guttorm Haugen, Gunilla Hedlin, Katarina Hilde, Christine M. Jonassen, Live S. Nordhagen, Björn Nordlund, Katrine D. Sjøborg, Håvard O. Skjerven, Anne C. Staff, Cilla Söderhäll, Riyas M. Vettukatil, and Karin C. Lødrup Carlsen. ERJ Open Res, 2019 Apr 8;5(2):00197-2018. doi: 10.1183/23120541.00197-2018. eCollection 2019 Apr. PMID: 30972353

II The effect of fetal sex and nicotine-containing products on circulating midpregnancy angiogenic biomarkers.

*Birgitte Kordt Sundet, *Ina Kreyberg, Anne Cathrine Staff, Kai-Håkon Carlsen, Berit Granum, Gunilla Hedlin, Guttorm Haugen, Christine M. Jonassen, Björn Nordlund, Katrine D. Sjøborg, Håvard O. Skjerven, Cilla Söderhäll, Riyas Vettukatil, Jens-Petter Berg, Corina Silvia Ruegg, Karin C. Lødrup Carlsen, and Meryam Sugulle. *Shared first authorship. Submitted September 8th 2020 to Acta Obstetrica et Gynecologica Scandinavica (AOGS).

III Snus in pregnancy and infant birth size: a mother–child birth cohort study.

Ina Kreyberg, Katarina Hilde, Karen Eline S. Bains, Kai-Håkon Carlsen, Berit Granum, Guttorm Haugen, Gunilla Hedlin, Christine M. Jonassen, Live S. Nordhagen, Björn Nordlund, Corina S. Rueegg, Katrine D. Sjøborg, Håvard O. Skjerven, Anne C. Staff, Riyas Vettukattil, and Karin C. Lødrup Carlsen. ERJ Open Research, 2019 Dec 2;5(4):00255-2019. doi: 10.1183/23120541.00255-2019. eCollection 2019 Oct. PMID: 31803771

2 Background

Globally cigarette smoking remains the most commonly used tobacco product among women [1, 2]. Nevertheless, smoking rates have generally declined in the past decades, also in women in the reproductive age [3, 4]. Epidemiological and experimental studies indicate that the beginning of life, marked by the peri-conceptional and intrauterine periods as well as infancy, represent stages sensitive to environmental factors with increased risk of later development of non-communicable diseases (NCDs) [5]. Cigarette smoke and nicotine from snus represent modifiable environmental risk factors. In line with compelling evidence that in-utero exposure of tobacco cigarettes increases the risk of adverse effects in the unborn child [6], maternal smoking has decreased, especially persistent and late-pregnancy smoking [7, 8]. However, today the extent of snus use appears to have a different development.

2.1 New trends in prevalence of tobacco and nicotine in Scandinavia

In parallel with the steady fall in smoking rates among women and men in all age groups there has been an increase in the use of snus (also called moist snuff) in Norway (Figure 2-1) and Sweden (Figure 2-3). In fact, in 2017 it was more common to use snus than to smoke cigarettes among the younger age groups (16-24 and 25-44 years) in Norway [9].

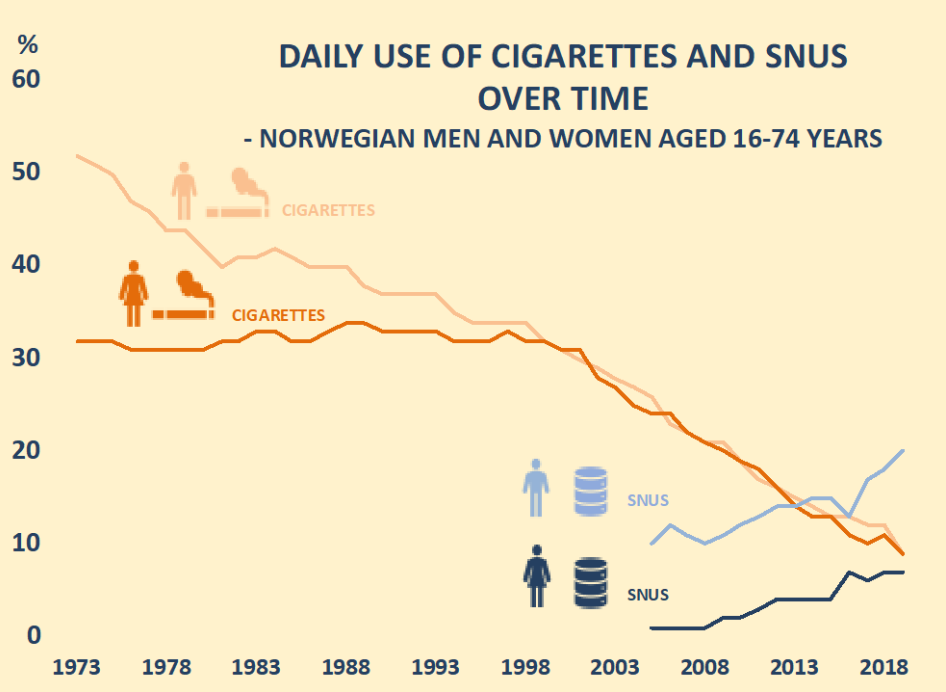

Figure 2-1. The prevalence of daily cigarette smoking and snus use over time among Norwegian men and women in all age groups (16-74 years). Data from Statistics Norway (2020).

In 2019 the cigarette smoking rates were around 9% for both women and men in Norway (Figure 2-1) [10]. The daily snus use among women was overall 7% (Figure 2-1) with the highest rate of 17% among women aged 25-34 years (Figure 2-2). The corresponding rates among men were 20% (Figure 2-1) and 30%, respectively [10]. In Sweden snus use is also common, but the prevalence has been relatively stable around 4% for women and 19 % for men between the period 2004 and 2018 (Figure 2-3). As has been the case in Norway, smoking rates continues to fall in Sweden, and the number of daily female smokers decreased from 10 to 7% from 2016 to 2018 (Figure 2-3) with the lowest prevalence of 5% among the youngest age groups (16-29 and 30-44 years of age) [11]. For Swedish men there was a decline from 8 to 7% in the same period (Figure 2-3) also with 5% among those 16-29 years of age [11].

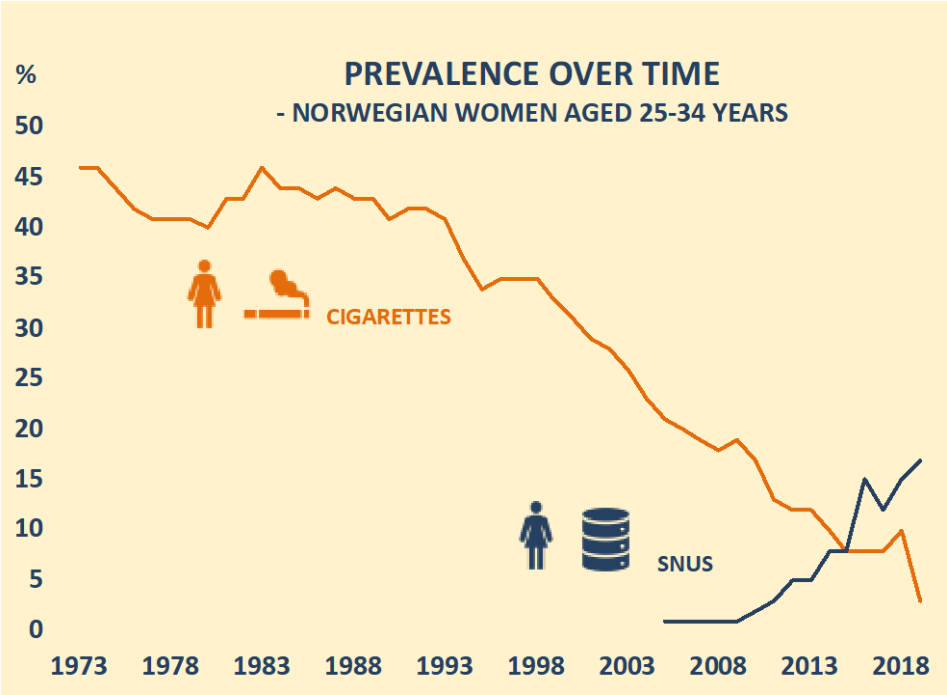

Figure 2-2. The prevalence of cigarette smoking and snus use over time among Norwegian women age group 25-34 years. Data from Statistics Norway (2020).

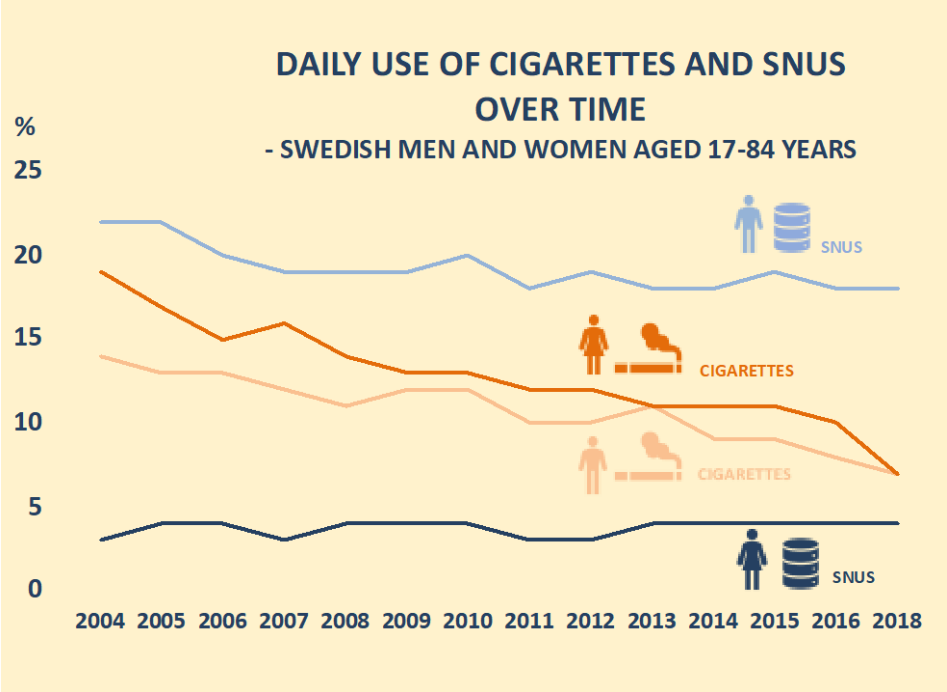

Figure 2-3. The prevalence of daily cigarette smoking and snus use over time among Swedish men and women in all age groups (17-84 years). Data from The Public Health Agency of Sweden (2020).

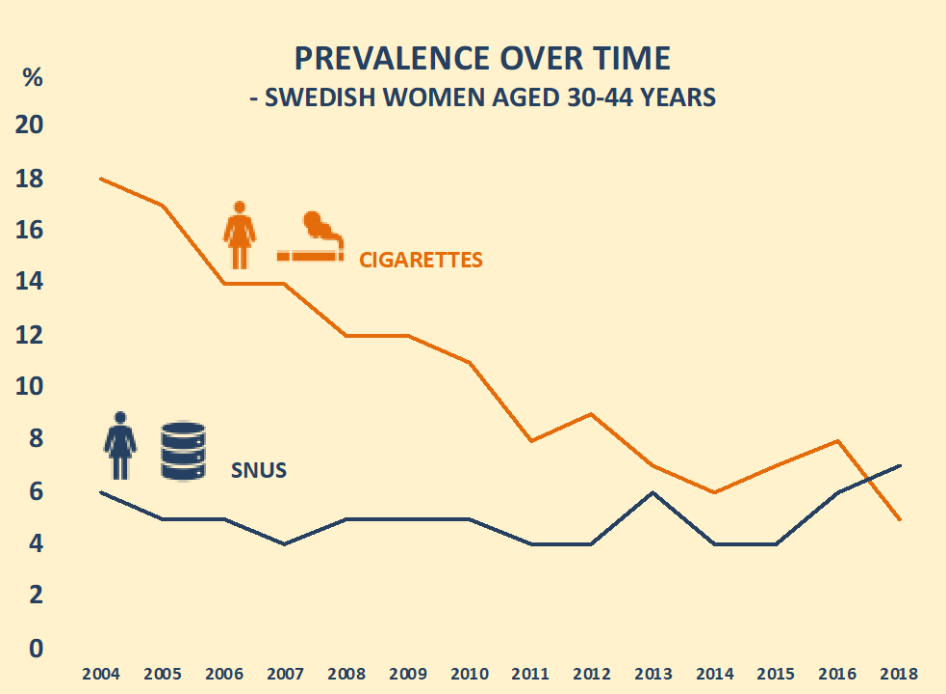

Figure 2-4. The prevalence of cigarette smoke and snus over time among Swedish women in the age group 30-44 years. Data from The Public Health Agency of Sweden (2020).

Among women in the reproductive age, the prevalence of snus is lower in Sweden compared to Norway and has remained unchanged over the past two decades. The overall daily snus use in 2018 was 4% (Figure 2-3) for women with a peak of 7% among the 30-44 years old (Figure 2-4). The equivalent numbers for Swedish men were 18% and 21%, respectively [11].

Electronic cigarettes (e-cigarettes), invented in 2003 [12], has become an increasingly common alternative nicotine product in some countries [13, 14]. In countries with legal and easy access to e-cigarettes, a shift towards other smokeless tobacco products has been observed [15]. Among the current users of tobacco products in high school students (aged 14-18) in the USA the use of e-cigarettes increased from 1.5% to 11.3% in a five year period from 2011 to 2016 [16]. In Scandinavia the use of e-cigarettes is still uncommon. The prevalence of daily or sometime use of e-cigarettes with and without nicotine in 2018 in Sweden was 2%, with rates from previous years missing [11]. In Norway national statistics on e-cigarettes are mainly lacking, however it was only in December 2016 that the

Norwegian parliament decided to abolish the prohibition of e-cigarette sales, and the change is expected to come into force in 2021 [9, 17].

The new trends in tobacco products are largely related to marketing, politics, and economic interests by the tobacco industry. Furthermore, there is little evidence of effective smoking cessation and a high numbers of young non-smokers becoming nicotine addicted by using e-cigarettes [15]. Smokeless tobacco products such as e-cigarettes have been heavily marketed towards adolescents and young adults, including through social media platforms like Twitter [18] promoting unregulated messages on its benefits [19]. Additionally many have the perception that these products are less harmful than conventional tobacco cigarettes [20], and snus is advocated as a cessation aid for smokers [21, 22].

2.2 Tobacco and nicotine during pregnancy

2.2.1 Prevalence

Although maternal smoking during pregnancy has decreased, both pregnant and non-pregnant women in younger age groups, and with lower socioeconomic status have a slower drop in prevalence [7, 8]. In studies performed between 2011-2017 the prevalence of smoking during pregnancy varies between 8,4% to 14,7% across the United States [23], England [24], Ireland (Dublin) [25], Poland [26] and Finland [27]. In 2005 16% of all pregnant Norwegian women smoked in the first trimester with a reduction to 9% in the third trimester according to data from the Medical Birth Register of Norway (MBRN). However, in 2019 the corresponding rates had fallen to 2.7% and 1.6%, respectively [28]. Data from the Swedish Medical Birth Register (MBR) in 2018 showed that 4.2% of the pregnant women reported smoking in the first trimester [29].

To my knowledge, there are few recent prospective birth cohort studies investigating the prevalence and effects of snus use during pregnancy [30, 31]. In Norway, the maternal snus use during pregnancy has been recorded in the hospital birth record Partus since 2012 [32]. However, only in 2017 a change in regulation

ensured that data on maternal snus use are included in the annual statistics of the MBRN. In Sweden, annual national statistics has been available already since year 1999.

In a register-based study from Southern Norway in the period 2012-2014 snus use during pregnancy was 2.4% in the first trimester and 1.7% in the third trimester, with higher rates among the youngest age groups [32]. In the follow-up study for the years 2015-2017 the corresponding prevalence were 2.8% and 2.0%, respectively [33].

In 2016 data from the Swedish MBR, the prevalence of snus use was 1.2% in early pregnancy and 0.7% in late pregnancy (week 30-32) [29], with a similar rate of 1.2% prevalence in the first trimester in 2018 albeit lacking information from late pregnancy [29]. To the best of our knowledge there are no available statistics on the use of snus in general, or during pregnancy, in other European countries.

2.2.2 Risk factors of nicotine use

Smoking during pregnancy is associated with younger age, lower educational level, being unmarried, receiving welfare benefits, multiparty, unemployment, unplanned pregnancy, and history of psychiatric problems, alcohol and illicit drug use [23, 25, 26, 34]. Lower education has been shown to be strongly associated with smoking in pregnancy [35]. A study using data from MBRN found a decline in pregnancy smoking rates among all social and demographic groups within and between the two periods 1999-2000 and 2013-2014. The decrease was nevertheless unevenly distributed as women with low and medium educational level and single mothers had the highest smoking prevalence and the lowest drop [36].

The main socio-economic risk factors for use of snus during pregnancy are to our knowledge unknown. In general, data from Sweden show no differences in educational level or level of household income among adult snus users [11], although Norwegian adolescents aged 13-16 years who reported poor experienced family economy showed higher rate of snus use [37].

2.3 Nicotine

Nicotine is a nitrogen-containing alkaloid made from the tobacco plant of the nightshade family called Solanoceae [38], and can also be produced synthetically [39]. It is the major chemical substance responsible for addiction to tobacco products. Under high (alkaline) pH conditions, nicotine easily crosses mucosal membranes in the mouth and airways and reaches systemic circulation [40]. Nicotine is metabolized in the liver into the major pharmacological active metabolite cotinine. Excretion is primarily renal, but accumulation of nicotine and its metabolites are also measured in feces, saliva, sweat, hair, nails and gastric juice [6, 40].

During pregnancy nicotine and cotinine passes the placenta into fetal circulation and is also excreted into breast milk [40]. The fetal blood levels of nicotine and cotinine probably reach similar levels as in the maternal plasma. Despite most of the metabolites returning to the maternal circulation, some nicotine and cotinine enters amniotic fluid via fetal urine. Thus, the potential accumulation in amniotic fluid might expose the fetus to higher levels of nicotine and cotinine even when decreasing in the maternal circulation [41] as it is shown to concentrate in fetal blood, urine, meconium and amniotic fluid [42, 43]. The extent of potential accumulation in the different fetal body tissues and the fetal ability to metabolize nicotine and cotinine are less well known. Additionally, because of increased maternal liver blood flow, enzymatic breakdown and clearance of nicotine and cotinine is faster in pregnant women. This indicates that maternal plasma cotinine levels observed during pregnancy do not necessarily reflect nicotine exposure [44].

2.4 Content and uptake in the different nicotine products

2.4.1 Tobacco cigarettes

A conventional tobacco cigarette is a roll of tobacco wrapped in paper with or without a filter that is lit in one end and puffed in the other. Both tobacco and tobacco smoke contain more than 5000 compounds; many are proven to be

carcinogens such as polycyclic aromatic hydrocarbons, polonium-210, nickel, arsenic, chromium, cadmium and tobacco-specific N-nitrosamines (TSNA). The cigarette aerosol is predominantly consisting of the combustion gas carbon monoxide, other vapor-phase components, the particulate matter tar and the highly addictive substance nicotine in addition to thousands of chemical constituents [38]. A single conventional cigarette typically contains 12-14 mg of nicotine/cigarette [37], but the concentration varies by type and brand often from 10 to 30 mg of nicotine [45]. Nicotine from tobacco cigarettes crosses mucosal membranes in the mouth and airways reaching the systemic circulation within 10 to 20 seconds after inhaling tobacco smoke [40].

2.4.2 Snus

Snus, also known as '(Swedish) moist snuff', is a non-combustible smokeless, ground tobacco product where a pinch (called a dip) or pre-made portion is placed in the mouth between the gum and the upper lip. Snus differs from dry snuff which comes in a powdered form often inhaled or sniffed nasally, but also applied in the oral cavity depending on type [37, 46]. Of the most important chemical constituents in snus are the highly addictive substance nicotine and the carcinogenic TSNA as well as small amounts of chromium, nickel, polonium210 and polycyclic aromatic hydrocarbons [37, 46]. The level of nicotine in snus varies between 8-30 mg/g snus [39]. Uptake of nicotine by absorption through the oral mucosa is slower for snus use than by smoking cigarettes. Although the nicotine levels reached in serum are higher for cigarettes initially, snus use provides a higher level over time with a prolonged decline, resulting in a greater systemic dose as illustrated in Figure 2-5 [46]. The amount of nicotine absorption and plasma levels depends on the time the snus is kept under the lip, the type of snus and the alkaline condition [39].

2.4.3 NRTs

Nicotine replacement therapies (NRTs) such as transdermal patches (from 7-25 mg nicotine/16-24 hours), chewing gum (2-4 mg) and lozenges (1-2 mg), generates lower nicotine concentrations (Figure 2-5) compared to smoking and use of snus as the nicotine concentrations and absorption are in general lower [47].

2.4.4 E-cigarettes

The product design and engineering of e-cigarettes, which are handheld devices used to heat, but not burn the contents, vary extensively [48]. E-cigarettes are available with and without nicotine, as well as with a variety of flavors [48]. The inhaled product from vaping e-cigarettes is an aerosolized mixture produced from the heated solution, typically containing nicotine, flavoring chemicals, and propylene glycol. However, due to the large variation in contents, the concentrations of nicotine are reported anywhere from 0 to 36 mg/mL [48]. In general however the serum cotinine levels of e-cigarettes are similar to that of conventional cigarettes after active and passive smoking, depending on the concentration in the solution [49].

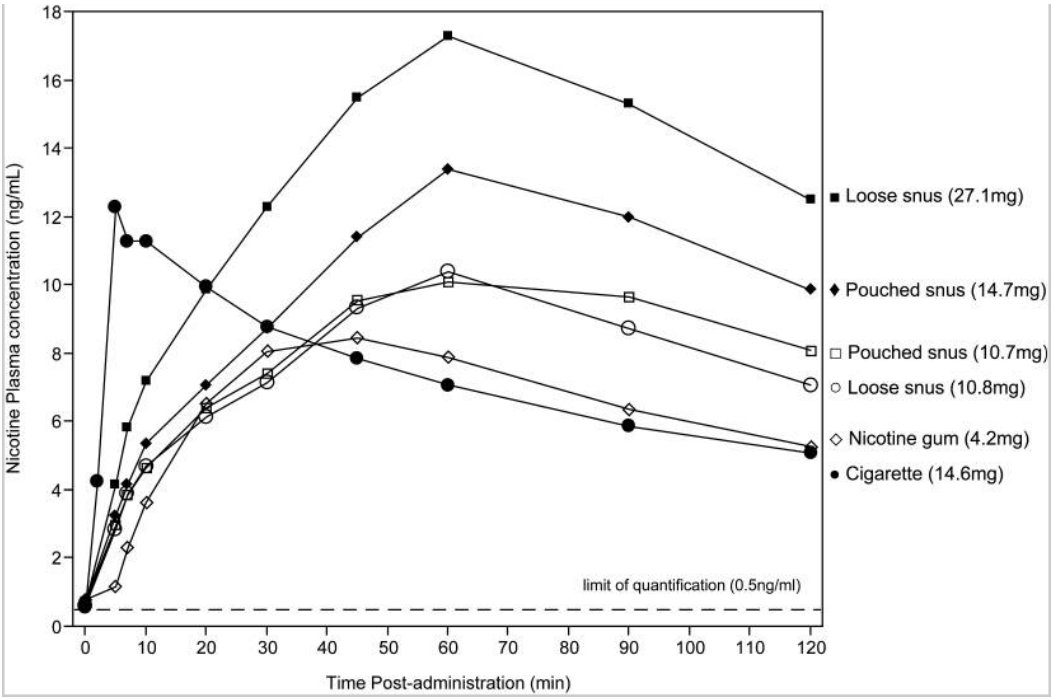

Figure 2-5. Mean plasma nicotine concentrations at each time point following single use of the different tobacco products and nicotine gum. *From the Nicotine and Tobacco Research, Digard H, Proctor C, Kulasekaran A, Malmqvist U, Richter A. Determination of nicotine absorption from multiple tobacco products and nicotine gum. 2013;15(1):255-261. doi:10.1093/ntr/nts123. Published by Oxford University Press on behalf of the Society for Research on Nicotine and Tobacco. Reprinted with permission.*

2.5 Adverse effects of tobacco and nicotine during pregnancy

2.5.1 Tobacco cigarettes

In-utero exposure to maternal smoking is associated with adverse health outcomes in the child and increases the risk of untoward pregnancy outcomes, including premature delivery [50], stillbirth and perinatal mortality [51], infant birth defects [52] and sudden infant death syndrome (SIDS) [53]. Maternal smoking during pregnancy might affect fetal lung development and growth [54], cause impaired lung function [55] and affect respiratory health in children [56, 57] as well as increase the risk of development of allergic disease including asthma [58], allergic rhinitis and atopic eczema [59]. Increased risk of impaired neurocognitive development and behavioral disorders has also been found [6, 60].

The observed increased risk of low birth weight, small for gestational age (SGA) [61-63] and diseases originating in-utero by tobacco exposure during pregnancy, points to effects on fetal growth throughout pregnancy. Maternal smoking during pregnancy has been associated with reduced birth length and head circumference [64, 65] as well as ponderal index [65]. There is an argument for a dose dependent effect as well, as studies have found a non-linear decrease in mean adjusted birth weight with increasing number of cigarettes smoked per day [66], but also with less than 5 cigarettes per day [67]. A meta-analysis reported small, but significant reductions in head size and femur length in the first trimester and reduced growth after the first trimester [68]. Selective reduction in fetal growth affecting abdominal circumference and muscle mass (thigh muscle area) has been observed in 65 exposed compared to 36 non-smoke-exposed fetuses in the third trimester [69].

2.5.2 Nicotine

Recent animal studies have provided evidence that many of the in-utero effects of maternal smoking are mediated by nicotine [54]. However, there are few studies to confirm these findings in humans, with the exception of register studies [31].

Nicotine functions primarily as a potent agonist on most nicotinic acetylcholine receptors (nAChRs) present in body tissues, including the central and peripheral nervous system, liver, kidney, spleen, skeletal muscle, lung, and skin [39, 40]. The

development of the brain and lung is considered to be especially susceptible to nicotine [70]. Nicotine exposure leads to release of signaling molecules involved in physiologic functions like cell proliferation, differentiation and cell death (apoptosis) through receptor interactions, and depending on the context of activation, this might result in adverse health outcomes [54, 70]. The effect of nicotine on the immune system has been reported to be both suppressive and stimulating, but with uncertain health consequences. Based on observed differences in adverse effects of nicotine exposure through tobacco cigarettes with combustion products compared to smokeless tobacco products, nicotine appears to play an important role on impaired fetal growth, and increased risk of preterm birth and stillbirth [6]. Animal models indicate that the risk of SIDS by fetal nicotine exposure might be explained by the impaired physiological protective autonomic and cardiorespiratory response against hypoxia [6, 71]. The response is decreased though nicotine induced altered expression of nAChRs in the brainstem areas involved in autonomic functions [72]. Pre- and postnatal nicotine exposure has also been shown to have a negative impact on brain development and cognitive functions [60, 73].

2.5.3 Snus

Most of the available information regarding the effect of snus during pregnancy is based on data from the Swedish MBR (Figure 2-6). These studies have reported that maternal use of snus in pregnancy increases the risk of spontaneous preterm birth [74], especially before 32 weeks [50, 75, 76] and stillbirth [51, 77]. There are indications that snus use in pregnancy is associated with increased risk of preeclampsia [74, 78], neonatal apnea [79], oral cleft malformation [80] and changed heart rate variability in the infant [81]. Nordenstam et al. found that 5-6 years old children exposed to snus in-utero had a higher systolic blood pressure and altered heart rate variability compared to non-exposed peers [82].

The effects of in-utero exposure to snus on birth weight are unclear. England et al. using data from the Swedish MBR found that adjusted mean birth weight among snus users during pregnancy was reduced with 39 g (95% CI (-72)-(-6) g) compared to

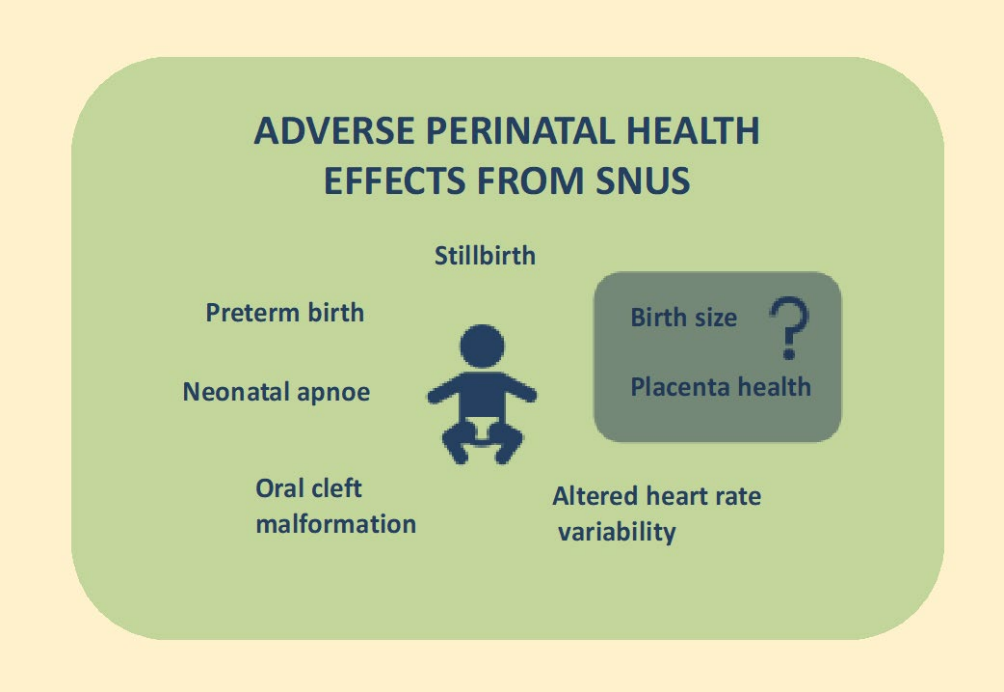

Figure 2-6. Adverse perinatal health effects of snus.

non-users [74]. However, due to possible confounding by familial factors linked with both snus use and birth weight, a sibling analysis by Juarez et al. on the same data found that snus exposure had a minor, but not significant reduction of 20 g (95% CI (-52)-12 g) on birth weight reduction [83]. Baba et al. conducted a study determining the risk of small for gestational age (SGA) births comparing snus users and smokers in early pregnancy to non-tobacco users. Snus users had increased adjusted OR of 1.26 (1.09-1.46) for SGA births, while smokers had an OR of 2.55 (2.43-2.67). The results from Baba and Juarez support the theory that combustion products play a central role in the risk of reduced birth weight and SGA, in addition to nicotine [84]. To our knowledge there are no prospective observational human studies exploring the effect of snus on infant birth size measures.

2.5.4 NRTs and e-cigarettes

Despite that NRTs and e-cigarettes are often marked as safer alternatives to smoking during pregnancy [85], the short and long term effects are largely unknown [31, 86]. Maternal use of NRTs during pregnancy is possibly associated with reduced birth weight and increased risk of preterm delivery [31]. Animal studies have shown that

nicotine is a major contributing factor to the adverse health effects of tobacco exposure [60], but human studies are lacking.

2.6 Placenta biomarkers

Normal fetal development and growth in-utero require a healthy placenta that provides sufficient nutrients and oxygen throughout pregnancy. The continuous growth of the placenta depends on a considerably widespread vascular network within the placental villi that are managed by vasculogenesis and angiogenesis [87, 88]. The processes of vasculogenesis involves de novo development of placental blood vessels, and angiogenesis represent vascular expansion with branching and elongation of capillaries in pre-existing blood vessels [89]. A balance of so-called pro-angiogenic and anti-angiogenic factors regulates the processes within the placenta [90] in addition to having distinct functions in the maternal circulation in relation to cardiovascular adaptation to pregnancy. Yet, the maternal circulating placenta-associated angiogenic proteins represent biomarkers that might be useful in predicting and identifying placental dysfunction in pregnancy, as a supplement to clinical signs and symptoms [91-93].

Several pro- and anti-angiogenic factors are involved in regulation of placental blood vessel development, including vascular endothelial growth factor (VEGF), soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF), angiopoietin-1 and -2, and endoglin. Further details of s-Flt-1 and PlGF will be outlined in more detail because of their role in this thesis.

Changes in the level of the pro-angiogenic PlGF and the anti-angiogenic sFlt-1 as well as the ratio sFlt-1/PlGF might be considered “markers of placenta health” that can be used to detect pregnancy disorders. The placenta secretes sFlt-1 and PlGF, which are both involved in angiogenesis and vasculogenesis, and circulating sFlt-1 binds free PlGF. When circulation in a free form PlGF is bioactive, but is inactivated when bound to sFlt-1 it [89]. Increasing gestational age, intermittent hypoperfusion leading to oxidative stress as seen in the preeclamptic placenta and/or dysfunctional placenta give a rise in sFlt-1 resulting in lower levels of PlGF (Figure 2-7) [94-96].

Reduced level of free circulating PlGF leads to maternal vascular inflammation and systemic endothelial dysfunction interceding new-onset hypertension and proteinuria also recognized as the clinical signs of preeclampsia [95, 96].

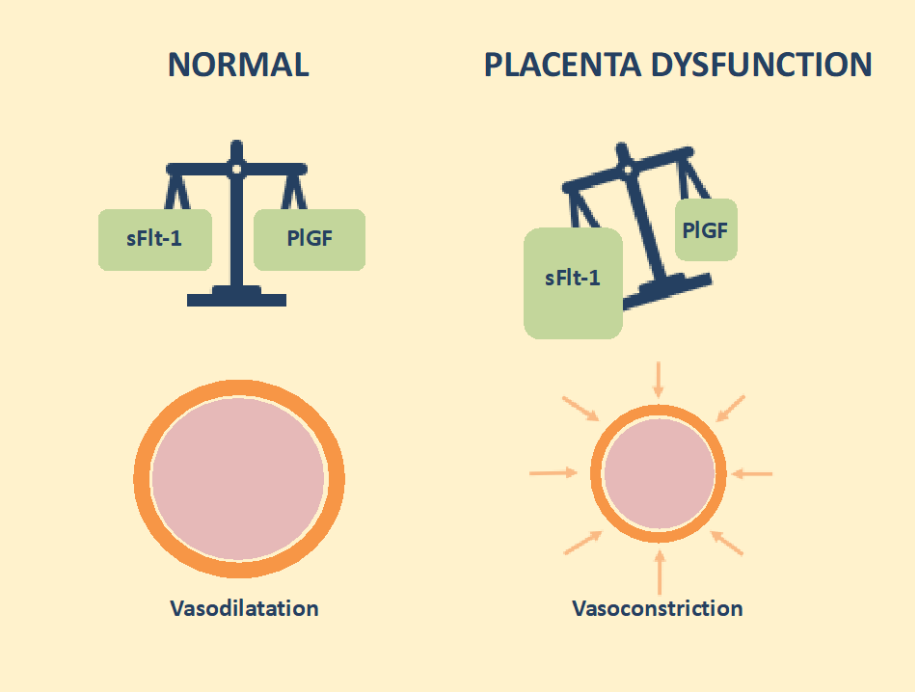

Figure 2-7. The vascular effect of angiogenic placentaderived biomarkers when sFlt-1 is increasaed resulting in lack of free PlGF.

Low PlGF, high sFlt-1, and high sFlt-1/PlGF ratio have been associated with increased risk of placenta dysfunction syndromes like preeclampsia, fetal growth restriction and preterm deliveries [95, 97-100]. Low PlGF and high sFlt-1 are considered valid predictors of preeclampsia [101], and a study published in the New England Journal of Medicine found that a sFlt-1/PlGF-ratio <38 was a useful predictor of short-term absence of preeclampsia when the syndrome was clinically suspected [98].

Maternal current smoking during pregnancy has been associated with lower sFlt-1 [102] and higher PlGF levels compared to non-smokers [103], giving a more favorable angiogenic profile [104]. The reduced risk of preeclampsia and the favourable angiogenic biomarker profile among smokers, are probably secondary to placental carbon monoxide effects [105] rather than effects of nicotine [78].

A register study from Sweden found increased risk of preeclampsia associated with snus use during pregnancy [78], but knowledge of the effect of maternal use of snus on midpregnancy angiogenic biomarker levels is lacking.

2.7 The role of fetal sex and nicotine in-utero

The term sex dimorphism refers to morphologic and biological differences between men and women [106]. Many of the non-communicable diseases (NCDs) display some degree of sex bias that can be explained by the differences between sex chromosomes; the altered regulatory pathways underlying sex-related development of organs as well as the influence of sex hormones throughout life [107]. Environmental factors such as chemical compounds like nicotine and tobacco might influence organ function differentially in males and females by epigenetic marking. The sex-specific response to environmental factors from early life might explain the different susceptibility to diseases between males and females [107].

Several studies, both animal and human, have explored the role of sex-specific differences on short- and long-term effects of maternal tobacco and nicotine use during pregnancy. A mouse model study showed that prenatal nicotine exposure induced sex-specific changes in cardio-respiratory integration, with a more prominent and persistent effect in males than females, potentially leading to a male dominated increased risk of cardiovascular diseases later in life [108]. The same study group reported that prenatal nicotine exposure to rats induced sex dependent changes in sleep-related autonomic regulation of heart rate postnatally, with males being significantly more susceptible [109]. Stevens et al. using data from the National Health and Nutrition Examination Survey (1999-2014) in the USA demonstrated that in-utero smoke exposure appeared to have long-term cardiometabolic impact on the unborn child. Male adolescents exposed to smoke in-utero, had an increased risk of metabolic syndrome compared to non-exposed adolescents (OR of 2.48 (1.19-5.20)). They also found associations between high waist circumference and body mass index (BMI) percentiles and prenatal smoke exposure especially among female adolescents [110].

An Indian study suggested sex differences of the in-utero effect of the maternal smokeless tobacco product 'mishri', a burnt tobacco tooth powder applied orally [111]. Among the 178 included women with singleton live births use of mishri during pregnancy was recorded postnatally by interview. Use of mishri was associated with reduced birth weight, and when stratifying by fetal sex, more so in girls. Additionally, they found a decreased male:female of 80.6:100 ratio in live newborns of maternal tobacco users compared to 105.5:100 in non-users [111]. However due to low sample size, potential recall bias and insufficient information about confounding factors, the results are not conclusive. Nevertheless, the reduced male:female ratio was confirmed in by England et al in a retrospective medical record review of 502 randomly selected deliveries in Alaska, where the percentage of male:female in continued smokeless tobacco users was 102.4:100 compared to 108.8:100 in non-tobacco users [112]. England et al have also investigated the effect of snus use specifically using data from the Swedish MBR, which did not confirm a change in gender ratio [74].

Angiogenic placenta biomarkers have also been shown to differ in male and female pregnancies. Among 6040 participants in a prospective cohort from the Netherlands higher sFlt-1 and PlGF were found in female fetus pregnancies compared to male fetus pregnancies in the first trimester [113]. In an observational, prospective population-based Danish cohort differences in biomarker levels were also found in the second-third trimester among 2110 women with higher sFlt-1 and sFlt-1/PlGF ratio in female fetus pregnancies [114].

We are unaware of studies assessing the effect of in-utero snus exposure on placenta angiogenic biomarkers or if fetal health effects are dependent on the fetal sex.

3 Objective and specific aims of the thesis

We are in the beginning of understanding the potential consequences of snus use during pregnancy, but there is an urgent need for more studies to fill the knowledge gaps. This is particularly important with the increased use of snus among women in reproductive age, and legislations opening up for alternative nicotine sources with largely unknown long-term effects, as well as the risk of a new epidemic of nicotine dependence.

The main objective of the thesis study was to determine the prevalence of nicotine use during pregnancy and the effects of in-utero exposure to snus use on placental health and fetal growth. These are the following specific aims:

Aim 1: To determine the maternal prevalence, pattern of use and cessation rate of snus and other nicotine products during pregnancy (paper 1)

Aim 2: To identify women at risk using snus in pregnancy (paper 1)

Aim 3: To determine if maternal snus use pre- and perinatally affect placental biomarkers in midpregnancy and/or neonatal anthropometric measures at birth (paper 2 and 3)

Aim 4: To explore if the effects of in-utero snus exposure differ by fetal sex, focusing on midterm angiogenetic placenta biomarkers (paper 2)

The use of snus for smoking cessation, or investigations into the societal attitudes regarding the use of snus today is outside the scope of this thesis. Although the results from snus exposure might be transferable to e-cigarettes and NRTs due to the common mediator nicotine, the potential effect of these products is not assessed in this thesis.

4 Methods and subjects

4.1 Study design

This thesis is uses data from the large international, general population-based, multi-center, prospective mother-child cohort Preventing Atopic Dermatitis and ALLergies in Children (PreventADALL) study including 2697 women with 2701 pregnancies, and 2397 mother-child pairs. The PreventADALL study has a 2x2 factorially, randomized controlled trial design with the two interventions; skin emollients from 0.5-9 months of age and early food introduction in infancy; as well as being an exploratory prospective study planning to follow the children into adulthood.

The two overall objectives of the PreventADALL study are to determine if it is possible to prevent allergic diseases by interventions, and secondarily to explore exposures and early life factors involved in the development of allergic diseases and NCDs. This thesis is placed in the exploratory study arm, exploring the extent and role of nicotine exposure in-utero.

Enrolment in the PreventADALL study had two steps; antenatal recruitment and enrolment of the pregnant mother, and secondly enrolling her child(ren) in the study at birth.

4.1.1 Inclusion criteria

The consenting women had a gestational age (GA) between 16-22 weeks with singleton or twin pregnancies at inclusion. In order to complete the electronic questionnaires, they needed sufficient Norwegian or Swedish language skills. Their healthy infants were included when delivered no earlier that GA 35.0 weeks.

4.1.2 Exclusion criteria

Exclusion criteria were three or more fetuses, severe maternal and/or fetal disease discovered before or at the 18-week ultrasound examination or plans to move from the study site areas within the baby's first year of life.

4.2 Recruitment

From December 2014 through October 2016 all pregnant women from the general population undergoing the routine ultrasound examination at pregnancy week 18 (between week 16-22) at Oslo University Hospital, Østfold Hospital Trust in Norway, and in the greater Stockholm area with plans to deliver the baby at Karolinska Institutet in Sweden were invited to participate in the study. Information brochures about the study were enclosed in the ultrasound appointment letter. Drop-in visits for more information were available and offered Monday to Friday nearby the antenatal clinics. Complementary information was provided by e-mail when requested. Infants born to included mothers were identified daily at the maternity wards and enrolled in the study. The final baby was included April 11th 2017.

An overview of the different time points for data collection relevant in this thesis is illustrated in Figure 4-1**.**

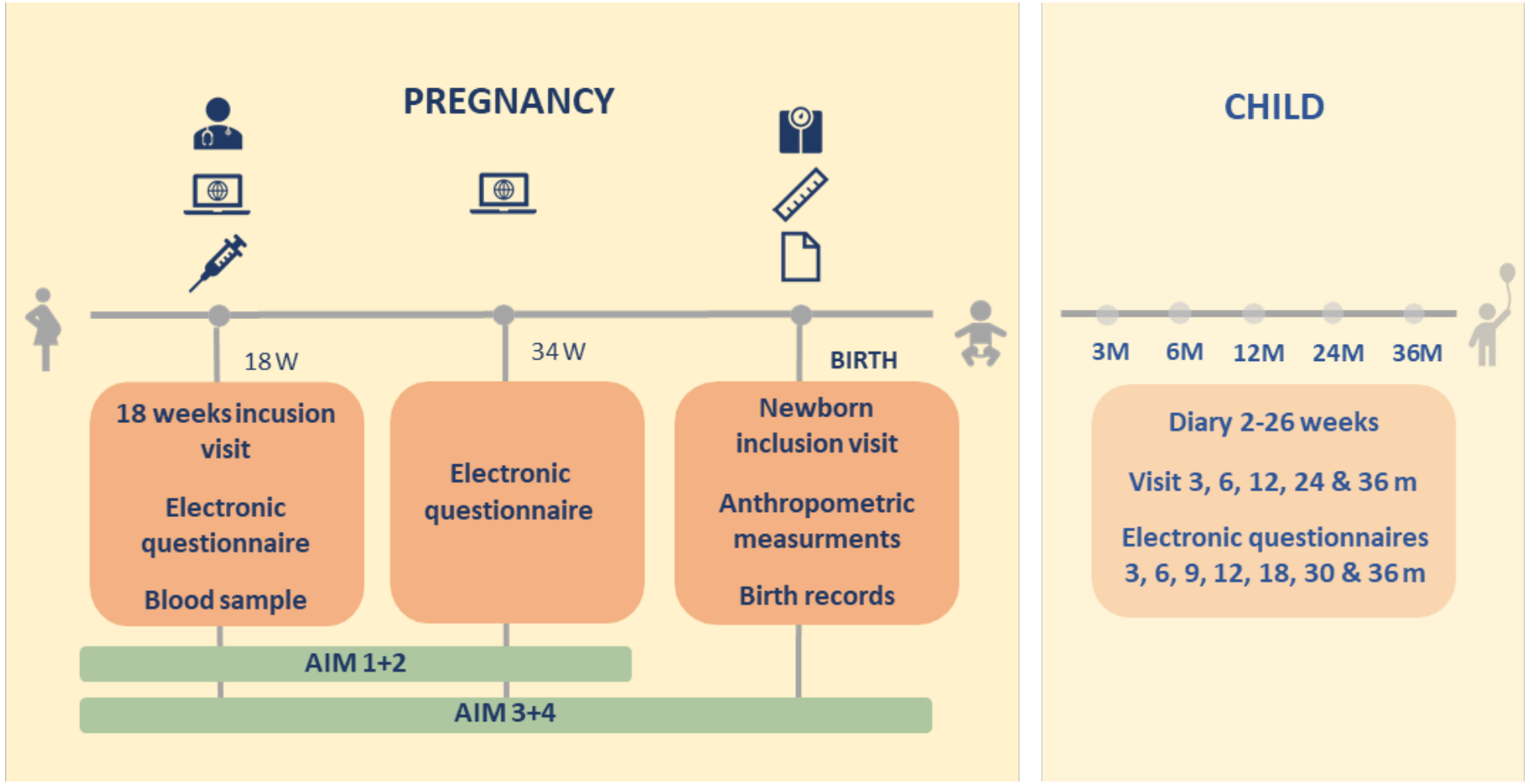

Figure 4-1. The aims of the thesis presented in a timeline related to data collection points and sources. The data collection points in the faded color box marked 'Child' are not included in the thesis, but are planned follow-up visits in the PreventADALL study.

4.3 Inclusion visit

At the routine ultrasound examination, all women were invited by their midwives to participate in the study. If the woman was interested, the midwives conducted additional ultrasound measurements with further referral to the inclusion visit arranged by the study personnel. After reading and signing the consent form, a brief structured interview was undertaken, including previous obstetric history as well as pre-pregnancy weight.

Maternal height, weight and blood pressure were measured, a non-fasting full blood sample was drawn and urine sample was collected.

Instructions were given on how to collect fecal and salivary specimen at home with a sampling kit provided by the PreventADALL study. Information on how to complete the electronic questionnaires at 18 and 34 weeks pregnancy was sent to the women via a link per e-mail, including information that they would receive an automatic reminder if the form was not completed within a week.

4.4 Electronic questionnaires at 18 and 34 weeks

The electronic questionnaires, as well as inclusion visit registration forms and diaries, were developed by the study team in collaboration with the University Center for Information Technology (USIT) at the University of Oslo. The encrypted responses of the questionnaires were securely collected, processed and stored in compliance with the Norwegian privacy regulation within the central Service for Sensitive Data (the TSD – Tjeneste for Sensitive Data) facilities owned by the University of Oslo.

The study participants received the questionnaires at fixed time points, the 18-week questionnaire related to the inclusion visit (between 16-22 weeks) and the 34-week questionnaire in pregnancy week 34.

The questionnaires collected general background information regarding maternal and paternal history of health and disease, maternal lifestyle and dietary habits during pregnancy, socio-demographic factors, living environment, previous and current exposure of nicotine and tobacco as well as stress and quality of life.

We collected information about ever use (daily or sometimes) of any tobacco or nicotine product prior to and during pregnancy up to the time of completing the 18-week questionnaire. In the 34-week questionnaire any use of tobacco or nicotine products since completing the 18-week questionnaire was recorded. The questions mapping previous and current tobacco and nicotine habits, where given separately for cigarette smoking and snus. The questions included stating the time of use in the categories 'stopped many years ago', 'stopped in time before pregnancy', 'stopped when recognized pregnancy' and 'current use'. If the women reported quitting snus and/or cigarette smoke prior to or at some time in pregnancy, she reported perceived pregnancy week of cessation. We did not specifically determine if cessation was in relation to antenatal visits. Further details in regards to frequency of use was gathered in the categories 'less than once a week', '1-2 days a week', '3-5 days a week' and 'daily'. Those who reported 'current use' received follow-up questions concerning the number of cigarettes or snus portions used and type/brand of product.

Questions assessing the use of NRTs and/or e-cigarettes were limited to time of use in the categories 'never', 'stopped before pregnancy/when recognized pregnancy', 'rarely', 'monthly', 'weekly', 'daily' or 'unknown'.

Gestational age at inclusion was based upon ultrasound corrected birth of date calculated by femur length.

The study participants registered their perceived GA when completing the questionnaires. It is unknown whether the GA reported was based upon ultrasound examination corrected GA or calculated from the last menstrual period. Detailed follow-up questions regarding the use of tobacco or other nicotine products in the period since completing the previous questionnaire were gathered in the 34-week questionnaire.

4.5 Blood sample and biomarker analyses

Standard Operating Procedures (SOPs) developed by the study personnel were used when drawing and managing the non-fasting blood sample from the women at the

18-week inclusion visit. The blood was collected into a Serum Separator Tube (SST), then centrifuged between 60 to 90 minutes and stored in the PreventADALL biobank before analyzing maternal serum concentrations of sFlt-1 (pg/mL) and PlGF (pg/mL). All analyses were performed at the Oslo University Hospital, Department of Medical Biochemistry from March-April 2018. The levels of s-Flt-1 and PlGF were determined using the fully automated Elecsys® sFlt-1 and Elecsys® PlGF assays on the cobas e801 electrochemiluminescence immunoassay platform (Roche Diagnostics GmbH, Mannheim, Germany). Subsequently these values were used to calculate the sFlt-1/PlGF ratio. The concentrations were within the detectable ranges of the Elecsys® assays of PlGF (3-10,000 pg/mL) and sFlt-1 (10-85,000 pg/mL). The coefficients of variation were ≤ 2.1% for PlGF and ≤ 1.8% for sFlt-1.

4.6 Newborn inclusion

Dedicated study personnel included the newborn baby at the maternity ward within the first 1-2 days of life, or as soon as possible and/or appropriate. Written consent was collected from both parents whenever possible. Anthropometric measures of the infant (details follow) were conducted at inclusion. Structured interview related to history of delivery and postpartum period was conducted in addition to collection of several biosamples including skin swabs for microbiome, meconium specimen, urine and vernix samples, cord blood and placenta swabs and biopsies [115] that are not relevant for this thesis.

4.7 Anthropometric measurements

Standardized anthropometric measures of the infant were performed by dedicated study personnel at the maternity ward at inclusion, within 1-2 days after delivery. The left upper arm circumference was measured midway between the acromial and olecranon process. Thoracic circumference was measured with the lower part of the measuring tape in line with the most caudal part of the xyphoid process. For the abdominal circumference, the measuring tape was placed with the lower part in line with the cranial part of the umbilicus. Both thoracic and abdominal circumferences

were measured at end-expiration when possible and repeated three times. The left upper arm circumference was measured twice. We used a non-elastic measuring tape held firmly, but not too tight in order to avoid depressing the skin. All values were denoted in centimeters with one decimal, reporting the mean value rounded up or down to one decimal.

4.8 Birth records

Birth weight, length (crown-heel) and head circumference as well as placenta weight were obtained from the hospital birth records Partus by three dedicated pediatricians working in the study. The midwives weighed and recorded the placenta within 30 minutes after delivery according to hospital guidelines.

4.9 Biobank

All PreventADALL biobank samples received a unique barcode and were electronically scanned and organized within the Oslo University Hospital system MedInsight before stored at -80° Celsius.

5 Statistical and analytic approaches

5.1 Outcomes, baseline variables, exposures and covariates

VARIABLES	DATA SOURCE	DENOMINATION & CATEGORIES	VARIABLE USE (AIM)
INFANT FACTORS			
Fetal sex	Newborn inclusion Birth records	Male, female	Baseline (3, 4) Covariate/ exposure (3, 4)
Gestational age at birth	Birth records	Weeks and days (continuous)	Baseline (3) Covariate (3)
Gestational age at blood sampling	18 W inclusion	Weeks (grouped; 16-17, 18, 19-20, 21-22)	Baseline (3, 4) Covariate (3, 4)
Birth weight	Birth records	g	Outcome (3)
Length	Birth records	cm	Outcome (3)
Head circumference	Birth records	cm	Outcome (3)
Thoracic circumference	Newborn inclusion	cm	Outcome (3)
Abdominal circumference	Newborn inclusion	cm	Outcome (3)
Left upper arm circumference	Newborn inclusion	cm	Outcome (3)
Placenta weight	Birth records	g	Outcome (3)
Thoracic/abdominal circumference	Calculated	Ratio	Outcome (3)
Thoracic/head circumference	Calculated	Ratio	Outcome (3)
Birth weight/placenta weight	Calculated	Ratio	Outcome (3)
MATERNAL FACTORS			
Age Years	18 W inclusion	Years (continuous and grouped; <30, >30-35, >35-40, >40)	Baseline (1-4) Covariate (2-4)
Pre-pregnancy BMI	Calculated	BMI (continuous and grouped; <18.5, 18.5-24.9, >25.0-29.9, >30.0 kg/m^2)	Baseline (1-4) Covariate (3, 4)
BMI at 18 weeks	Calculated	BMI scale	Baseline (3)
Height	18 W inclusion	cm	Baseline (1-3)
Weight pre-pregnancy	18 W inclusion	kg	Baseline (1-3)
Weight at inclusion	18 W inclusion	kg	Baseline (1-3)
Gestational weight gain up to 18 weeks	Calculated	kg	Covariate (3)
sFlt-1	Blood sample 18 W inclusion	pg/mL	Outcome (3, 4)
PlGF	Blood sample 18 W inclusion	pg/mL	Outcome (3, 4)
sFlt-1/PlGF	Blood sample 18 W inclusion	ratio	Outcome (3, 4)
Current in vitro fertilization	18 W inclusion	Yes, no	Baseline (3)
Miscarriage(s) <12 weeks	18 W inclusion	0, 1, >1	Baseline (3)
Miscarriage(s)/stillbirths 12-23 weeks	18 W inclusion	0, 1, >1	Baseline (3)
Previous pregnancies*	18-week Q		Baseline (1, 2)
Parity (based on previous deliveries)	18-week Q	0, 1, >1	Baseline (3, 4) Covariate (3, 4)

VARIABLES	DATA SOURCE	DENOMINATION & CATEGORIES	VARIABLE USE (AIM)
SOCIODEMOGRAFIC FACTORS			
Education	18-week Q	Preliminary school only High school only Higher education <4 years Higher education ≥ 4 years Other	Baseline (1-4)
Country of origin	18-week Q	Norway and Sweden Rest of the world	Baseline (1-4) Covariate (2)
Marital status	18-week Q	Married, cohabitants, single, divorced/separated, other	Baseline (1-3) Covariate (2)
Living area	18-week Q	City, densely populated City, less densely populated Suburb Countryside, village Countryside, outside village	Baseline (1-3) Covariate (2)
Household income	18-week Q	Low, middle, high, not reported	Baseline (3)
NICOTINE			
Previous smoking	18-week Q	Yes, no	Baseline (3) Covariate (2-4)
Snus	18-week Q 34-week Q	Never Stopped years before pregnancy Stopped in time before pregnancy Stopped when recognizing pregnancy Current use at 18 and 34 weeks	Baseline (3, 4) Covariate/ exposure (3, 4) Outcome (1, 2)
Smoke	18-week Q 34-week Q	Never Stopped years before pregnancy Stopped in time before pregnancy Stopped when recognizing pregnancy Current use at 18 and 34 weeks	Baseline (3, 4) Covariate/ exposure (3, 4) Outcome (1, 2)
NRTs and e-cigarettes	18-week Q 34-week Q	Never Stopped before/when recognizing pregnancy Rarely Monthly Weekly Daily Unknown	Outcome (1, 2)
In-utero exposure to cigarette smoke**	18-week Q	No, yes, do not know	Baseline (3) Covariate (2)

Table 5-1 presents a detailed overview of the different variables used in this thesis. *Excluding current pregnancy. **The index woman being exposed to maternal smoking in-utero. G=gram, cm=centimeter, W=week, BMI=body mass index, kg=kilogram, m=meter, pg/mL=picogram/milliliter, Q=questionnaire.

5.1.1 Tobacco and nicotine

The prevalence of any tobacco and snus use, as well as other nicotine products such as NRTs (patches and lozenges) and e-cigarettes during pregnancy were *outcome* variables when determining maternal prevalence and cessation rate during pregnancy in aim 1 with details presented in Table 5-1.

Snus use during pregnancy was also an *exposure* variable when assessing the in-utero effect on the biomarker and birth size outcomes:

To determine the effect on biomarkers for aim 3 and 4, the snus *exposure* group was re-categorized into *ever use in life* by 'never' (reference group), 'stopped before pregnancy', 'stopped when recognized pregnancy' and 'current use'. The dual users of cigarette smoke and snus were removed from the snus categories in order to isolate the effect of snus use alone during pregnancy.

For assessing the effect on birth size for aim 3, the snus *exposure* group was re-categorized into *ever use in pregnancy* by 'never' (reference group), 'snus only' and 'smoke/dual'. We also divided the 'snus only' group into 'snus only 18 weeks' and 'snus only 34 weeks' to differentiate between early and late snus exposure.

5.1.2 Angiogenic biomarkers

Biomarker *outcomes* for aim 3 and 4 were the midpregnancy levels of the biomarkers sFlt-1 (pg/mL), PlGF (pg/mL), and sFlt-1/PlGF ratio. The *exposure* variables used in the analyses of biomarkers levels included use of snus during pregnancy (as described in the previous section) as well as fetal sex.

5.1.3 Birth size

The *outcome* variables for aim 3 were the anthropometric measures and the calculated proportional size ratios listed in Table 5-1.

5.1.4 Covariates

An overview of covariates included in the multivariable linear regression analyses in the preliminary and final models for aims 2, 3 and 4 are presented in Table 5-2.

PRELIMINARY COVARIATES	AIM 2 RISK FACTORS SNUS	AIM 3 & 4 BIOMARKERS	AIM 3 BIRTH SIZE
Maternal age	x	x	x
Educational level	x		x
Marital status	x		x
Country of origin	x		x
Living area	x		x
Household income	x		
Previous or current smoking history	x	x*	x
Household cigarette smoking	x		
In-utero exposure to cigarette smoking	x		x
Previous pregnancies (excl. current)	x		
Parity (based on previous deliveries)	x	x	x
Pre-pregnancy BMI	x	x	x
Gestational weight gain up to 18 weeks			
GA at birth			x
Fetal sex		x	x
GA at 18 weeks blood sampling		x	

*The model with snus use as main exposure was additionally adjusted for previous smoking history.

Table 5-2. Overview of covariates included in the multivariable regression analyses in the preliminary models.

Covariates included in the biomarker analyses for aim 2 were based on factors previously shown to be associated with cigarette smoking, and for aim 3 and 4 the covariates were based on those previously shown to be associated with snus use during pregnancy [116] as well as factors possibly associated with birth size and angiogenic biomarkers.

5.2 Statistical methods

5.2.1 Aim 1 and 2 – prevalence and risk factors

Categorical variables are presented in numbers and percentages, and continuous variables as means with maximum and minimum values.

Differences between categorical variables were analysed by the Chi-Square test and numerical data by the Student's t-test or by One-Way ANOVA tests. To determine

factors associated with snus use during pregnancy, binary logistic regression was used by first excluding potential covariates with a p-value above 0.25. The covariates included in the preliminary model are listed in Table 5-2.

5.2.2 Aim 3 and 4 – biomarkers

Categorical variables are presented in numbers and percentages, and continuous variables as means ± standard deviation (SD) or medians with interquartile ranges (IQR) as appropriate. Differences between the outcome variables were analysed by the Mann-Whitney U test or by Kruskal-Wallis tests stratified by exposure variables including fetal sex.

The midpregnancy angiogenic biomarkers were log transformed due to non-normality before assessment of the effect of snus exposure for all linear regression models. These effect estimates were back translated using the exponential function before presenting the results. The preselected covariates tested are presented in Table 5-2, all with potential biological effect on the biomarkers. The interaction fetal sex*tobacco exposure were included and effect modification analyses were conducted by entering the interaction term into the multivariable regression model when significant.

5.2.3 Aim 3 – birth size

The categorical variables are presented in numbers and percentages, and continuous variables as means with SD or 95% confidence intervals (CI). Categorical variables were recoded into binary variables when needed. Differences in categorical data were analysed by Chi-Square test and numerical data by One-Way ANOVA tests.

To explore if snus exposure and birth size outcomes were associated, general linear regression models were used with birth outcomes as dependent and snus exposure as independent variable. After preliminary analyses (Table 5-2) significant covariates were kept for each outcome in the final multivariable models. Sensitivity analyses and adjustment for maternal gestational weight gain from pre-pregnancy to 18 weeks of pregnancy were performed in case of significant associations between snus exposure and the respective birth size outcome post hoc.

The significance level was set to 5 per cent. All analyses were performed by IBM© SPSS© statistics version 25 (Chicago, IL, U.S.A.).

5.3 Subjects

In the PreventADALL study 2697 women with 2701 pregnancies were included at 18-week gestational age at the routine ultrasound examination, 2149 in Norway and 552 in Sweden between December 2014 and October 2016. Four of the study participants were included twice, and there were in total 17 twin pregnancies.

In the mother-child cohort in total 2397 mother-child pairs were included. In the course of the study one study participant withdrew after newborn inclusion.

Mean (range) age of the participating women was 32 (18-42) years, 57.1% had more than 4 years of higher education, they were predominantly native born in Norway or Sweden (89%), and most were urban living (76.8%) together with their husbands (41.2%) or cohabiting partner (55.9%). At birth the mean (range) infant estimated GA was 39.2 (35.6 - 42.9) weeks and 52.7% of the included infants were boys [115].

The final sub-study populations in regard to the specific aims are illustrated in Figure 5-1, Figure 5-2 and Figure 5-3 and background characteristics are presented in Table 5-3. Twin pregnancies (n=12) were excluded from the biomarker and birth size analyses because of the detrimental different effect on both placenta and fetal growth (significantly different mean birth weight of singletons compared to twins [117]) compared to singleton pregnancies.

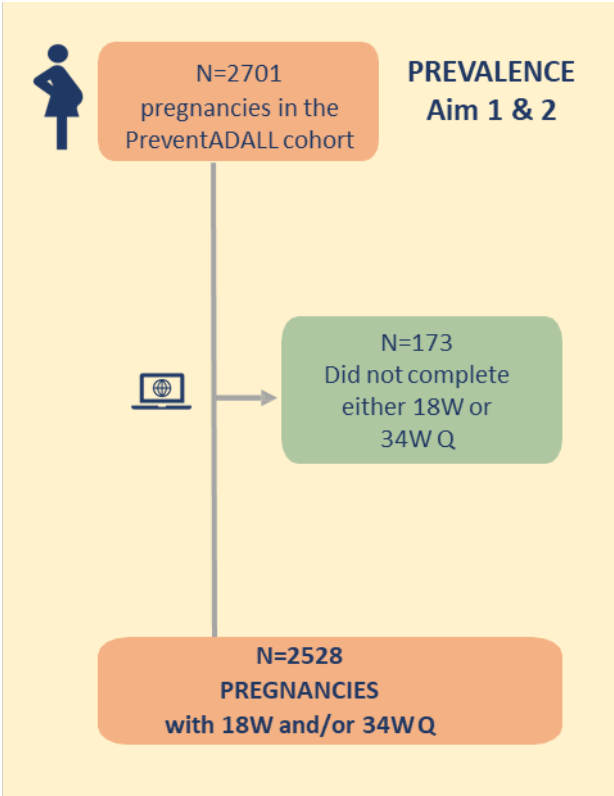

Figure 5-1. N=number, W=week, Q=questionnaire

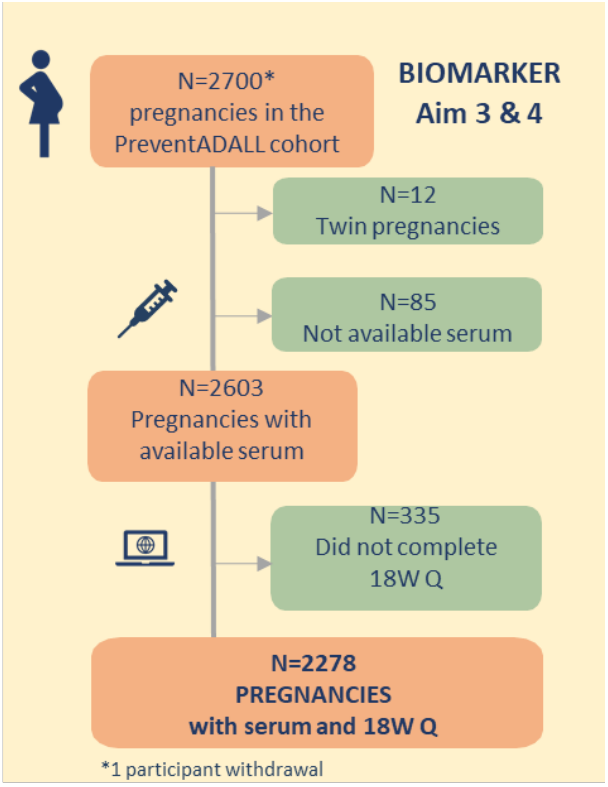

Figure 5-2. N=number, W=week, Q=questionnaire

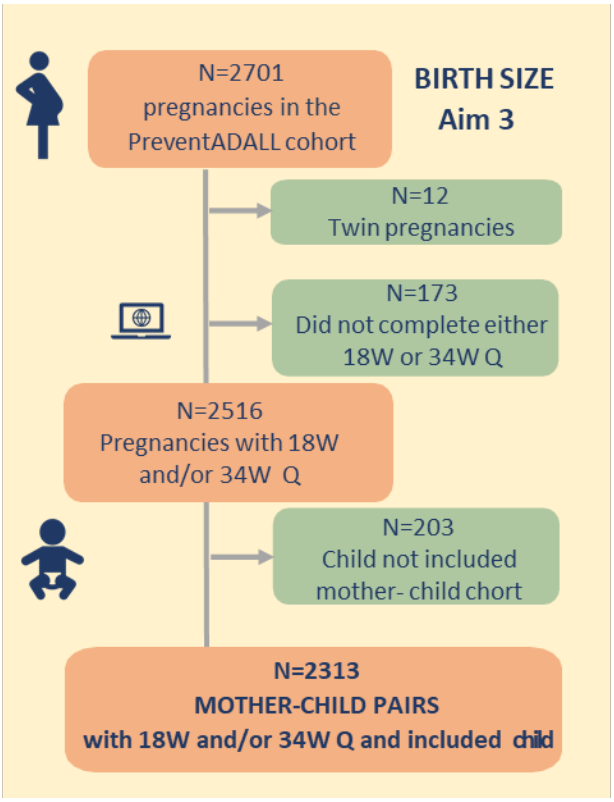

Figure 5-3. N=number, W=week, Q=questionnaire

Baseline characteristics for the different study populations are presented in Table 5-3.

In the population for aim 1 and 2 the baseline characteristics between those who completed at least one of the 18- and/or 34-week questionnaires (n=2528) and those who did not (n=173), were similar except slightly higher age among the included.

Of the 2603 women who had available serum compared to those who also had available 18-week questionnaire (n=2278) for the biomarker aim 3 and 4, the study populations were similar in terms of baseline characteristics.

For aim 3 regarding birth size the 2313 included mother-child pairs with at least one available questionnaire as well as anthropometric measures of the newborn; only marital status, previous smoking history and gestational age at birth differed from the excluded group.

BASELINE CHARACTERISTICS	Aim 1+2 PREVALENCE (N=2528)	Aim 3+4 BIOMARKER (N=2278)	Aim 3 BIRTH SIZE (N=2313)
Fetal male sex, n (%)	N/A	1201 (53.0)	1216 (52.6)
GA at delivery (weeks), mean (±SD)	N/A	N/A	39.3 (1.7)
GA at delivery (days), mean (±SD)	N/A	N/A	274.8 (11.7)
GA at blood sampling (weeks), mean (±SD)	N/A	19.8 (6.1)	N/A
GA at blood sampling (weeks), n (%)	N/A		N/A
16-17		168 (7.5)	
18		695 (30.8)	
19-20		859 (38.1)	
21-23		532 (23.6)	
Maternal age at inclusion (years), mean (min-max)/(±SD)	32.4 (20.0-48.0)	32.4 (4.2)	32.4 (4.1)
Maternal age at inclusion (years), n (%)	N/A		N/A
<30		770 (33.8)	
30 – 35		993 (43.6)	
>35 – 40		441 (19.4)	
>40		74 (3.2)	
Maternal pre-pregnancy BMI (kg/m^2), mean (min-max)/(±SD)	23.2 (13.8-45.7)	24.8 (3.7)	23.1 (3.6)
Maternal pre-pregnancy BMI (kg/m^2), n (%)	N/A		N/A
Underweight <18.5		76 (3.4)	
Normal weight 18.5 – 24.9		1669 (75.1)	
Overweight 25.0 – 29.9		361 (16.3)	
Obese ≥30.0		115 (5.2)	
Height (cm), mean (min-max)/(±SD)	168.0 (147.0-187.0)	N/A	168.0 (6.2)
Weight pre-pregnancy (kg), mean (min-max)/(±SD)	65.4 (42.5-126.0)	N/A	65.4 (11.2)
Weight at inclusion (kg), mean (min-max)/(±SD)	70.2 (44.8-132.7)	N/A	70.1 (11.2)
BMI at 18 weeks (kg/m^2), mean (±SD)	N/A	N/A	24.8 (3.7)
Current in vitro fertilization, n (%)	N/A	N/A	177 (7.7)
Miscarriage(s) <12 weeks, n (%)	N/A	N/A	
0			1737 (75.2)
1			408 (17.7)
>1			165 (7.1)
Miscarriage(s)/stillbirths 12-23 weeks, n (%)	N/A	N/A	
0			2250 (97.8)
1			48 (2.1)
>1			2 (0.1)
Previous pregnancies (excluding current), n (%)		N/A	N/A
0	1236 (48.9)		
1	652 (25.8)		
>1	640 (25.3)		

BASELINE CHARACTERISTICS	Aim 1+2 PREVALENCE (N=2528)	Aim 3+4 BIOMARKER (N=2278)	Aim 3 BIRTH SIZE (N=2313)
Parity (previous deliveries), n (%)	N/A		
0		1369 (60.1)	1290 (60.0)
1		717 (31.5)	677 (31.5)
>1		192 (8.4)	183 (8.5)
Country of origin, n (%)			
Norway or Sweden	2085 (88.7)	2020 (88.7)	1914 (89.0)
Rest of the world	265 (11.3)	258 (11.3)	236 (11.0)
Education (years), n (%)			
Preliminary school only (9-10 years)	18 (0.8)	18 (0.8)	16 (0.7)
High school only	239 (10.2)	230 (10.1)	219 (10.2)
Higher education <4 years	758 (32.2)	730 (32.2)	682 (31.9)
Higher education >4 years	1324 (56.3)	1289 (56.8)	1222 (57.1)
Other	2 (0.1)	2 (0.1)	2 (0.1)
Unknown	9 (0.4)	N/A	N/A
Marital status, n (%)		N/A	
Married	968 (41.2)		885 (41.2)
Cohabitants	1313 (55.9)		1210 (56.3)
Single	44 (1.9)		37 (1.7)
Divorced/separated	1 (0.04)		1 (0.0)
Other	24 (1.0)		17 (0.8)
Living environment, n (%)		N/A	
City, densely populated	916 (39.0)		834 (38.8)
City, less densely populated	882 (37.5)		817 (38.0)
Suburb	373 (15.9)		343 (16.0)
Countryside, in village	127 (5.4)		111 (5.1)
Countryside, outside village	52 (2.2)		46 (2.1)
Household income, n (%)	N/A	N/A	
Low			25 (1.2)
Middle			1150 (53.5)
High			938 (43.6)
Not reported			37 (1.7)
Previous smoking, n (%)	N/A	N/A	475 (22.1)
Smoke, n (%)	N/A		N/A
Never		1762 (77.3)	
Stopped before pregnancy		404 (17.7)	
Stopped when recognizing pregnancy		97 (4.3)	
Current use at 18 and 34 weeks		15 (0.7)	
Snus, n (%)	N/A		N/A
Never		1762 (77.3)	
Stopped before pregnancy		343 (15.1)	
Stopped when recognizing pregnancy		160 (7.0)	
Current use at 18 and 34 weeks		13 (0.6)	
Grand maternal smoking during pregnancy, n (%)	N/A	N/A	
No			1623 (75.5)
Yes			306 (14.2)
Do not know			221 (9.5)

Table 5-3. Baseline characteristics for the study populations categorized by aims. N=number, GA=gestational age, SD=standard deviation, min=minimum, max=maximum, BMI=body mass index, kg=kilograms, m=metre, cm=centimetre. There are missing data for several of the baseline characteristics for each aim with further details described in the articles published/submitted in the appendix.

6 Ethical considerations

The PreventADALL 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), and the study was registered at ClinicalTrials.gov (number NCT02449850). Written informed consent was obtained from the pregnant woman at 18 weeks inclusion, and by both parents when possible at newborn inclusion.

7 Results

The covariates used in the final statistical models for aim 2, 3 and 4 are presented in Table 7-1.

	AIM 2 RISK FACTORS SNUS		AIM 3 & 4 BIOMARKERS		AIM 3 BIRTH SIZE	
COVARIATES	Preliminary	**Final**	Preliminary	**Final**	Preliminary	**Final**
Maternal age	x	**x**	x	**x**	x	**x**
Educational level	x				x	
Marital status	x	**x**			x	
Country of origin	x	**x**			x	
Living area	x	**x**			x	
Household income	x					
Previous or current smoking history	x	**x**	x*	**x***	x	
Household cigarette smoking	x					
In-utero exposure to cigarette smoking	x	**x**			x	
Previous pregnancies (excl. current)	x					
Parity (based on previous deliveries)	x		x	**x**	x	**x**
Pre-pregnancy BMI	x		x	**x**	x	**x**
Gestational weight gain up to 18weeks						**x (post hoc)**
GA at birth					x	**x**
Fetal sex			x	**x**	x	**x**
GA at 18W blood sampling			x	**x**		

*The model with snus use as main exposure was additionally adjusted for previous smoking history.

Table 7-1. Overview of covariates included in the multivariable regression analyses in the preliminary and final models for each aim.

7.1 Aim 1: To determine the maternal prevalence, pattern of use and cessation rate of snus and other nicotine products during pregnancy

Overall, 286 out of 2528 participants, 11.3% reported use of any nicotine product at some time during pregnancy. The most commonly used product was snus alone, reported by 6.5% of the women, followed by 4.1% for cigarette smoke, 0.6% dual snus and cigarette users and finally 0.2% use of NRTs/e-cigarettes.

To address the specific prevalence of use of nicotine products in early and late pregnancy, we used data from the 18-week (n=2350) and 34-week (2365) questionnaire respectively. To assess the cessation and relapse rate throughout pregnancy, we included data from all 2187 women who completed both questionnaires.

Further details on number of completed questionnaires stratified by country are given in Table 7-2. When stratifying the study population by snus exposure groups the baseline characteristics differed significantly in regard to gestational age at birth, maternal age, maternal gestational weight gain, parity and socioeconomic factors.

Based on the 18-week questionnaire (n=2350), the prevalence was higher with snus use alone of 6.9%, cigarette smoke of 4.3% and dual use of 0.6%. Adding the dual users to snus users, 7.6% reported snus use at some time including current use, during pregnancy. When stratified by country, there was a significant increased snus use among Norwegian compared to Swedish women (8.2% versus 5.5%, p-value 0.035) (Table 7-2).

Overall 1.4% of those completing the 18-week questionnaire reported current use of nicotine at 18 weeks pregnancy, with 0.6% reporting snus use alone and 0.8% reporting cigarette smoking. In all, 0.3% reported continued use of snus or cigarette smoke, respectively at 34 weeks pregnancy, while none reported continued dual use.

The prevalence of NRTs (only nicotine gum reported) and e-cigarettes was 0.2% at some time until 18 weeks, 0.04% between 18 and 34 weeks, and no current users at 34 weeks pregnancy. Among the daily current snus users at 18 weeks pregnancy, 77% reported use of three to six snus portions a day.

Parameter	Total	Norway	Sweden	p-value[#]
Questionnaire adherence				
Completed at 18 weeks	2350 (100.0)	1818 (77.4)	532 (22.6)	
Completed 34 weeks	2365 (100.0)	1853 (78.4)	512 (21.7)	
Completed one or more	2528 (100.0)	1985 (78.5)	543 (21.5)	
Completed both	2187 (100.0)	1686 (77.1)	501 (22.9)	
Tobacco habits				
Ever use in life (n=2350)				
All nicotine-containing products	840 (35.7)	680 (37.4)	160 (30.1)	**0.002**
Snus	529 (22.5)	434 (23.9)	95 (17.9)	**0.003**
Cigarette smoke	531 (22.6)	423 (23.3)	108 (20.3)	0.15
Use before pregnancy (n=2350)				
Stopped years before pregnancy				
Snus	213 (9.6)	165 (9.1)	48 (9.0)	0.97
Cigarette smoke	338 (14.4)	276 (15.2)	62 (11.7)	**0.04**
Stopped in the time before pregnancy				
Snus	138 (5.9)	120 (6.6)	18 (3.4)	**0.005**
Cigarette smoke	77 (3.3)	65 (3.6)	12 (2.3)	0.13
Snus in pregnancy (including dual users)				
At some time up to 18 weeks (n=2350)	178 (7.6)	149 (8.2)	29 (5.5)	**0.035**
Current use at 18 weeks	13 (0.6)	11 (0.6)	2 (0.4)	0.81
Stopped when recognising pregnancy	165 (7.0)	138 (7.6)	27 (5.1)	
At some time from 18–34 weeks (n=2365)	18 (0.8)	14 (0.8)	4 (0.8)	0.95
Current use at 34 weeks	6 (0.3)	4 (0.2)	2 (0.4)	
Stopped between 18 and 34 weeks	12 (0.5)	10 (0.5)	2 (0.4)	
Cigarette smoke in pregnancy (including dual users)				
At some time up to 18 weeks (n=2350)	116 (4.9)	82 (4.5)	34 (6.4)	0.08
Current use at 18 weeks	18 (0.8)	14 (0.8)	4 (0.8)	0.84
Stopped when recognising pregnancy	98 (4.2)	68 (3.7)	30 (5.6)	
At some time from 18–34 weeks (n=2365)	14 (0.6)	10 (0.5)	4 (0.8)	0.53
Current use at 34 weeks	8 (0.3)	6 (0.3)	2 (0.4)	
Stopped between 18 and 34 weeks	6 (0.3)	4 (0.2)	2 (0.4)	
Dual snus and cigarette smoke in pregnancy				
At some time up to 18 weeks (n=2350)	15 (0.6)	7 (0.4)	8 (1.5)	**0.004**
Current use at 18 weeks	0	0	0	
Stopped when recognising pregnancy	15 (0.6)	7 (0.4)	8 (1.5)	
At some time from 18–34 weeks (n=2365)	1 (0.04)	0	1 (0.2)	
Current use at 34 weeks	0	0	0	
Stopped between 18 and 34 weeks	1 (0.04)	0	1 (0.2)	
NRTs/e-cigarettes in pregnancy				
At some time up to 18 weeks (n=2350)	5 (0.2)	3 (0.2)	2 (0.4)	
Current use at 18 weeks	2 (0.1)	1 (0.1)	1 (0.2)	
Stopped when recognising pregnancy	3 (0.1)	2 (0.1)	1 (0.2)	
At some time from 18–34 weeks (n=2365)	1 (0.1)	0	1 (0.2)	
Current use at 34 weeks	1 (0.1)	0	1 (0.2)	
Stopped between 18 and 34 weeks	0	0	0	

[#]: p-values in bold (p<0.05) are significant.

Table 7-2. Reported use of snus, cigarette smoke and nicotine-replacement therapies (NRTs)/e-cigarettes in Norway and Sweden. *From ERJ Open Research, Kreyberg I, Bains KES, Carlsen KH, et al. Stopping when knowing: use of snus and nicotine during pregnancy in Scandinavia. 2019;5(2):00197-2018. Published 2019 Apr 8. doi:10.1183/23120541.00197-2018. Reprinted with permission.*

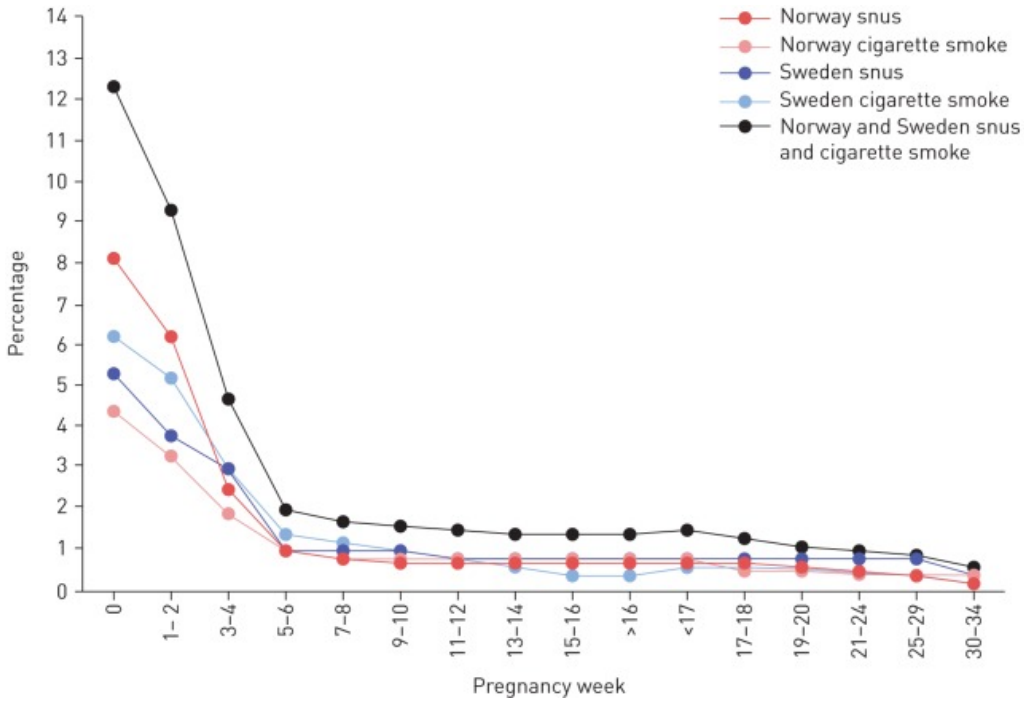

Figure 7-1. Percentage of women using snus and/or cigarette smoke during pregnancy based on self-reported time for stopping from those who completed both 18-week and 34-week questionnaires (n=2187). Time for stopping categories were from 1–2 weeks to >16 weeks and from <17 weeks to 30–34 weeks, respectively. Among the women who reported the use of snus, cigarette smoke or dual use at some time during pregnancy, most stopped when they recognized their pregnancy (mean pregnancy week three to four). Week 0 reflects the total percentage of women who reported any use during pregnancy. E-cigarettes and nicotine replacement therapies were not included since the rates were low. *From ERJ Open Research, Kreyberg I, Bains KES, Carlsen KH, et al. Stopping when knowing: use of snus and nicotine during pregnancy in Scandinavia. 2019;5(2):00197-2018. Published 2019 Apr 8. doi:10.1183/23120541.00197-2018. Reprinted with permission*

Nicotine use some time in pregnancy was reported by 269 of the 2187 women who completed both the 18- and 34-week. While 245/269 (91.1%) reported cessation of smoke and/or snus at the time of recognizing the pregnancy, 143 of the 164 snus users (87.2%) and 83 of the 105 smokers (79.1%) reported cessation already within pregnancy week 6 (Figure 7-1). Relapse of nicotine use after cessation by 18 weeks of pregnancy was reported by six (0.03%) women for snus use and one woman to cigarette smoking in the period from 18 to 34 weeks pregnancy. Additionally, three women relapsed to snus or cigarettes after 18 weeks of pregnancy after not reported use of nicotine products earlier in pregnancy.

7.2 Aim 2: To identify women at risk of using snus in pregnancy

All the factors investigated, including those not found significantly associated, are illustrated in Figure 7-2.

In the final multiple logistic regression model we identified the following factors associated with snus use during pregnancy; an inverse association with age, a positive association with being native born in Norway or Sweden, urban living, a cohabitant relationship, previous and/or current smoking history as well as the study participant herself being exposed to cigarette smoking in-utero with details given in Figure 7-3. The associated predictors could explain 15% of the snus use.

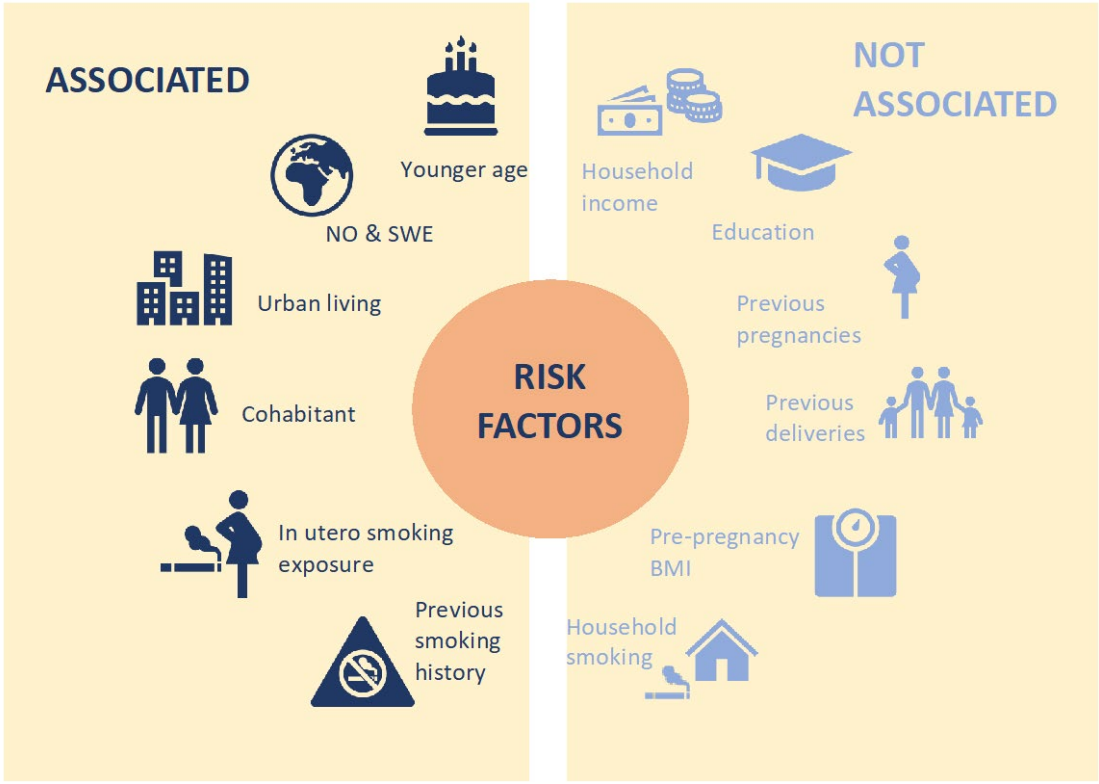

Figure 7-2. The different risk factors assessed to determine if associated with snus use during pregnancy, significance level set to 0.05 for associated factors.

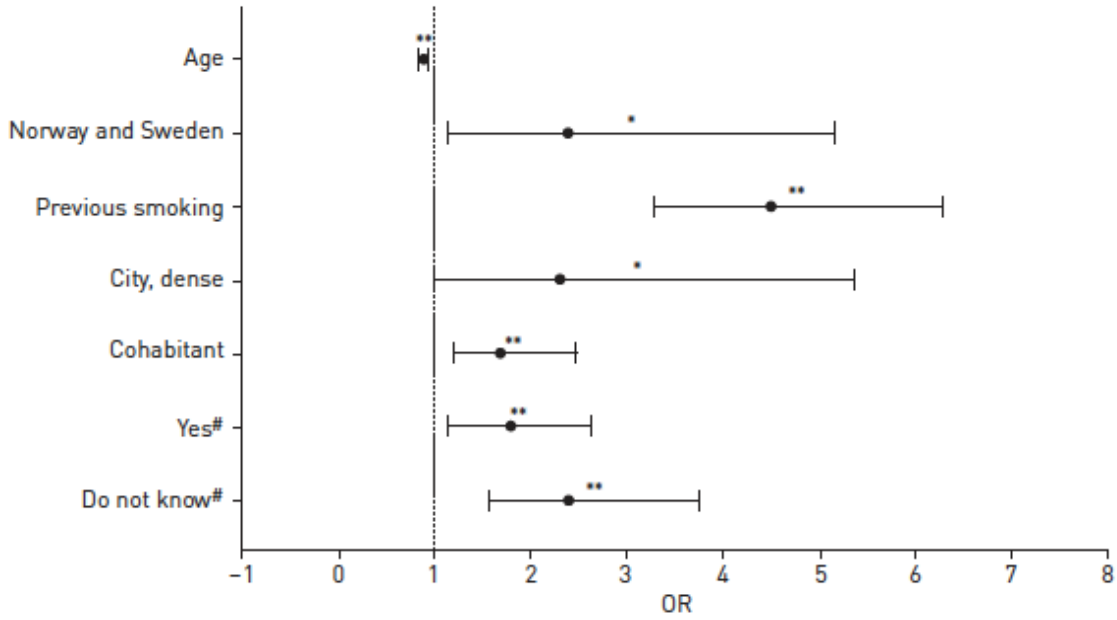

Figure 7-3. Factors associated with snus use during pregnancy. In multivariate analysis, the following factors were identified as associated with the use of snus at some time during pregnancy: living area (countryside (village) *versus* city (densely populated), city (less densely populated), suburb, countryside (outside village)); grand maternal cigarette smoking (no *versus* yes, do not know); country of origin (rest of the world and other Nordic countries combined *versus* Norway or Sweden); previous and/or current smoking (no *versus* yes); marital status (married *versus* cohabitant, single, divorced/separated, other). Age was used as a continuous variable. OR: odds ratio. #: *in utero* smoking exposure of the index woman; *: p<0.05; **: p<0.01. *From ERJ Open Research, Kreyberg I, Bains KES, Carlsen KH, et al. Stopping when knowing: use of snus and nicotine during pregnancy in Scandinavia. 2019;5(2):00197-2018. Published 2019 Apr 8. doi:10.1183/23120541.00197-2018. Reprinted with permission.*

7.3 Aim 3: To determine if maternal snus use pre- and perinatally affect placental biomarkers in midpregnancy and/or neonatal anthropometric measures at birth

Due to the cessation rate of 87.2% amongst snus users in early pregnancy, the exposure was mainly based on snus use at some time up to 18 weeks with the prevalence of 7.0%. In order to isolate the effect of snus alone, the 0.6% dual snus and cigarette users were excluded.

7.3.1 Angiogenic biomarkers

Among the 2603 women with available serum the median maternal serum concentration of sFlt-1 was 1258 pg/mL (IQR 938.0-1754.0), of PlGF 192 pg/mL (IQR 142.0-260.0) whereas sFlt-1/PlGF ratio was 6.8 (IQR 4.5-9.7).

For 2278 women with available serum and 18-week questionnaire the medians were similar compared to the women with only available serum with median sFlt-1 of 1257 pg/mL (IQR 973.8-1753.0), PlGF 193 pg/mL (IQR 143.0-261.0), and sFlt-1/PlGF ratio 6.7 (IQR 4.5-9.7).

In the univariable linear regression models we found that snus exposure compared to never snus exposed was significantly (p-value 0.023) associated with lower maternal circulating PlGF levels only (Table 7-3). The association remained significant (p=0.020) in the multivariable model after adjusting for gestational age, maternal age, pre-pregnancy BMI and parity, as well as when adding previous smoking to the multivariable model (Table 7-3). Either sFlt-1 or the sFlt-1/PlGF ratio was significantly associated with snus exposure.

		sFlt-1			PlGF			sFlt-1/PlGF-ratio		
SNUS EXPOSURE	**N**	**B**	**95% CI**	**P**	**B**	**95% CI**	**P**	**B**	**95% CI**	**P**
					UNIVARIABLE					
	2090[#]									
Never	1622	Ref.		0.091	Ref.		**0.023**	Ref.		0.072
Stopped before pregnancy	313	0.02	-0.01-0.04		-0.02	-0.05-0.00		0.04	0.01-0.07	
Stopped when recognized pregnancy	143	-0.01	-0.05-0.03		-0.03	-0.06-0.01		0.02	-0.03-0.06	
Current	12	-0.12	-0.2-(-0.00)		-0.13	-0.2-(-0.01)		0.01	-0.14-0.15	
					MULTIVARIABLE**					
	2090[#]									
Never	1622	Ref.		0.157	Ref.		**0.020***	Ref.		0.271
Stopped before pregnancy	313	0.00	-0.02-0.03		-0.03	-0.05-0.00		0.03	0.00-0.06	
Stopped when recognized pregnancy	143	-0.02	-0.06-0.01		-0.03	-0.06-0.01		0.004	-0.04-0.05	
Current	12	-0.11	-0.2-0.01		-0.12	-0.2-0.00		0.007	-0.14-0.15	

N=number, sFlt-1=Soluble Fms-like tyrosine kinase receptor 1, PlGF= Placental Growth Factor, B=beta coefficient, CI=confidence interval, P=p-value, Ref.=reference group. *Significant global p-value. **Multivariable regression analysis (log transformed) adjusted for fetal sex, gestational age, maternal age, pre-pregnancy BMI and parity. Additional adjusting for history of smoking in the model on snus exposure did not change the results (data not shown). #Missing data: to isolate the effect on snus alone in pregnancy, dual users of smoking and snus at some time in pregnancy (current and stopped when recognizing pregnancy smokers) were removed from the snus group.

Table 7-3. Univariable and multivariable linear regression analyses (log transformed): the effect of nicotine exposure on midpregnancy circulating angiogenic biomarkers. *Table from paper submitted, not published.*

7.3.2 Birth size

Of all the anthropometric measures, head circumference only was significantly associated with tobacco and nicotine exposure. Infants in the 'snus only' group had larger mean (cm) head circumference compared to 'never' snus use and 'smoke/dual' users in unadjusted analyses (Table 7-4).

	Subjects	Tobacco exposure during pregnancy#			
		Never	Snus only	Smoke/dual	p-value
Anthropometric measures		2061 (89.1)	150 (6.5)	102 (4.4)	
Birthweight g	2252	3577 (3556–5598)	3662 (3591–3733)	3575 (3472–3678)	0.11
Length cm	2181	50.5 (50.4–50.6)	50.8 (50.4–51.1)	50.4 (49.9–50.9)	0.26
Head circumference cm	2238	35.2 (35.1–35.3)	35.5 (35.3–35.8)	35.3 (34.9–35.6)	0.029
Thoracic circumference cm	2157	34.0 (33.9–34.1)	34.2 (33.9–34.5)	34.2 (33.7–34.7)	0.30
Abdominal circumference cm	2156	32.8 (32.7–32.8)	32.9 (32.5–33.3)	32.7 (32.2–33.2)	0.79
Left mid upper arm circumference cm	2166	11.1 (11.1–11.2)	11.3 (11.2–11.5)	11.2 (11.0–11.4)	0.15
Proportional size					
Abdominal/head circumference	2102	0.94 (0.93–0.94)	0.92 (0.92–0.94)	0.93 (0.91–0.94)	0.45
Thoracic/abdominal circumference	2151	1.04 (1.037–1.04)	1.04 (1.04–1.05)	1.05 (1.04–1.06)	0.14
Thoracic/head circumference	2103	0.97 (0.965–0.97)	0.96 (0.96–0.97)	0.97 (0.96–0.98)	0.68
Birthweight/placenta weight	1729	5.6 (5.5–5.7)	5.5 (5.4–5.7)	5.7 (5.5–6.0)	0.54

Data are presented as n (%), n or mean (95% CI), unless otherwise stated. The reference group "never" includes all females who did not report use of tobacco or nicotine during pregnancy. The "smoke/dual" group includes dual smokers and snus users during pregnancy. Most of these subjects (>90%) quit snus use or smoking by 6 weeks of pregnancy. #: includes four females who answered "yes" to ever-use of other nicotine products (nicotine replacement therapy or electronic cigarettes); one was a daily user during pregnancy at 18 weeks and three quit when recognising pregnancy.

Table 7-4. The anthropometric measures and proportional size are given by tobacco exposure group for 2313 newborn infants. *From ERJ Open Research, Kreyberg I, Hilde K, Bains KES, et al. Snus in pregnancy and infant birth size: a mother-child birth cohort study. 2019;5(4):00255-2019. Published 2019 Dec 2. doi:10.1183/23120541.00255-2019. Reprinted with permission.*

In the multivariable regression analyses adjusted for the significant covariates parity, GA at birth, fetal sex, pre-pregnancy BMI and maternal age, we found a significantly higher head circumference and birth weight in infants exposed to 'snus only 18 weeks' (Table 7-5). However, neither head circumference nor birth weight remained significantly associated with 'snus only 18 weeks' when adding maternal weight gain from pre-pregnancy to gestational week 18, to the multivariable sensitivity analyses

(Table 7-5). Lower birth weight was observed for the children of the 11 subjects reporting ‘snus only 34 weeks’ in all models as shown in Table 8-4, but this was not statistically significant. None of the other birth size outcomes (both anthropometric and proportional size measures) were significantly associated with snus exposure in univariable or multivariable analyses.

	Univariable			Multivariable[#] (1–5)			Univariable sensitivity analyses			Multivariable sensitivity analyses (1–6)		
	Subjects n	β (95% CI)	p-value	Subjects n	β (95% CI)	p-value	Subjects n	β (95% CI)	p-value	Subjects n	β (95% CI)	p-value
Tobacco exposure			0.085			0.113			0.110			0.550
Never	1772	Ref.		1772	Ref.		1694	Ref.		1694	Ref.	
Snus only	143	91.3 (10.1–172.5)		143	78.1 (4.7–151.5)		137	88.8 (5.6–172.0)		137	36.3 (–37.4–110.0)	
Smoke/dual	97	–6.5 (–103.9–90.9)		97	11.0 (–76.6–98.6)		90	–5.0 (–106.4–96.4)		90	–20.1 (–109.1–68.9)	
Tobacco exposure			0.120			0.032			0.180			0.250
Never	1772	Ref.		1772	Ref.		1694	Ref.		1694	Ref.	
Snus only up to 18 weeks	132	102.0 (17.7–186.3)		132	100.0 (23.9–176.1)		127	96.8 (10.6–183.0)		127	53.8 (–22.6–130.1)	
Snus only up to 34 weeks	11	–36.4 (–318.9–246.1)		11	–183.1 (–436.5–70.3)		10	–13.1 (–310.3–284.1)		10	–180.6 (–440.6–79.5)	
Smoke/dual	97	–6.5 (–103.9–90.9)		97	10.9 (–76.7–98.4)		90	–5.0 (–106.4–96.4)		90	–20.0 (–108.9–69.0)	

The reference group "never" includes all females who did not report use of tobacco or nicotine during pregnancy. The "smoke/dual" group includes dual smokers and snus users during pregnancy, of whom most quit before 6 weeks of pregnancy. The nonsignificant global p-values for snus-only and smoke/dual indicate that no significant associations were observed with birthweight. Covariates used in multivariable analyses: 1=parity, 2=gestational age at birth, 3=fetal sex, 4=pre-pregnancy body mass index, 5=maternal age, 6=gestational weight gain up to 18 weeks of pregnancy. Ref.: reference value. [#]: the results of the multivariable analyses restricted by the same study population as in the sensitivity analyses without adjusting for gestational weight gain, were similar in both populations (data not shown).

Table 7-5. Linear regression analyses: effect of tobacco exposure during pregnancy on birth weight (grams). *From ERJ Open Research, Kreyberg I, Hilde K, Bains KES, et al. Snus in pregnancy and infant birth size: a mother-child birth cohort study. 2019;5(4):00255-2019. Published 2019 Dec 2. doi:10.1183/23120541.00255-2019. Reprinted with permission.*

7.4 Aim 4: To explore if the effects of in-utero snus exposure differ by fetal sex, focusing on midterm angiogenetic placenta biomarkers

The 160 (7%) women who reported snus use during pregnancy included the group ´stopped when recognized pregnancy' (n=148) and 'current' use (n=12) were compared to the 1671 (74%) women who reported never use. Among the snus group 82/160 (51%) were male pregnancies.

Assessing the effect of snus exposure on the biomarker levels, the multivariable linear regression models showed significant results on the level of the biomarker PlGF (p=0.020), but not for sFlt-1 and the SFlt-1/PlGF-ratio.

As a significant interaction (p=0.031) was observed for fetal sex*snus exposure in the multivariable linear regression models, analyses were subsequently stratified by fetal sex.

The midpregnancy PlGF levels were significantly lower among snus using women carrying a male fetus who reported 'stopped before pregnancy' (n=167, median 189.0 pg/mL, IQR (136.0-245.0)) and among the group reporting 'stopped when recognized pregnancy' (n=76, median 177.5 pg/mL, IQR (134.5- 249.8) compared to women who never used snus (n=899, median 205.0 pg/mL (151.1-276.0) with p=0.008 and p=0.020, respectively (Mann-Whitney U test). Furthermore, among women carrying female fetuses, the PlGF was significantly lower (p=0.036) among the 'current' users (n=6, median 104 pg/mL, IQR (91.0-213.8)) compared to the 'never' users (n=772, median 183.0 pg/mL, IQR (139.3-249.8)). There were no significant differences observed in any of the other exposure groups for either sFlt-1 or sFlt-1/PlGF ratio.

In the multivariable regression analysis adjusted for gestational age, maternal age, BMI and parity a general, significant trend (p=0.002) towards lower PlGF in women carrying male fetuses and who reported snus use at any time before or during early pregnancy compared to women who had never used snus were found (Figure 7-4). Among women carrying female fetuses we found no significant associations between PlGF levels and snus exposure (Figure 7-4).

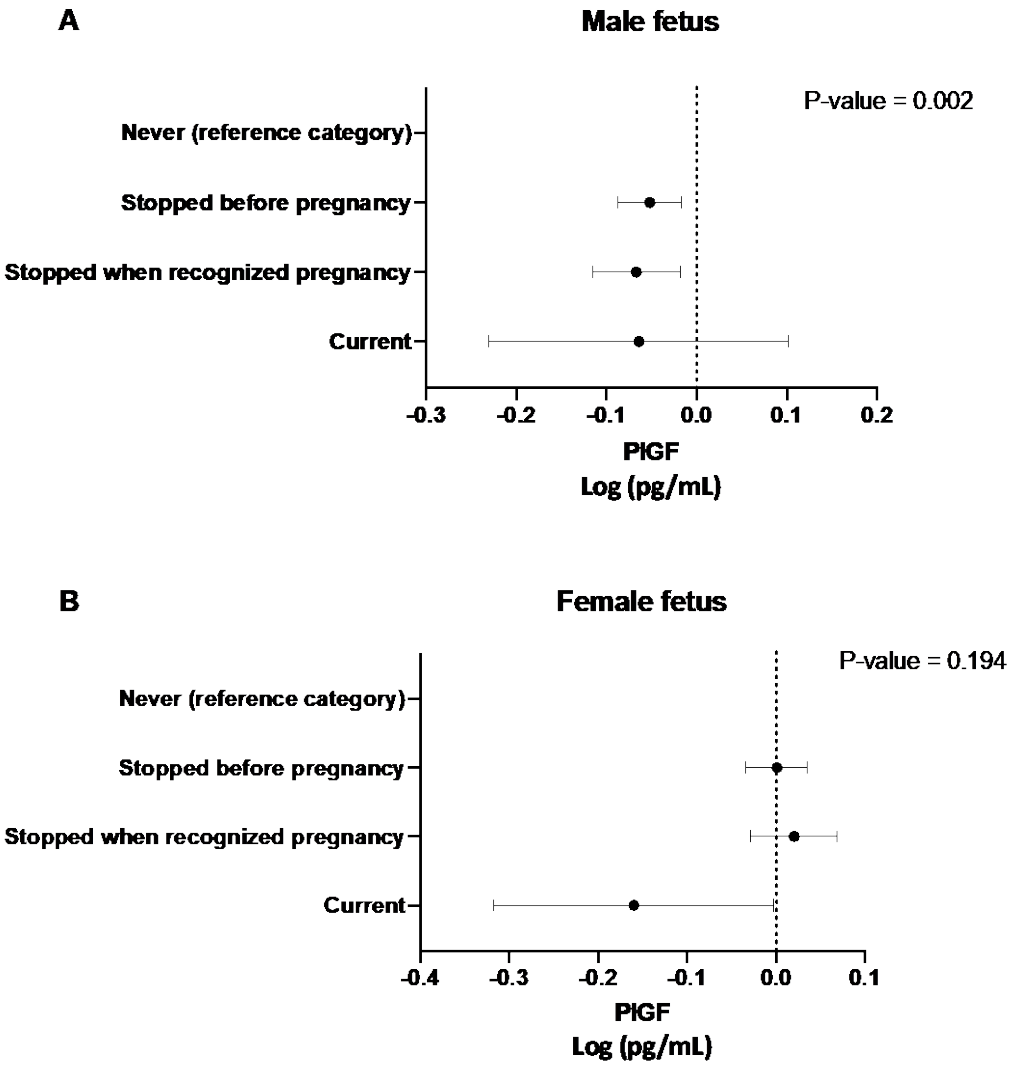

Figure 7-4. P-value represents the results from the multivariable linear regression analyses demonstrating the effect of snus use on circulating midpregnancy maternal angiogenic biomarker levels (log transformed) by fetal sex (male fetus n= 1148, female fetus n= 1007). *Figure from paper submitted, not published.*

8 Discussion

8.1 Aim 1: To determine the maternal prevalence, pattern of use and cessation rate of snus and other nicotine products during pregnancy

Snus alone was the most frequently used nicotine product during pregnancy (6.5%), in the PreventADALL study including Norwegian and Swedish women, followed by cigarette smoke (4.1%), dual snus and smoking (0.6%) and other NRT products among 0.2%. Snus was more commonly reported in Norway than in Sweden (8.2% versus 5.5%, p-value 0.035). Among snus users and smokers 91.1% of the women reported quitting when recognizing their pregnancy.

Finding that snus alone is the most commonly used nicotine product during pregnancy, as well as an observed increase in the pre-pregnancy use of snus from 5.1% (CI; 4.6 to 5.5) to 8.4% (CI; 7.8 to 8.9) in 2012-14 to 2015-17 from the registry study from Southern Norway [33], mirrors the general trend observed among young Norwegian women the last decade [118]. The reported cigarette smoking decreased significantly both before and during pregnancy throughout both periods [33].

The 8.2% observed snus use prevalence (including dual users) in the first trimester among the Norwegian women in the PreventADALL study is higher than the 2.8% reported first trimester (2015-2017) snus use in the register study from Southern Norway [33]. Similarly, the overall 7.6% prevalence of snus some time during pregnancy, as well as the 5.5% among the Swedish participants in our study, exceed the Swedish national statistics showing 1.2% of women using snus in the first trimester in 2018 [29]. However, the 0.3% prevalence of snus use alone in late pregnancy was closer to, although lower than the reported prevalence of 2.0% in late pregnancy from the register studies from Norway [33]. National statistics from late pregnancy use of snus in Sweden are not available.

One explanation for the higher prevalence in early pregnancy in our cohort compared to national registers in Norway and Sweden is likely to be the cessation rate of more than 90% when recognizing pregnancy in our study. To our knowledge this high cessation rate has not been previously reported. Compared to 87.2%

quitting snus by pregnancy week six in our study, the registry study from South Norway reported that 66.4% quit snus by the first trimester and 76.7% by the third trimester [33]. Further, the reported smoking cessation of 79.1% by pregnancy week six among smoking women in PreventADALL was higher than that reported in the Southern Norway pregnancy register by the first and third trimester, with rates of 52.6% and 60.5%, respectively [33]. The higher cessation rate among women in the PreventADALL study compared to the register study from Southern Norway may be explained by the general educational level, as approximately 57% had at least four years higher educational attainment level in our cohort [115]. This is in line with the findings in the register study from Southern Norway showing that higher education, being primiparous and aged 25-34 years were powerful predictors of both snus and smoke cessation during pregnancy [33].

At 18 weeks, 0.6% reported current use of snus and 0.8% current cigarette smoking, both being lower than the prevalence reported from register data from Southern Norway in 2017, with snus use of 2.8% in the first trimester and 2.0% in the third trimester [33].

As was found in our study, snus use among women in general, and in the youngest age groups in particular, was higher in Norway compared to Sweden [9, 11]. Sweden has a longer tradition of snus use compared to the rest of the world including Norway, and the majority of studies on maternal use of snus during pregnancy are Swedish [31]. Snus habits among pregnant women have been registered as part of antenatal care in Norway only in the recent years, whereas in Sweden this has been reported since 1999. The perception that use of snus is less harmful than smoking, also during pregnancy, might be more widespread in Norway explaining the higher prevalence.

The 0.2% prevalence of NRTs and e-cigarette use during pregnancy in our study is to our knowledge novel, with previous data lacking from Norway and Sweden.
To our knowledge there is little updated data on the prevalence of NRTs during pregnancy [31]. Many studies have investigated the use of NRTs as an aid in smoking

cessation during pregnancy, and dual use as well as relapse to cigarette smoke is common. In a study from the USA including 1365 pregnant women interviewed by telephone (2015-2018), the participants using NRTs were categorized in the group together with the women who reported no tobacco use giving no representative numbers for NRT use alone. However, in total 4.0% reported use of e-cigarette, 27.3% use of tobacco cigarettes and 68.8% reported no tobacco or NRTs during the preconception period and/or pregnancy [119]. A literature review from 2017 summarizing reported prevalence rates of e-cigarettes during pregnancy included studies from Ireland and the USA and found a prevalence that ranged from 0.6% to 15% [85]. The latest results were from an online national survey of 445 pregnant women that showed that 5.6% used only tobacco cigarettes, 6.5% used only e-cigarettes, and 8.5% were dual users of tobacco cigarettes and e-cigarettes [120]. Another study from the US using survey data from 2015 found a prevalence of electronic vaping products, including e-cigarettes, of 10.4% before pregnancy and 7.0% around the time of pregnancy, including 1.4% during the last 3 months of pregnancy [121].

Most of the literature on snus is based on studies and national statistics available primarily from Sweden and Norway [31]. Studies investigating other smokeless tobacco products from other parts of the world [122] may differ from, or not be directly comparable to snus, therefore mainly Norwegian and Swedish data form the basis for this thesis. The use of snus still represents the preferred nicotine product among young Scandinavian women, but the shift towards e-cigarettes cannot be excluded in the future. This is an increasing trend in other European countries [13, 123] as well as the USA [124, 125]. Additionally, there is an increased availability of e-cigarettes by over the counter purchase as well as online [126].

8.2 Aim 2: To identify women at risk of snus use in pregnancy

The identified risk factors associated with women using snus during pregnancy such as urban living, lower age, being cohabitant, previous smoking history and being exposed to smoke in-utero differed from the previously reported association with maternal smoking.

The lack of association between snus use in pregnancy and educational attainment is in contrasts to the lower educational level previously seen among pregnant smokers [127]. Yet our findings are in line with recent data showing positive associations between snus use and intermediate educational level in Norway [128].

In our analyses the woman being cohabitants, which in Scandinavia is as common as being married, was associated with snus use, while single women were more likely to smoke during pregnancy [127]. Our risk factors for snus use differed from characteristics typically observed among daily smokers during pregnancy like lower educational level, lower household income, higher pre-pregnancy BMI, higher order birth, and being unmarried which all are shown not to be associated with snus use in our analyses [129]. Our observation of the inverse association with age is however in line with the trend of alternative nicotine products, such as e-cigarettes, generally being common among the young [16], as well as findings of increased risk of persistent smoking during pregnancy among younger women [7].

Previous smoking history and being exposed to maternal smoking in-utero were significantly associated with snus use in pregnancy. This is in line with a population-based birth cohort study from the USA linking birth records between mothers and daughters delivering in Washington state, where an intergenerational association was demonstrated between mother and daughter smoking habits during pregnancy [130]. Daughters exposed to tobacco cigarettes in-utero were more likely to smoke during their own pregnancy than unexposed daughters. This suggests an in-utero nicotine susceptibility affecting the risk of smoking later in life. Animal models indicate that fetal nicotine exposure may have an impact on the neural pathways that affect lifetime sensitivity to nicotine [131]. Furthermore, Rydell et al reported in a Swedish study that maternal smoking during pregnancy was consistently

associated with snus use rather than smoking in the offspring [132]. However, in further sibling analyses, the association was no longer significant when adjusting for shared confounding variables such as genetic and environmental factors [133, 134]. This suggest that behavioural, (epi)genetic and environmental factors are all important and influential for future snus dependence [130].

Household smoking, although not significantly associated with snus use during pregnancy, was more frequent among snus users than non-users in our study. Knowing that a smoking partner is a risk factor for maternal smoking and exposure during pregnancy [127], the same might be applicable to partner use of snus. Unfortunately, we did not obtain systematic tobacco habits of the baby's father or co-mother, excluding the possibility to examine potential associations to partner use of snus.

8.3 Aim 3: To determine if maternal snus use pre- and perinatally affect placental biomarkers in midpregnancy and/or neonatal anthropometric measures at birth

8.3.1 Angiogenic biomarkers

We found that snus exposure was significantly associated with lower maternal PlGF levels during pregnancy, but not with sFlt-1 or the ratio PlGF/sFlT-1. Our study is to the best of our knowledge, the first study to determine the effect of maternal snus use on placenta function at midpregnancy evaluated by the circulating maternal placenta-associated angiogenic biomarkers, sFlt-1, PlGF and the sFlt-1/PlGF ratio. In general our study population had similar sFlt-1 levels, but slightly higher PlGF levels, and therefore lower sFlt-1/PlGF ratio compared to the study by Verlohren et al. [135] including 15-19 gestational week pregnancies. Noteworthy our cohort was larger than the study by Verlohren et al. (n=2603 versus n=1149).

In the adjusted multivariable linear regression analyses, we found that snus exposure in pregnancy was only significantly associated with the pro-angiogenic factor PlGF. The lack of potential general effect of snus on the angiogenic factors might be due to low prevalence of current snus use at midterm, specifically around

18 weeks. A previous study suggested that the effect of smoke might be more pronounced towards late pregnancy since the alterations of circulating angiogenic biomarker levels are more distinct in that stage of pregnancy [103]. The same might be similar for snus, but the effect of continued snus use throughout pregnancy was not possible to determine in our study because of the high cessation rate.

8.3.2 Birth size

In our study early snus exposure during pregnancy did not significantly affect infant birth size based on the anthropometric or proportional size measures when adjusting for relevant covariates including gestational weight gain. However, we were not able to assess the potential effect of continued snus use throughout pregnancy due to the high cessation rate.

To our knowledge this is the first prospective birth cohort study assessing snus exposure in-utero in relation birth size by composite anthropometric measures. Our results are in contrast to a Swedish register study finding that snus use was associated with birth weight reduction [74]. This study included in total 789 snus users, 11.240 smokers and 11.495 non-users born in Sweden from 1999 through 2000. As the results from the register study might have been confounded by familiar factors linked with both snus and infant birth weight, Juarez et al. conducted a sibling analysis in the attempt to provide stronger causal evidence using the same register data. They explored a subsample of siblings with discordant snus use habits between pregnancies i.e., women who had at least one pregnancy during which they used snus. The analyses were adjusted for gestational age, birth order, fetal sex, maternal age and marital status. The result is more in line with ours, finding that snus use is associated with a minor (20 g), but not statistically significant birth weight reduction [83].

Although we did see a trend of decreased birth weight among the infants exposed to snus throughout pregnancy, we were unable to conclude as this group consisted of 11 women only. A Swedish register study found increased risk of SGA in the general population with continued use of snus during pregnancy [84], however snus use with

early cessation showed no increased risk of SGA compared to non-users in accordance with our results. In the study based on register data from Southern Norway the authors compared mean birth weights for infants of snus users to infants of the non-tobacco users in the third trimester. They found that mean birth weight of infants exposed to snus daily or occasionally in the third trimester (n=201) was 3418 g with a statistically significant (P-value <0.006) reduction in average birth weight of 106 g [33]. Based on the evidence available today we have reason to believe that continued snus use might affect birth size.

The anthropometric measures chosen provide detailed information on infant birth size. The size of the newborn child is determined by genetic predisposition and by the intrauterine environment. According to the hypothesis on Developmental Origins of Health and Disease (DOHaD) unfavourable environmental exposures resulting in restricted nutrients and oxygen supply in-utero might affect the fetal development and growth [136] and increase the risks for NCDs later in life [137, 138]. The rationale behind including several measures of infant birth size was that each measure adds different and complementary information providing a more nuanced and complete measure of the size and body proportions of the neonate. Birth weight and length are typical predictors of lean mass [139]. Abdominal circumference might indicate level of fat and, or size of the liver [136]. Finally, upper mid arm circumference predicts muscle mass [140]. In addition, several of the chosen measures are previously used in studies investigating the effects of cigarette smoke on fetal growth [69] and neonatal size such as birth weight, length and head circumference [64, 65], providing the opportunity to compare the effects.

Concerning tobacco and nicotine exposure in-utero and associations with reduced birth weight, there are some conflicting results as to potential underlying mechanisms. One hypothesis is that the main effect of nicotine is through impaired placenta function [54, 141]. Others suggest that toxic combustion products from tobacco cigarettes and/or familial confounding may be the culprit, rather than nicotine per se [83]. An animal study showed that carbon monoxide was responsible

for the effect on fetal weight in rats, while nicotine was linked to reduced maternal weight gain during pregnancy [142].

When we examined the possible effect of snus exposure during pregnancy on infant birth size by multivariable linear regression, we initially included many potential covariates, illustrated in Table 5-2. In the final models, however, only the significant covariates parity, gestational age at birth, fetal sex, pre-pregnancy BMI and maternal age for each outcome were included. The finding of apparent higher birth weight in infants born to mothers using snus up to, but not beyond 18 weeks of pregnancy in multivariable analysis was in contrast to the Swedish registry studies [74, 83, 84]. However, this was no longer significant after adjusting for maternal weight gain up to 18 weeks of pregnancy. In sensitivity analyses we found significantly higher maternal gestational weight gain in those stopping snus use compared to non-users. Adjusting for weight gain, the difference disappeared. To our knowledge none of the other studies exploring potential effects of in-utero snus exposure on fetal growth include maternal gestational weight gain in their adjusted models. Although we are unaware of studies assessing the maternal gestational weight gain after snus cessation in pregnancy, several studies have found an increased risk of excess gestational weight gain from pre-pregnancy to delivery when quitting smoking in pregnancy compared to non-smokers [143, 144]. Thus, we believe that gestational weight gain, not only up to 18 weeks, but also until term, should be included in future analyses when assessing the effect of snus on infant birth size. Also, one would prefer to obtain objective measures of pre-pregnancy weight rather than base the weight on self-report at inclusion visit as there is a risk of recall bias and error [145].

8.4 Aim 4: To explore if the effects of in-utero snus exposure differ by fetal sex, focusing on midterm angiogenetic placenta biomarkers

The level of PlGF was significantly lower among women carrying a male fetus who reported use of snus prior to (p=0.008) or in early pregnancy (p=0.020) compared to non-users. A lower PlGF was also found among the six women carrying a female fetus who reported current snus use during pregnancy (p=0.036). However, only a

significant lower PlGF in male fetus pregnancies was sustained in multivariable analyses when adjusting for relevant covariates suggesting a sexual dimorphic effect of snus.

To our knowledge we are the first to assess the effect of in-utero snus exposure on maternal anti- and proangiogenic profiles measured by placenta biomarkers in a sex dimorphic manner. Our contribution to the field is the finding of a significant trend towards lower midpregnancy PlGF levels among the women carrying a male fetus who reported snus use at any time before pregnancy or those who stopped when recognizing pregnancy compared to the women who had never used snus. Thus, our results argue towards a potential persistent "antiangiogenic" effect of snus in women carrying a male fetus. The finding is in line with other studies on fetal sex-specific differences where male fetuses are more susceptible to environmental stressors than female fetuses [146]. To determine the effect of snus by fetal sex, we chose the objective biomarkers as outcomes.

In retrospect it would have been interesting to analyze the effect of nicotine on anthropometric and proportional birth size measures by testing effect modification by fetal sex. As we only adjusted for fetal sex in the analyses, we cannot assess whether there is a different effect on male and female fetuses.

8.5 Ethical perspectives

The enrolment procedure to the PreventADALL study included a strong focus on informing the participants of the study goals, the implications of participating in a study with many follow-up visits and data collections, as well as subjecting their infants to interventions in a randomized manner. Further, all participants were informed that they could withdraw from the study at any time without any queries or consequences. They did not undergo any invasive procedures or interventions of risk apart from blood sampling of the pregnant women at 18 weeks inclusion. We believe that the potential benefits of participation in this study outweighed the burden and risks to the subjects, and the study followed the international Good Clinical Practice guidelines. The participants did not receive any individual

information about the blood serum analyses, but were informed about other results immediately available at 18-week inclusion. The published results from our analyses have been made easily accessible for the participants, and the society at large, through our PreventADALL website.

8.6 Strengths and limitations

The PreventADALL study has several strengths, including recruiting pregnant women from the general population at three different locations providing a more diverse demographic profile, environmental exposures and living areas. Furthermore, with the long-term goal of identifying factors in early life that may impact NCD development, antenatal recruitment provides opportunities to study exposures and clinical measures in the mother and fetus prospectively for impact on child health.

Another strength is the prospective study design with high follow-up rate. Detailed information about previous and current tobacco- and nicotine use as well as frequency, type of product and dosage were collected at the 18-week questionnaire with a high follow-up rate at 34 weeks, providing complete information in more than 2000 participants.

The electronic questionnaires had compulsory response fields with respect to nicotine use, ensuring that no fields were missing. Data was reported twice, both early and late in pregnancy providing detailed information regarding use, cessation and relapse specified with two weeks intervals of the different products during pregnancy.

The focus on biological sampling in early life provided available midpregnancy serum for approximately 84% of the women, for whom we had detailed information about socio-economic, lifestyle and health characteristics, as well as measures at the inclusion visit including height and weight, tobacco and nicotine information.

Information about fetal sex was collected from Partus and gestational age was based upon ultrasound measurements of femur length which is much more accurate than self-reports based on last menstrual period. Anthropometric measurements were

performed within the first 24 hours of delivery from the vast majority of the participants by trained, dedicated staff using SOP based on international studies and recommendations. Close collaboration between the three study sites also ensured standardized measuring methods.

Further, an important strength is that the maternal blood samples were analysed for placenta health-related biomarkers in midpregnancy in a quite homogenous population providing opportunity to explore the impact of nicotine exposure and fetal sex on anigogenic biomarkers. Additionally, the women were enrolled within a rather small gestational age range, as compared to other studies studying placenta biomarkers [103] representing another strength when analysing the snus effect on biomarkers.

However, generalizability of our study population has some limitations. Most participants were ethnically Scandinavian women born in Norway or Sweden, resulting in low ethnic diversity. Nevertheless, the PreventADALL study is not unlike other large prospective cohort studies biased towards a more homogenous ethnic population as well as higher educational attainment level. For instance, in a biomarker study by Andersen et al. only 3.5% originating from non-Western countries in their Danish population [114], making our observations comparable to their findings. The high educational attainment and high mean maternal age in our study [115] compared to the general population, might potentially lead to an underestimation of nicotine use in the society as a whole. However, as maternal education has not been found to be associated with snus use it is less probable it has affected the reported prevalence. In contrast, since use of snus in pregnancy has been shown to be inversely associated with age [116], and younger women are in general more likely to use snus prior to pregnancy [37], the rates might be undervalued.

Another limitation of this study is that for those who reported cessation of nicotine and/or tobacco use at some time during pregnancy detailed information about the pattern of use like frequency and number of snus portions, and/or cigarettes smoked, were not collected. Hence determining the dose-response effect is difficult in these subgroups. Although separating dual snus and smokers from snus groups to

evaluate the effect of snus use alone, we recognize that there might have been prior smokers in all snus categories, potentially affecting the effects through e.g. epigenetic changes. However, with the broad spectrum of exposures and life-style factors collected in the PreventADALL study for analyses versus later health effects, it was deemed necessary to restrict the level of details for many of the factors, in questionnaires that could take up to 45 minutes to complete.

Giving the fact that our data is based on self-reports with no objective validation of nicotine or cotinine levels during pregnancy, there is a risk of misreporting and recall bias. Gunnerbeck et al. found in a prospective Swedish cohort study (n=474) that self-reported snus use through questionnaires had high validity with sensitivity and specificity values of 98% and 96%, respectively. They found from the Swedish MBR that 45% of the snus users were misclassified as non-users in late pregnancy [147], thereby underestimating actual prevalence and potential harmful effects when using register data. In contrast other studies have shown that self-reports represent valid markers for tobacco exposure assessed by blood cotinine [148, 149]. Mattsson et al. found high agreement between cotinine levels and MBR smoking data ($\kappa = 0.82$) as well as correlation between cotinine levels in maternal and umbilical cord serum indicating that MBR data were valid [150]. Based on this, we have reason to believe that the results from our study are likely to be adequately accurate.

The time of ceasing use of snus, cigarettes or other NRTs was reported based upon the participants' self-reported pregnancy week. When completing the 18-week questionnaire they had already received the ultrasound corrected length of pregnancy, which is considered the best predictor of estimated date of delivery [151]. However, we do not know if the women used the ultrasound corrected date of delivery to report her pregnancy week. If the reported pregnancy week was relying on the first day of the last menstrual period, the first two pregnancy weeks might actually be before conception. Hence, the in-utero nicotine exposure might be limited during pregnancy, but rather more prominent around the beginning of life.

There are limitations related to the timing and biometric method used in this study to estimate GA at the routine ultrasound examination performed at inclusion

between week 16-22. Because of lower biological variation in fetal size in the first trimester, ultrasound measures in this period are regarded superior to measures later in pregnancy [152], as recommended by the The International Society of Ultrasound in Obstetrics and Gynecology (ISUOG). Despite this, Norwegian and Swedish women are not generally offered routine ultrasound scans before the second trimester [153-155]. Second trimester head circumference measurements and biparietal diameter are considered appropriate to predict estimated date of delivery, particularly if 283 days are used for the length of pregnancy [156]. In PreventADALL we used the GA calculated by biometric measures of head size (head circumference or biparietal diameter) and femur length. However, the biometric measures of fetal head were assessed differently between the three study locations in the two countries due to different regional and national standards [155, 157]. Femur length was measured in the same way at all three study locations and therefore the estimated date of delivery was based on this measure [115].

As one of our exclusion criteria was delivery before gestational week 35.0, potential effects from snus exposure related to infants born prior to 35 weeks were not analysed. Given the fact that we know that there is an association between snus use and preterm birth [76], this subgroup of preterm infants could potentially have provided further results. However, only 10 babies were born before week 35 with tobacco exposure similar to the entire cohort. With the increased risk of associated morbidity and confounding in this group, we do not consider excluding them a limitation of our study.

When the linear regression analyses were performed for aim 3 and 4, the potential covariates such as preeclampsia, gestational diabetes or other relevant maternal diseases that possibly could affect some of our outcomes were unavailable.

9 Implications of the study

This thesis has contributed to the international scientific knowledge with updated data on prevalence of snus and nicotine use during pregnancy, and confirmed that snus is the most commonly used product reflecting the general trend among younger women today. We also found that although 7% reported use of snus ever in pregnancy, the cessation rates were almost 90% by 18 weeks pregnancy. The women most likely to use snus during pregnancy also differed from women who smoke, suggesting that snus using pregnant women represent a more diverse population than do smokers. This is important to take in consideration when providing antenatal care.

Our novel finding that snus has an antiangiogenic effect on biomarkers for placenta health in male fetus pregnancies, suggesting that in-utero snus exposure has effect in a sex dimorphic manner. As antiangiogenic placental profiles are associated with placenta dysfunction and subsequently increased risk of pregnancy related complications, midpregnancy biomarkers in addition to clinical signs might represent a valuable tool in the future to identify women at risk of adverse pregnancy outcomes.

There is growing evidence that maternal snus use is harmful for the unborn child and should be avoided during pregnancy. The good news is that early cessation is likely to attenuate the effects of snus on impaired somatic fetal growth. This is in accordance with other studies investigating the effect of snus [84] and smoke [66], and confirms that stopping when knowing might reverse the adverse effects. The potential consequences of late pregnancy snus exposure demand further investigations.

10 Conclusions

Aim 1

Snus is the most commonly used nicotine product at some time during pregnancy, reported by 7% of women in a large general population-based prospective mother-child birth cohort, with 87% stopping snus use when knowing about their pregnancy within pregnancy week six.

Aim 2

Younger, cohabitant, urban living women originally born in Norway or Sweden with previous smoking experience and mothers who smoked during their pregnancy were at risk of using snus during pregnancy. In contrast to lower educational level and household income being known risk factors associated with smoking during pregnancy, these did not represent risk factors for snus use.

Aim 3

Snus exposure during pregnancy was significantly associated with lower maternal PlGF when adjusted for relevant covariates when including all pregnancies.
Further, early snus exposure did not affect infant anthropometric or proportional size measures at birth. However, as with an almost 90 % cessation rate in early pregnancy, the potential adverse effects of snus use in late pregnancy are still unclear.

Aim 4

A positive interaction was found between fetal sex and snus use prior to, and in early pregnancy in relation to PlGF suggesting a sex dimorphic effect of snus exposure. When stratifying by fetal sex, maternal proangiogenic level of PlGF was lower among the snus using women carrying a male fetus, supporting the perception that male fetuses are more susceptible to environmental factors already from the time of conception.

11 Future investigation and research

The work outlined in this thesis has contributed to updated knowledge on the prevalence of snus use during pregnancy, as well as reported novel information on identifying the women at risk or maybe more precisely, the women not at risk of using snus during pregnancy.

The high cessation rate is undoubtedly good news from a health perspective. Whether the women relapse postpartum is another question that needs to be explored. It would also be of interest to explore why Norwegian women use snus more than Swedish women. One hypothesis is to assess whether Norwegian women perceive snus less harmful during pregnancy, and investigate potential differences in cessation pattern between women from the two countries.

Because of the around 90% cessation rate in early pregnancy in this study, we could only determine the effects of early snus exposure. The potential adverse consequences to the unborn child of persistent maternal snus use during pregnancy are still uncertain and needs further investigation.

Further, the potential differences in health effects of in-utero snus exposure between males versus females may provide further insight into how nicotine affects early life growth and development.

It would be of interest to follow the infants into childhood and examine if early snus exposure peri- and prenatally have any other health effects although the exposure groups are probably too small to provide results with enough strength.

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13 Papers

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ORIGINAL ARTICLE
NICOTINE AND PREGNANCY

Stopping when knowing: use of snus and nicotine during pregnancy in Scandinavia

Ina Kreyberg[1,2], Karen E.S. Bains[1,2], Kai-H. Carlsen[1,2], Berit Granum[3], Hrefna K. Gudmundsdóttir[1,2], Guttorm Haugen[1,4], Gunilla Hedlin[5,6], Katarina Hilde[1,4], Christine M. Jonassen[7,8], Live S. Nordhagen[1,2,9], Björn Nordlund[5,6], Katrine D. Sjøborg[10], Håvard O. Skjerven[1,2], Anne C. Staff[1,4], Cilla Söderhäll[6], Riyas M. Vettukatil[1,2] and Karin C. Lødrup Carlsen[1,2] on behalf of the PreventADALL study group[11]

Affiliations: [1]Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, Oslo, Norway. [2]Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway. [3]Dept of Toxicology and Risk Assessment, Norwegian Institute of Public Health, Oslo, Norway. [4]Division of Obstetrics and Gynaecology, Oslo University Hospital, Oslo, Norway. [5]Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden. [6]Dept of Women's and Children's Health, Karolinska Institute, Stockholm, Sweden. [7]Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway. [8]Genetic Unit, Centre for Laboratory Medicine, Østfold Hospital Trust, Kalnes, Norway. [9]VID Specialized University, Oslo, Norway. [10]Dept of Obstetrics and Gynaecology, Østfold Hospital Trust, Kalnes, Norway. [11]For a list of the PreventADALL study group members and their affiliations see the Acknowledgements section.

Correspondence: Ina Kreyberg, Division of Paediatrics and Adolescent Medicine, Oslo University Hospital and the University of Oslo, Postboks 4956 Nydalen, 0424 Oslo, Norway. E-mail: inakreyberg@hotmail.com

ABSTRACT In young women, the use of snus increases in parallel with decreasing smoking rates but the use in pregnancy is unclear. Our aims were to determine the prevalence of snus use, smoking and other nicotine-containing product use during pregnancy, and to identify predictors for snus use in pregnancy.

Prevalence was determined for 2528 women in Norway and Sweden based on the Preventing Atopic Dermatitis and ALLergies (PreventADALL) study, a population-based, mother–child birth cohort. Electronic questionnaires were completed in pregnancy week 18 and/or week 34, and potential predictors of snus use were analysed using logistic regression models.

Ever use of any snus, tobacco or nicotine-containing products was reported by 35.7% of women, with similar rates of snus use (22.5%) and smoking (22.6%). Overall, 11.3% of women reported any use of nicotine-containing products in pregnancy up to 34 weeks, most often snus alone (6.5%). Most women (87.2%) stopped using snus by week 6 of pregnancy.

Snus use in pregnancy was inversely associated with age and positively associated with urban living and personal or maternal history of smoking. While 11.3% of women used snus or other nicotine-containing products at some time, most stopped when recognising their pregnancy. Younger, urban living, previously smoking women were more likely to use snus in pregnancy.

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Of the 11.3% of women using any nicotine products in pregnancy (most often snus (6.5%)), the majority stop within pregnancy week 6. Snus use is associated with urban living, previous smoking, *in utero* smoking exposure of the index woman and lower age. http://ow.ly/Gyg230nmxIo

Cite this article as: Kreyberg I, Bains KES, Carlsen K-H, *et al*. Stopping when knowing: use of snus and nicotine during pregnancy in Scandinavia. *ERJ Open Res* 2019; 5: 00197-2018 [https://doi.org/10.1183/23120541.00197-2018].

This article has supplementary material available from openres.ersjournals.com.

Received: Oct 24 2018 | Accepted after revision: Dec 26 2018

This study is registered at www.clinicaltrials.gov with identifier number NCT02449850. No individual participant data will be available for sharing.

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

Introduction

The risk of adverse health effects in offspring due to maternal tobacco smoking during pregnancy is well documented [1], including epigenetic changes persisting in the offspring after prenatal exposure [2]. The effects of fetal exposure to nicotine from non-combustible tobacco products, such as snus or nicotine-replacement therapies (NRTs), are less clear. Use of snus leads to systemic absorption of nicotine into the maternal circulatory system, with subsequent exposure of the foetus through the placenta [3]. Recent register studies suggest that compared to non-tobacco users snus use alone in pregnancy increases the risk of stillbirth [4], preterm delivery [5] and small size for gestational age (GA) [6]. In addition, fetal nicotine exposure in animal models has shown altered pulmonary development and health in offspring [7], as well as epigenetic changes [8].

A substantial shift in the use of nicotine-containing products has recently been observed in many parts of the world, particularly among the young. In the United States the overall use of any tobacco or nicotine products reported by high school students in 2016 (20.2%) was similar to 5 years earlier, however, the use of electronic cigarettes (e-cigarettes) increased from 1.5% to 11.3% while combustible tobacco use decreased from 21.8% to 13.8% [9]. Similar shifts from smoking towards the use of other nicotine products are observed in many Western countries [10–12], as shown by 29.9% of Polish youths reporting the use of e-cigarettes in 2014 [11] and e-cigarettes being almost twice as common as smoking among youths in Wales [12]. While e-cigarettes have been banned [13, 14] and infrequently used [15] in Norway and Sweden, the shift from smoking has rather been towards snus which has been available for many years. Snus, also termed moist snuff, is smokeless, ground tobacco placed between the gum and the lip [3]. Compared to cigarettes, snus gives a slower rise in nicotine plasma levels but a higher total concentration over time [16]. In 2010, a 10-fold higher rate of snus use was reported in Sweden compared to other European countries, where snus sales are limited [17]. From 2008 to 2017 the use of snus increased among women in Norway, with daily snus use increasing from 5% to 14% among 16–24 year olds and from 1% to 12% among 25–34 year olds [18].

The use of nicotine-containing products among young women is likely to be reflected in their use during pregnancy, as seen with declining smoking rates in some countries which are also being reported during pregnancy [19, 20]. Apart from register studies from Sweden [5], Norway [21] and the United States [22], there is limited updated information on the use of other nicotine products (such as snus and NRTs) in pregnancy. A recent study from Norway demonstrated a doubling of "daily" or "sometimes" snus use at the start of pregnancy between 2012 and 2014, from 1.7% to 3.4%, with the highest rates among the 16–24 year olds [21].

In pregnancy, single women smokers with lower educational levels have the highest smoking prevalence [23, 24] and the lowest smoking decline since 1999 [24]. While risk factors for snus use during pregnancy are largely unknown, educational level has not in general been clearly associated with snus use [25, 26]. However, lower economic status [27], younger age, higher alcohol consumption, number of sexual partners and heavy physical activity [26] have been.

Limited knowledge of the potential harmful effects of snus use in pregnancy, the alleged harm reduction of snus use compared to smoking and animal studies suggesting that fetal exposure to nicotine is similar in both snus and cigarette use, raise concerns about the continuing, albeit changing, patterns of nicotine use by young women. The primary aim of the present study was therefore to determine the prevalence of snus and other nicotine-containing product use during pregnancy. The secondary aim was to identify factors associated with the use of snus.

Subjects and methods

Study design

The present study used data from the Preventing Atopic Dermatitis and ALLergies (PreventADALL) study [28], a large population-based, prospective mother–child birth cohort. Briefly, between December 2014 and October 2016, 2697 women with a total of 2701 pregnancies were recruited from general, nonselected populations in Norway (2149 from Oslo University Hospital and Østfold Hospital Trust) and Sweden (552 from the Karolinska Institutet, Stockholm) at the routine ultrasound screening examination in gestational week 18. Inclusion criteria were single and twin pregnancies at weeks 16–22 and sufficient Scandinavian language skills. Exclusion criteria were severe maternal or fetal disease or the parents planning to move away from the area within the baby's first year of life. Healthy infants born at a GA of at least 35.0 weeks were enrolled at birth, constituting 2397 mother–child pairs [28].

The enrolment visit included obtaining written informed consent, measures of height and weight, a brief interview on the present and previous pregnancies, and information on how to complete the electronic questionnaires at 18 and 34 weeks.

The PreventADALL study was approved by the Regional Committee for Medical and Health Research Ethics in South-Eastern Norway (2014/518) and in Sweden (2014/2242–31/4), and the study was registered at ClinicalTrials.gov (number NCT02449850).

Subjects

The present study included all 2528 women who completed at least one of two electronic questionnaires. The overall mean age among the 1985 women enrolled in Norway and the 543 women in Sweden was 32.4 years (range 20–48 years) as shown in table 1 and supplementary table S1.

Methods

The electronic questionnaires, developed in collaboration with the University Center for Information Technology (USIT) at the University of Oslo [28], included detailed questions about all types of tobacco

TABLE 1 Background characteristics of the study population, comparing those women who were included in the present analysis *versus* those who were not

Characteristics	Inclusion in the present analysis	
	Yes[#] (n=2528)	No[#] (n=173)
Maternal age years	32.4 (20–48)[¶]	31.1 (18–44)[¶]
Maternal data		
BMI before pregnancy (n=2460)	23.2 (13.8–45.7)	23.6 (17.1–44.1)
Height cm (n=2491)	168.0 (147.0–187.0)	167.4 (147.0–186.0)
Weight before pregnancy kg (n=2471)	65.4 (42.5–126.0)	64.2 (42.0–116.0)
Weight at inclusion kg (n=2498)	70.2 (44.8–132.7)	68.0 (45.8–117.0)
Previous pregnancy	1292 (51.1)	
Previous pregnancies[+] (n=2528)		
0	1236 (48.9)	
1	652 (25.8)	
2	377 (14.9)	
3	143 (5.7)	
>3	120 (4.8)	
Education (n=2350)		
Preliminary school only (9–10 years)	18 (0.8)	
High school only	239 (10.2)	
Higher education <4 years	758 (32.2)	
Higher education ⩾4 years	1324 (56.3)	
Other	2 (0.1)	
Unknown	9 (0.4)	
Country of origin (n=2350)		
Norway	1562 (66.5)	
Sweden	523 (22.3)	
Other nordic	31 (1.3)	
Rest of the world	234 (10.0)	
Marital status (n=2350)		
Married	968 (41.2)	
Cohabitant	1313 (55.9)	
Single	44 (1.9)	
Divorced/separated	1 (0.04)	
Other	24 (1.0)	
Living environment (n=2350)		
City (densely populated)	916 (39.0)	
City (less densely populated)	882 (37.5)	
Suburb	373 (15.9)	
Countryside (in village)	127 (5.4)	
Countryside (outside village)	52 (2.2)	

Data is presented as mean (min–max) or n (%). BMI: body mass index. [#]: subjects were included in the present analysis if one or more questionnaires (from 18 weeks and 34 weeks) was completed. Subjects were excluded if questionnaires were not completed. Total pregnancies at 18 weeks available for inclusion was 2701. Total questionnaires completed at 18 weeks was 2350. [¶]: p<0.001; [+]: number of reported pregnancies excluding the current pregnancy.

and nicotine use prior to and during pregnancy. The questionnaires were sent at a fixed time related to the inclusion date (between the 16th and 22nd weeks of pregnancy), with an automatic reminder in case of no response. The women specified their perceived week of pregnancy when completing the questionnaires. The 18-week questionnaire included questions about ever use (daily or sometimes) of any tobacco or nicotine product prior to and during pregnancy up to the time of completing the questionnaire, while the 34-week questionnaire asked about any use in the period since completing the previous questionnaire.

The questions regarding ever use of tobacco- or nicotine-containing products had separate detailed follow-up questions for cigarette smoking and snus use, included the time of use ("stopped years before pregnancy", "stopped in the time before pregnancy", "stopped when recognising pregnancy" or "current use in pregnancy"), with further details of use in pregnancy or the week of pregnancy when stopping as outlined in the supplementary material. There were no questions specifically determining if cessation was in relation to antenatal visits.

Outcomes, definitions and explanatory variables

The main outcome was use of any nicotine-containing products such as snus, tobacco cigarettes, NRTs and e-cigarettes during pregnancy. The main outcome was further reported for snus, smoking or dual users reported as mutually exclusive categories, unless otherwise specified. Additional nicotine-containing products included "e-cigarettes", "cigars/cigarillos", "pipes" or "other", while NRTs were categorised as "patches" or "gums/lozenges/sprays".

To explore factors related to the use of snus in pregnancy, we included variables that have been associated with smoking: country of origin, living environment, marital status, education level, total household income, previous or current cigarette smoking, the index woman being exposed to maternal smoking *in utero*, household cigarette smoking, age, pre-pregnancy body mass index (BMI), and number of previous pregnancies (excluding current pregnancy) and deliveries.

Statistical analysis

Categorical variables are presented in numbers and percentages, and continuous variables as mean values with maximum and minimum ranges. To address the rates of stopping use of nicotine-containing products in pregnancy, we used data from all 2187 subjects who completed both questionnaires.

Differences between categorical variables were analysed by Chi-squared tests and numerical data by t-tests or one-way ANOVA tests. Binary logistic regression was used to determine factors that might be associated with the use of snus in pregnancy by first excluding potential covariates with a p-value greater than 0.25. The significance level was set to 0.05 and all analyses were performed using IBM SPSS statistics version 25 (Chicago, IL, USA).

Results

Of the 2528 women who completed at least one questionnaire, 2350 completed the 18-week questionnaire, 2365 completed the 34-week questionnaire and 2187 completed both. The included women were similar to those who did not complete any of the questionnaires, with the exception of a slightly higher age (table 1). Background characteristics given by study location are described in supplementary table S1. The use of nicotine-containing products prior to pregnancy is given in table 2, in the supplementary material and in supplementary figure S1.

Prevalence

Based on the women who completed at least one of the 18- or 34-week questionnaires, any use of tobacco- or nicotine-containing products at some time during pregnancy was reported by 286 out of 2528 subjects (11.3%). Snus use alone was reported in 6.5% of cases, cigarette smoking alone in 4.1% of cases, dual use in 0.6% of cases and use of NRTs/e-cigarettes in 0.2% of cases.

From the 18-week questionnaire, any use of tobacco- or nicotine-containing products until pregnancy week 18 was reported by 284 out of 2350 of subjects overall (12.1%), with 12.5% reported in Norway and 10.7% reported in Sweden. Use of Snus alone was the most common form of nicotine-containing product use, reported by 6.9% of subjects, while 4.3% reported smoking alone and 0.6% were dual users. Snus use (including dual use) at some time during pregnancy was 7.6% in total and significantly more common in Norway than in Sweden (8.2% *versus* 5.5%; p=0.035), as shown in table 2 and supplementary figure S1.

Current use of any nicotine-containing products at pregnancy week 18 was reported by 33 out of 2350 subjects (1.4%), with 0.6% using only snus and 0.8% being current smokers only. None reported current dual use, as shown in table 2 and supplementary figure S1. Most current snus users were daily users of three to six portions, a portion being a sealed pouch of snus (see the supplementary material for further

TABLE 2 Reported use of snus, cigarette smoke and nicotine-replacement therapies (NRTs)/ e-cigarettes in Norway and Sweden

Parameter	Total	Norway	Sweden	p-value[#]
Questionnaire adherence				
Completed at 18 weeks	2350 (100.0)	1818 (77.4)	532 (22.6)	
Completed 34 weeks	2365 (100.0)	1853 (78.4)	512 (21.7)	
Completed one or more	2528 (100.0)	1985 (78.5)	543 (21.5)	
Completed both	2187 (100.0)	1686 (77.1)	501 (22.9)	
Tobacco habits				
Ever use in life (n=2350)				
All nicotine-containing products	840 (35.7)	680 (37.4)	160 (30.1)	**0.002**
Snus	529 (22.5)	434 (23.9)	95 (17.9)	**0.003**
Cigarette smoke	531 (22.6)	423 (23.3)	108 (20.3)	0.15
Use before pregnancy (n=2350)				
Stopped years before pregnancy				
Snus	213 (9.6)	165 (9.1)	48 (9.0)	0.97
Cigarette smoke	338 (14.4)	276 (15.2)	62 (11.7)	**0.04**
Stopped in the time before pregnancy				
Snus	138 (5.9)	120 (6.6)	18 (3.4)	**0.005**
Cigarette smoke	77 (3.3)	65 (3.6)	12 (2.3)	0.13
Snus in pregnancy (including dual users)				
At some time up to 18 weeks (n=2350)	178 (7.6)	149 (8.2)	29 (5.5)	**0.035**
Current use at 18 weeks	13 (0.6)	11 (0.6)	2 (0.4)	0.81
Stopped when recognising pregnancy	165 (7.0)	138 (7.6)	27 (5.1)	
At some time from 18–34 weeks (n=2365)	18 (0.8)	14 (0.8)	4 (0.8)	0.95
Current use at 34 weeks	6 (0.3)	4 (0.2)	2 (0.4)	
Stopped between 18 and 34 weeks	12 (0.5)	10 (0.5)	2 (0.4)	
Cigarette smoke in pregnancy (including dual users)				
At some time up to 18 weeks (n=2350)	116 (4.9)	82 (4.5)	34 (6.4)	0.08
Current use at 18 weeks	18 (0.8)	14 (0.8)	4 (0.8)	0.84
Stopped when recognising pregnancy	98 (4.2)	68 (3.7)	30 (5.6)	
At some time from 18–34 weeks (n=2365)	14 (0.6)	10 (0.5)	4 (0.8)	0.53
Current use at 34 weeks	8 (0.3)	6 (0.3)	2 (0.4)	
Stopped between 18 and 34 weeks	6 (0.3)	4 (0.2)	2 (0.4)	
Dual snus and cigarette smoke in pregnancy				
At some time up to 18 weeks (n=2350)	15 (0.6)	7 (0.4)	8 (1.5)	**0.004**
Current use at 18 weeks	0	0	0	
Stopped when recognising pregnancy	15 (0.6)	7 (0.4)	8 (1.5)	
At some time from 18–34 weeks (n=2365)	1 (0.04)	0	1 (0.2)	
Current use at 34 weeks	0	0	0	
Stopped between 18 and 34 weeks	1 (0.04)	0	1 (0.2)	
NRTs/e-cigarettes in pregnancy				
At some time up to 18 weeks (n=2350)	5 (0.2)	3 (0.2)	2 (0.4)	
Current use at 18 weeks	2 (0.1)	1 (0.1)	1 (0.2)	
Stopped when recognising pregnancy	3 (0.1)	2 (0.1)	1 (0.2)	
At some time from 18–34 weeks (n=2365)	1 (0.1)	0	1 (0.2)	
Current use at 34 weeks	1 (0.1)	0	1 (0.2)	
Stopped between 18 and 34 weeks	0	0	0	

[#]: p-values in bold (p<0.05) are significant.

details). The use of NRTs or e-cigarettes was uncommon until 18 weeks (0.2%), with two pregnant women using nicotine gum and three using e-cigarettes. Of these women, four were previously cigarette smokers or snus users.

Overall, 1.3% of the 2365 women completing the 34-week questionnaire reported use of any tobacco- and nicotine-containing products in late pregnancy (defined as from 18 to 34 weeks). At pregnancy week 34, 0.3% were current snus users only and 0.3% were current cigarette smokers only (see table 2 and supplementary figure S1). None of the women reported current use of NRTs and only one woman (0.04%) reported use of e-cigarettes at some time between 18 and 34 weeks of pregnancy.

Based on reports from the 2187 women who completed both questionnaires, 245 out of 269 (91.1%) of the women using snus (n=164) and/or cigarettes (n=105) at some time while pregnant stopped as soon as they

became aware of their pregnancy. As illustrated in figure 1 for snus and smoking, 143 out of 164 (87.2%) quit snus and 83 out of 105 (79.1%) quit smoking by pregnancy week six. Only five women reported use of NRTs or e-cigarettes until pregnancy week 18, of whom three reported quitting at recognised pregnancy. Relapse was generally uncommon, with details given in the supplementary material.

Factors associated with use of snus in pregnancy

From our final multiple logistic regression analyses (Nagelkerke), we could explain 15% of snus use (including dual cigarette smoking) in pregnancy based upon the significant predictors shown in figure 2. The adjusted, significant predictors included inverse association with age, a positive association with urban living (see supplementary figure S2 for details), cohabiting with the partner rather than being married (as is common in Norway and Sweden), being native born in Norway or Sweden compared to elsewhere, previous or current smoking history and the index women being exposed to smoking *in utero*. Maternal education, household income, household smoking, pre-pregnancy BMI and the number of reported previous pregnancies and deliveries were not significantly associated with the use of snus during pregnancy.

Discussion

In this prospective mother–child birth cohort study from 2014, 11.3% of the women reported use of any type of tobacco- or nicotine-containing product at some time during pregnancy up to 34 weeks. Use of snus alone was most common (reported by 6.5% of women), followed by smoking only (4.1%), dual use (0.6%) and NRTs or e-cigarettes (0.2%). However, most women stopped when recognising their pregnancy, with 87.2% of snus users and 79.1% of smokers quitting by pregnancy week six. Snus use in pregnancy was inversely associated with age and positively associated with urban living, being a cohabitant compared to being married or a single mother, being native born in Norway or Sweden compared to other countries, having a personal smoking history and being exposed to cigarettes *in utero*.

The 4.7% of women smoking during pregnancy (including 0.6% dual use) is much lower than rates in similar birth cohort studies from Norway and Sweden around 25 years ago, with a 27% smoker rate in the Environment and Childhood Asthma (ECA) study [29] and a 13% rate in the Swedish BAMSE study [30].

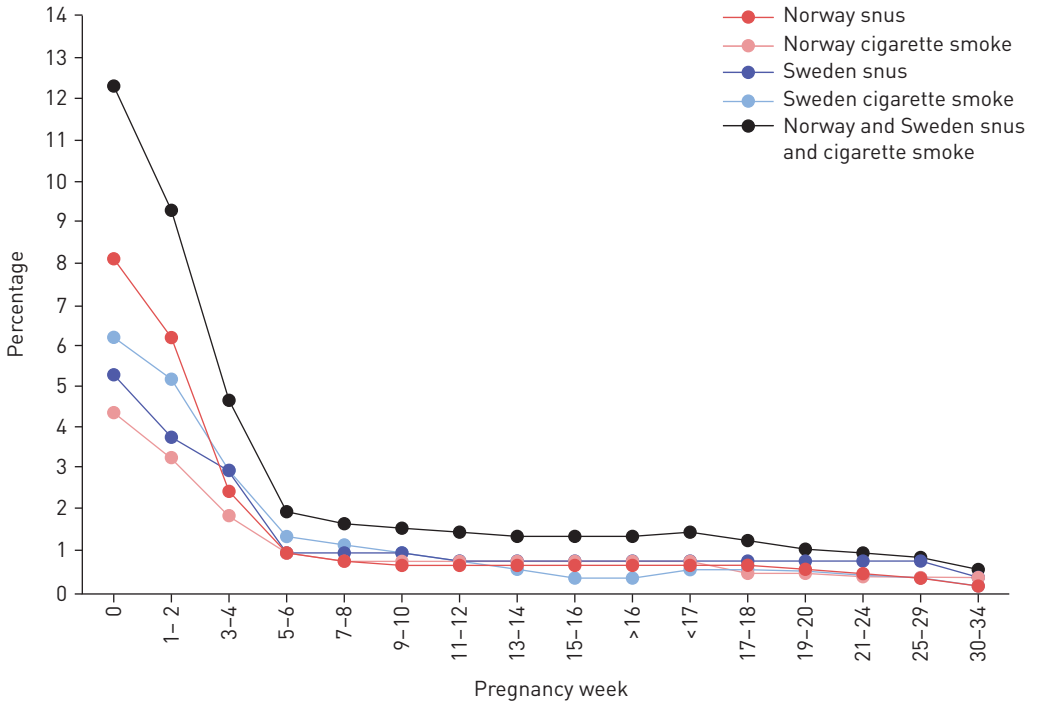

FIGURE 1 Percentage of women using snus and/or cigarette smoke during pregnancy based on self-reported time for stopping from those who completed both 18-week and 34-week questionnaires (n=2187). Time for stopping categories were from 1–2 weeks to >16 weeks and from <17 weeks to 30–34 weeks, respectively. Among the women who reported the use of snus, cigarette smoke or dual use at some time during pregnancy, most stopped when they recognised their pregnancy (mean pregnancy week three to four). Week 0 reflects the total percentage of women who reported any use during pregnancy. E-cigarettes and nicotine replacement therapies were not included since the rates were low.

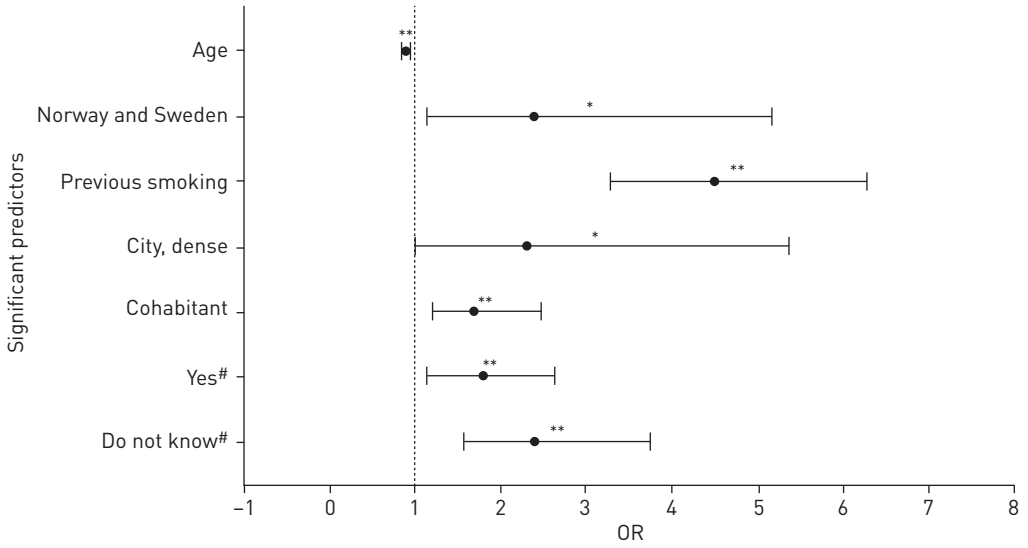

FIGURE 2 Factors associated with snus use during pregnancy. In multivariate analysis, the following factors were identified as associated with the use of snus at some time during pregnancy (reference categories are presented in italic for each factor): living area (countryside (village) *versus* city (densely populated), city (less densely populated), suburb, countryside (outside village)); grand maternal cigarette smoking (no *versus* yes, do not know); country of origin (rest of the world and other nordic countries combined *versus* Norway or Sweden); previous and/or current smoking (no *versus* yes); marital status (married *versus* cohabitant, single, divorced/separated, other). Age was used as a continuous variable. OR: odds ratio. #: *in utero* smoking exposure of the index woman; *: p<0.05; **: p<0.01.

This favourable change harmonises with the decrease in overall smoking rates among pregnant women and the population in general, a trend seen in some [19, 20] but not all [31, 32] countries in the past decades. In contrast, snus use in pregnancy has been largely unknown until recently. The 7.6% of women using snus at some time until week 18 of pregnancy (including 0.6% dual use) is somewhat higher than recent registry studies from Norway [21] and Sweden [33]. The 8.2% of pregnant Norwegian women reporting snus use in our study (including 0.4% dual use) is substantially higher than the 3.4% of first trimester snus use recorded in 2014 that was obtained from a hospital register in Southern Norway [21]. Likewise, the 5.5% of snus use during pregnancy in the Swedish part of our study (including 1.5% dual use) exceeded the 1.2% of snus use in early pregnancy reported in the Swedish Birth Register in 2016 [33]. However, the rate of snus use alone in late pregnancy (0.3%) was similar to the Norwegian and Swedish registries [21, 33]. The higher rates among pregnant women reported in our study, with data recorded from 2014 to 2016, probably reflect the recent rapid increase in snus use among young women [27]. The low prevalence of NRT and e-cigarette use in the present study is, to the best of our knowledge, not previously reported. It should also be noted that we collected data only on tobacco and nicotine products available in Scandinavia and thus products such as "hookah" are not reported.

That the majority of women stopped using snus or stopped smoking by week six of pregnancy is good news and has to our knowledge not been reported previously. It is in line with the reported drop in the prevalence of snus use from early to late pregnancy in the Swedish and Norwegian birth registries [21, 33]. A growing body of evidence demonstrates that the beginning of life, including the time before conception, during pregnancy and in infancy, is a critical period in setting the trajectory towards an increased risk of non-communicable diseases [34]. As epigenetic changes are likely to impact developmental programming in the context of Developmental Origins of Health and Disease (DOHaD) [35], avoiding nicotine overall, even before recognising pregnancy, is important. In this study, the early "stopping when knowing" that is likely to occur even before the first antenatal visit suggests an active choice to abstain from potentially harmful exposure to the unborn child.

The inverse association of snus use with age, the positive association with urban living and lack of association with educational attainment in our study differ from the lower educational level seen among smokers [36]. However, our predictors for snus use appear more similar to non-daily smokers than daily smokers in terms of educational level, income, marital status, weight and the rate of spontaneously quitting in early pregnancy [37]. The explained variation in predictors of snus use in pregnancy was only 15%, but was quite similar to predictors of smoking in pregnancy [38]. In this study, previous smoking history and exposure to maternal prenatal smoking were factors associated with using snus in pregnancy.

This corresponds with a study demonstrating an intergenerational association of mothers' and daughters' smoking habits during pregnancy, indicating that daughters exposed *in utero* were more likely to smoke when pregnant than unexposed daughters [39]. Though the mechanism behind this is multifactorial and likely includes behavioural, (epi)genetic and environmental factors [39], it suggests a nicotine susceptibility affecting the risk of use in pregnancy.

The present study is strengthened by its prospective design, with a high follow-up rate where detailed information on tobacco and nicotine use is reported twice during pregnancy. The higher educational attainment level in our study compared to society at large [28] is unlikely to impact the observed prevalence rates, as maternal education was not associated with snus use and our findings largely reflect the rapidly changing societal use of snus. The under-reporting of the use of tobacco products cannot be excluded in this questionnaire-based study, nor did we have biological assays to confirm nicotine exposure. However, self-reported data may be sufficiently accurate as a high degree of association between self-reported tobacco use and nicotine exposure, as assessed by blood cotinine drawn at delivery, has been found [40]. Our data showed a higher prevalence than previously reported from a national health register [21], suggesting that under-reporting may be limited in this prospective study.

Conclusion

In the present study, the 11.3% use of tobacco- and nicotine-containing products during pregnancy was lower than two decades ago, but the prevalence of snus use alone (6.5%) was higher than in recent registry reports. Of snus users, 87.2% quit by week six of pregnancy. Snus use in pregnancy was inversely associated with age and was more common among urban living, cohabitating women with a personal or maternal history of smoking.

In order to give appropriate advice to pregnant women there is an urgent need for large prospective studies investigating potential harmful effects on offspring following the use of non-combustible nicotine products in pregnancy. In the meanwhile, the precautionary principle should be advocated for all types of tobacco and nicotine products in pregnancy.

Acknowledgements: We sincerely thank the study participants and the health personnel who contributed to the recruitment and inclusion of the subjects.

The PreventADALL study group: Anna Asarnoj (Astrid Lindgren Children's Hospital, Karolinska University Hospital, and Dept of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden), Oda C. Lødrup Carlsen (Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway), Kim A. Endre (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, and Division of Paediatric and Adolescent Medicine, and Dept of Dermatology, Oslo University Hospital, Oslo, Norway), Peder A. Granlund (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, and Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway), Henrik Holmstrøm (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, and Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway), Geir Håland (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, and Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway), Caroline-Aleksi O. Mägi (Astrid Lindgren Children's Hospital, Karolinska University Hospital, and Dept of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden), Unni C. Nygaard (Dept of Toxicology and Risk Assessment, Norwegian Institute of Public Health, Oslo, Norway), Eva M. Rehbinder (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, and Division of Paediatric and Adolescent Medicine, and Dept of Dermatology, Oslo University Hospital, Oslo, Norway), Carina M. Saunders (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, and Division of Paediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway), Ingebjørg Skrindo (Dept of Ear, Nose and Throat, Akershus University Hospital, Lørenskog, Norway), Sandra G. Tedner (Astrid Lindgren Children's Hospital, Karolinska University Hospital, and Dept of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden), Magdalena R. Værnesbranden (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, Oslo, and Dept of Obstetrics and Gynaecology, Østfold Hospital Trust, Kalnes, Norway) and Johanna Wiik (Dept of Obstetrics and Gynaecology, Østfold Hospital Trust, Kalnes, Norway, and Dept of Obstetrics and Gynaecology, Institute of Clinical Sciences, Sahlgrenska Academy, Gothenburg, Sweden).

Author contributions: All authors have contributed substantially to the design and/or clinical follow-up of the PreventADALL study, have revised the work critically for important intellectual content and approved the final version before submission. All members of the PreventADALL study group have contributed to the design and/or data collection, have revision the paper and approved the last version before submission.

Conflict of interest: B. Nordlund has nothing to disclose. K-H. Carlsen has received an honorarium for a lecture from Boehringer Ingelheim, outside the submitted work. A.C. Staff has nothing to disclose. C.M. Jonassen has nothing to disclose. L.S. Nordhagen has nothing to disclose. K.D. Sjøborg has nothing to disclose. G. Hedlin has nothing to disclose. H.K. Gudmundsdóttir has nothing to disclose. R.M. Vettukatil has nothing to disclose. C. Söderhäll has nothing to disclose. K. Hilde has nothing to disclose. H.O. Skjerven has nothing to disclose. K.E.S. Bains has nothing to disclose. B. Granum has nothing to disclose. G. Haugen has nothing to disclose. I. Kreyberg has nothing to disclose. K.C. Lødrup Carlsen reports grants as declared in the support statement. She has also received an honorarium for a lecture at the EAACI conference in 2018 from ThermoFisher Scientific, as well as a speaker honorarium from ThermoFisher Scientific outside the submitted work.

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

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III

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Snus in pregnancy and infant birth size: a mother–child birth cohort study

Ina Kreyberg[1,2], Katarina Hilde[1,3], Karen Eline S. Bains[1,2], Kai-Håkon Carlsen[1,2], Berit Granum[4], Guttorm Haugen[1,3], Gunilla Hedlin[5,6], Christine M. Jonassen[7,8], Live S. Nordhagen[1,2,9], Björn Nordlund[5,6], Corina S. Rueegg[10], Katrine D. Sjøborg[11], Håvard O. Skjerven[1,2], Anne C. Staff[1,3], Riyas Vettukattil[1], Karin C. Lødrup Carlsen[1,2] and the PreventADALL Study Group[12]

ABSTRACT

Rationale: While recent studies show that maternal use of snus during pregnancy is increasing, the potential effects on infant birth size is less investigated, with conflicting results.

Objectives: We aimed to determine if maternal use of snus during pregnancy influences the infant anthropometric and proportional size measures at birth.

Methods: In 2313 mother–child pairs from the population-based, mother–child birth cohort PreventADALL (Preventing Atopic Dermatitis and ALLergies) in Norway and Sweden, we assessed nicotine exposure by electronic questionnaire(s) at 18 and 34 weeks of pregnancy, and anthropometric measurements at birth. Associations between snus exposure and birth size outcomes were analysed by general linear regression.

Results: Birthweight was not significantly different in infants exposed to snus in general, and up to 18 weeks of pregnancy in particular, when adjusting for relevant confounders including maternal age, gestational age at birth, pre-pregnancy body mass index, parity, fetal sex and maternal gestational weight gain up to 18 weeks. We found no significant effect of snus use on the other anthropometric or proportional size measures in multivariable linear regression models. Most women stopped snus use in early pregnancy.

Conclusion: Exposure to snus use in early pregnancy, with most women stopping when knowing about their pregnancy, was not associated with birth size. We were unable to conclude on effects of continued snus use during pregnancy because of lack of exposure in our cohort.

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Snus use in pregnancy, reported by 7.1% of 2313 women, was not associated with infant birth size. As most women stopped snus use by 6 weeks gestational age, it was not possible to assess potential birth size effects of persistent use during pregnancy. http://bit.ly/2IG8Vnk

Cite this article as: Kreyberg I, Hilde K, Bains KES, *et al*. Snus in pregnancy and infant birth size: a mother–child birth cohort study. *ERJ Open Res* 2019; 5: 00255-2019 [https://doi.org/10.1183/23120541.00255-2019].

This article has supplementary material available from openres.ersjournals.com

Received: 18 Sept 2019 | Accepted after revision: 7 Oct 2019

Introduction

Smoking during pregnancy is well established as one of the most modifiable risk factors for adverse pregnancy and infant related health effects [1], with effects related to infant birth size including increased risk of low birthweight, length and head circumference [2, 3], ponderal index [3] and being small for gestational age (SGA) [4–6]. Nonlinear decrease in mean adjusted birthweight has been observed with increasing number of cigarettes smoked per day during pregnancy [7, 8]. Tobacco exposure may influence fetal growth throughout pregnancy, with small but significant reductions in head size and femur length in the first trimester, reduced growth after the first trimester [9] and selective reduction in abdominal circumference and muscle mass in fetuses exposed in the last trimester [10]. However, it is unclear whether these findings may be extended to smokeless tobacco.

The use of snus, a smokeless tobacco product also known as moist snuff, and other smokeless nicotine products such as electronic cigarettes have increased in recent years [11, 12], paralleling decreased smoking rates among young women in many countries [13]. A similar increased use of snus in women of reproductive age in Norway and Sweden [12, 14] is also shown during pregnancy [14, 15]. We recently showed in the Preventing Atopic Dermatitis and Allergies in Children (PreventADALL) study that 11.3% of pregnant women reported use of any tobacco or nicotine products by 34 weeks of pregnancy; most commonly as snus only in 6.5%, followed by cigarette smoking only in 4.1% and dual smoking and snus in <1%. Most women stopped snus use or cigarette smoking early in pregnancy, usually within pregnancy week six [16]. Nicotine from snus readily crosses the placenta into the fetal compartments and, together with its metabolites such as cotinine, concentrates in fetal blood, urine, meconium and amniotic fluid [17, 18]. Despite substantial documentation in animal models showing adverse effects in the offspring of nicotine exposure by the pregnant female, there are few studies to verify these findings in humans [19, 20]. Exposure to snus during pregnancy increased the risk of preterm birth [21], stillbirth [22, 23], oral cleft malformation [24] and neonatal apnoea [25] in Swedish Medical Birth Register studies, while altered infant heart rate variability was observed in a prospective observational study [26]. While a study from India showed an average of 87 g reduced birthweight (adjusted for gestational age) in infants born to women who used smokeless tobacco regularly in pregnancy [27], no significant effect of snus use was observed on birthweight in the Swedish registry study [28]. Thus, the effect of snus on birthweight is unclear, nor are we aware of studies assessing potential effects of snus use on other infant size measures at birth [19].

Therefore, we aimed to determine if maternal use of snus in pregnancy might affect infant anthropometric and proportional size measures at birth.

Subjects and methods

Study design

This study is based on the large population-based, prospective mother–child birth cohort PreventADALL [29], enrolling 2697 women with 2701 pregnancies and their 2397 infants born at a gestational age of ⩾35.0 weeks without serious neonatal disease. The main objectives of the PreventADALL study are to determine whether primary prevention of allergic diseases is possible through a 2×2 factorially designed, randomised trial of two interventions. Additionally, early life exposures and factors involved in allergic diseases and noncommunicable diseases are assessed. Pregnant females were recruited at the routine ultrasound screening at second trimester between gestational weeks 16 and 22, in hospitals from the general, nonselected population from the greater area of Oslo and southeast Norway as well as from the Stockholm area in Sweden between December 2014 and October 2016 [29]. Details of recruitment, inclusion and exclusion criteria are given in the supplementary material.

Affiliations: [1]Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, Oslo, Norway. [2]Division of Paediatrics and Adolescent Medicine, Oslo University Hospital, Oslo, Norway. [3]Division of Obstetrics and Gynaecology, Oslo University Hospital, Oslo, Norway. [4]Dept of Toxicology and Risk Assessment, Norwegian Institute of Public Health, Oslo, Norway. [5]Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden. [6]Dept of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden. [7]Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway. [8]Genetic Unit, Centre for Laboratory Medicine, Østfold Hospital Trust, Kalnes, Norway. [9]VID Specialized University, Oslo, Norway. [10]Oslo Centre for Biostatistics and Epidemiology, Oslo University Hospital and University of Oslo, Oslo, Norway. [11]Dept of Obstetrics and Gynaecology, Østfold Hospital Trust, Kalnes, Norway. [12]A full list of PreventADALL study group members and their affiliations can be found in the Acknowledgements section.

Correspondence: Ina Kreyberg, Division of Paediatrics and Adolescent Medicine, Oslo University Hospital and the University of Oslo, Postboks 4956 Nydalen, 0424 Oslo, Norway. E-mail: inakreyberg@hotmail.com

The present study included all 2313 mother–child pairs with available exposure data of any type of nicotine or tobacco product from electronic questionnaires at 18 and/or 34 weeks of pregnancy and whose included singleton newborn babies had anthropometric measurements performed at birth (supplementary figure S1).

Maternal written consent was obtained upon primary enrolment and signed by both parents at newborn inclusion. The PreventADALL 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).

Subjects

Baseline characteristics among the included and the excluded study participants were similar, except for marital status, previous smoking history and gestational age at birth as shown in supplementary table S1.

Methods

From electronic questionnaires completed by the women at 18 and 34 weeks of pregnancy, we collected detailed information about snus, cigarette smoking, nicotine replacement therapy (NRT) or electronic cigarettes: never, ever, prior to pregnancy, and during pregnancy up to 18 weeks and from 18 weeks to 34 weeks [16]. Background characteristics and history of previous and present pregnancies were obtained from the 18-week questionnaire and a brief interview at enrolment. Whether the women reported the ultrasound-corrected gestational age or calculated gestational age from their last menstrual period is not known. Study personnel measured maternal weight and height at the 18-week inclusion visit. Pre-pregnancy weight was obtained by self-report rather than by objective measures.

Dedicated trained study personnel using a non-elastic measuring tape performed infant anthropometric measures within the first 24 h after delivery. We report the mean of two left upper arm circumference measures midway between the acromial and olecranon process, and the mean of three thoracic and abdominal circumference measures performed at end-expiration when possible. For the thoracic circumference measures we placed the lower part of the measuring tape in line with the most caudal part of the xyphoid process. For the abdominal circumference measures the lower end of the measuring tape was placed in line with the cranial part of the umbilicus. All values were recorded in centimetres with one decimal, and mean values were calculated and rounded when appropriate. Background for the methods used is described in the supplementary material. Birthweight, length (crown–heel) and head circumference as well as placenta weight was collected from the hospital records. Placenta was weighed and recorded by the midwives within 30 min of delivery according to hospital guidelines.

Outcomes, exposures and covariates

The main outcome was birthweight (in grams). Secondary anthropometric outcomes were birth length, head circumference, thoracic circumference, abdominal circumference and left mid upper arm circumference (all in centimetres), while the ratios of abdominal circumference to head circumference, thoracic circumference to head circumference, thoracic circumference to abdominal circumference and finally birthweight to placenta weight were proportional size outcomes.

The main exposure variables were based on any use of snus and/or smoke in pregnancy categorised into never in pregnancy ("never"), snus alone in pregnancy ("snus only") and smoking including dual snus users in pregnancy ("smoke/dual"). To differentiate between early and late snus exposure, we also separated the snus only group into "snus only 18 weeks" and "snus only 34 weeks". Women who reported ever-use of snus and/or smoking before pregnancy were included in the never group representing never-use during pregnancy. As only four women reported using NRT or electronic cigarettes, of whom three stopped when recognising pregnancy, they were included in the analyses based upon their use of snus and cigarette smoking.

Potential covariates were based upon factors previously shown to be associated with snus use in pregnancy [16] including maternal age, marital status, previous smoking history, *in utero* smoking exposure of the index women and living area. Additionally, we included factors possibly associated with birth size of the baby such as maternal education, pre-pregnancy body mass index (BMI), parity, fetal sex and gestational age at delivery (days). Further, as smoking cessation during pregnancy may cause weight gain [30, 31], we performed sensitivity analyses including adjustment for maternal gestational weight gain from pre-pregnancy to 18 weeks of pregnancy. The gestational age was based on femur length obtained at the routine second trimester ultrasound, as described previously [29].

Statistical analysis

Categorical variables are presented as numbers and percentages, and continuous variables as means with standard deviation or 95% confidence intervals. Differences between categorical variables were analysed by Chi-squared test and numerical data by one-way ANOVA tests.

Associations between snus exposure and birth size outcomes were analysed using univariable and multivariable linear regression models with the birth outcomes as dependent variable and snus use as independent variable. The group of never snus users were defined as the reference group. In the multivariable models we included all covariates that were known potential confounders associated with snus use from our previous study [16] and the literature in general. Significant covariates with a p-value <0.05 were kept in the final models, as appropriate for each outcome, with details given in supplementary table S3. Sensitivity analyses including adjustment for maternal gestational weight gain from pre-pregnancy to 18 weeks of pregnancy were performed in case of significant associations between snus use and the respective birth outcome.

The significance level was set to 5%. Because of low numbers of missing data we performed complete case analysis only. All analyses were performed using SPSS Statistics (version 25; IBM, Chicago, IL, USA).

Results

Most women (89.1%) reported never-use of tobacco products during pregnancy, while 150 (6.5%) reported snus only and 102 (4.4%) reported cigarette smoking, including 15 (0.6%) dual users. Up to 18 weeks of pregnancy 138 (6.0%) women used snus only at some time or current, and 12 (0.6%) up to 34 weeks. The majority (>90%) of the snus and smoking/dual-using women stopped within pregnancy week six. Exposure to nicotine products was similar in term and preterm infants, as described in detail in the supplementary material and table S2.

The exposure groups never, snus only and smoke/dual differed significantly from each other with respect to gestational age at birth, maternal age, maternal gestational weight gain, parity and socioeconomic factors, as listed in table 1.

Unadjusted analyses showed no significant difference in birthweight among the women who used snus only compared to never and smoke/dual users (table 2). We observed significantly higher birthweight in infants exposed to snus only up to 18 weeks (table 3) after adjusting for parity, gestational age at birth, fetal sex, pre-pregnancy BMI and maternal age in multivariable regression analyses. However, after adjusting for maternal gestational weight gain (from pre-pregnancy to gestational week 18), the associations were no longer statistically significant in sensitivity analyses (table 3). We found no significant interaction between gestational weight gain and the tobacco exposure groups.

For the "snus up to 34 weeks" group, there was a nonsignificant trend of decreased birthweight.

The only other anthropometric measure with significant associations to snus exposure in unadjusted (table 2) and adjusted regression analyses (supplementary table S4) was head circumference. However, after adjusting for maternal gestational weight gain (from pre-pregnancy to gestational week 18) in sensitivity analyses, the association was no longer significant. We found no other significant associations between snus exposure in the univariable or multivariable regression analyses on anthropometric (supplementary table S4) or proportional size outcomes (supplementary table S5).

Discussion

In our cohort, in which 90% of the women stopped snus use at recognised pregnancy, snus exposure was not significantly associated with birthweight or other anthropometric or proportional size measures.

To our knowledge, this is the first prospective mother–child cohort study showing that the use of snus in pregnancy was not associated with infant birth size. Our results are supported by registry studies showing that women who quit snus early in pregnancy had the same risk of SGA or low birthweight of the baby as non-snus users [28, 32]. This is in contrast to an Indian cohort study of 1217 women interviewed during months 3–7 of pregnancy at house-to-house visits showing an average of 105 g lower birthweight among the 17% reporting daily use of chewable tobacco for ⩾6 months [27]. However, there are important differences between the studies in regards to types of smokeless tobacco products, prevalence of exposures as well as probable cultural and sociodemographic differences. In view of the presumably low total *in utero* nicotine exposure in our study, with most women stopping in early pregnancy, our findings are in line with other studies showing that early cessation attenuates the effects of snus [32] or smoking [7].

The apparently higher birthweight in infants born to mothers using snus up to 18 weeks of pregnancy only, before adjusting for maternal weight gain, is in contrast to registry studies from Sweden, where nonsignificant reductions in birthweight were observed in sibling analyses [28]. However, sensitivity analyses showed that the significantly higher maternal gestational weight gain in those stopping snus use compared to non-users largely explained the difference in the model not adjusting for weight gain. We are unaware of studies including maternal gestational weight gain in their adjusted models, but propose that maternal gestational weight gain should be included in analyses exploring potential effects of nicotine

TABLE 1 Background characteristics of the study population (n=2313) stratified by tobacco exposure during pregnancy

	Subjects	Tobacco exposure during pregnancy[#]			
		Never	Snus only	Smoke/dual	p-value
Subjects	2313	2061 (89.1)	150 (6.5)	102 (4.4)	
Fetal sex male		1097 (53.0)	73 (48.7)	50 (48.5)	0.37
Gestational age at birth	2274				
Weeks		39.2±1.7	39.6±1.7	38.9±1.7	0.001
Days		274.7±11.6	277.5±11.7	272.0±11.8	0.001
Placenta weight g	1740	656±135.1	679±110.2	643±144.9	0.12
Maternal factors					
Age years	2313	32.6±4.1	30.9±3.2	32.0±4.8	<0.001
Pre-pregnancy BMI	2252	23.1±3.6	22.8±3.5	23.9±4.7	0.05
BMI at 18 weeks	2278	24.8±3.6	24.8±3.6	25.8±4.8	0.016
Weight pre-pregnancy kg	2263	65.4±11.1	64.8±11.1	66.4±13.4	0.55
Weight at inclusion kg	2288	70.1±11.1	70.4±11.4	71.6±13.5	0.37
Gestational weight gain up to 18 weeks kg	2259	4.7±3.2	5.4±3.5	5.3±3.8	0.002
Pregnancy history					
Current *in vitro* fertilisation	2300	173 (8.4)	4 (2.7)	0 (0.0)	<0.001
Miscarriage(s) <12 weeks	2300				0.08
0		1531 (74.3)	126 (84.0)	73 (70.9)	
1		371 (18.0)	15 (10.0)	20 (19.4)	
>1		146 (7.1)	9 (6.0)	10 (9.7)	
Miscarriage(s)/stillbirths 12–23 weeks	2300				0.006
0		2006 (97.9)	148 (98.7)	96 (94.1)	
1		41 (2.0)	2 (1.3)	5 (4.9)	
>1		1 (0.0)	0 (0.0)	1 (1.0)	
Parity	2150				0.008
0		1124 (59.2)	107 (71.8)	59 (57.8)	
1		612 (32.2)	36 (24.2)	29 (28.4)	
>1		163 (8.6)	6 (4.0)	14 (13.7)	
Sociodemographic factors					
Education	2141				<0.001
Preliminary school only		13 (0.7)	1 (0.7)	2 (2.0)	
High school only		175 (9.3)	19 (12.8)	25 (24.5)	
Higher education <4 years		580 (30.7)	60 (40.5)	42 (41.2)	
Higher education ⩾4 years		1122 (59.3)	67 (45.3)	33 (32.4)	
Other		1 (0.1)	1 (0.7)	0 (0.0)	
Country of origin	2150				0.005
Norway and Sweden		1683 (88.6)	144 (96.6)	87 (85.3)	
Rest of the world		216 (11.4)	5 (3.4)	15 (14.7)	
Marital status	2150				<0.001
Married		816 (43.0)	39 (26.2)	30 (29.4)	
Cohabitants		1037 (54.6)	107 (71.8)	66 (64.7)	
Single		30 (1.6)	2 (1.3)	5 (4.9)	
Divorced/separated		1 (0.1)	0 (0.0)	0 (0.0)	
Other		15 (0.8)	1 (0.7)	1 (1.0)	
Living area	2150				0.026
City, densely populated		716 (37.7)	76 (51.0)	42 (41.2)	
City, less densely populated		732 (38.5)	52 (34.9)	33 (32.4)	
Suburb		311 (16.4)	15 (10.1)	17 (16.7)	
Countryside, village		100 (5.3)	5 (3.4)	5 (4.9)	
Countryside, outside village		40 (2.1)	1 (0.7)	5 (4.9)	
Household income	2150				<0.001
Low		18 (0.9)	3 (2.0)	4 (3.9)	
Middle		991 (52.2)	87 (58.4)	72 (70.6)	
High		857 (45.1)	57 (38.3)	24 (23.5)	
Not reported		33 (1.7)	2 (1.3)	2 (2.0)	
Smoking history					
Previous smoking	2150	300 (14.6)	75 (50.0)	100 (98.0)	<0.001

Continued

TABLE 1 Continued

	Subjects	Tobacco exposure during pregnancy[#]			
		Never	Snus only	Smoke/dual	p-value
In utero exposure to cigarette smoke	2150				<0.001
No		1463 (77.0)	87 (58.4)	73 (71.6)	
Yes		256 (13.5)	29 (19.5)	21 (20.6)	
Do not know		180 (9.5)	33 (22.1)	8 (7.8)	

Data are presented as n, n (%) or mean±SD, unless otherwise stated. BMI: body mass index. [#]: includes four females who answered "yes" to ever-use of other nicotine products (nicotine replacement therapy or electronic cigarettes); one was a daily user during pregnancy at 18 weeks and three quit when recognising pregnancy.

exposure *in utero* on fetal growth. This is supported by studies showing an increased risk of excess gestational weight gain from pre-pregnancy to delivery when quitting smoking in pregnancy compared to non-smokers [30, 31] and substantially lower rate of neonatal birthweight below the 10th percentile [33]. However, we are unaware of studies on maternal gestational weight gain after snus cessation during pregnancy.

We were unable to conclude on the effects of continuous snus exposure through pregnancy up to 34 weeks, with only 11 subjects in this exposure group. Thus, the potential effect of continued use of snus throughout pregnancy is still uncertain. We did see a nonsignificant trend of decreased birthweight in this group, which is in line with the Indian study of smokeless tobacco [27], as well as in conventional [32], but not in sibling analyses in the Swedish birth registry study [26].

TABLE 2 Anthropometric measures and proportional size are given by tobacco exposure groups for 2313 newborn infants

	Subjects	Tobacco exposure during pregnancy[#]			
		Never	Snus only	Smoke/dual	p-value
Anthropometric measures		2061 (89.1)	150 (6.5)	102 (4.4)	
Birthweight g	2252	3577 (3556–5598)	3662 (3591–3733)	3575 (3472–3678)	0.11
Length cm	2181	50.5 (50.4–50.6)	50.8 (50.4–51.1)	50.4 (49.9–50.9)	0.26
Head circumference cm	2238	35.2 (35.1–35.3)	35.5 (35.3–35.8)	35.3 (34.9–35.6)	0.029
Thoracic circumference cm	2157	34.0 (33.9–34.1)	34.2 (33.9–34.5)	34.2 (33.7–34.7)	0.30
Abdominal circumference cm	2156	32.8 (32.7–32.8)	32.9 (32.5–33.3)	32.7 (32.2–33.2)	0.79
Left mid upper arm circumference cm	2166	11.1 (11.1–11.2)	11.3 (11.2–11.5)	11.2 (11.0–11.4)	0.15
Proportional size					
Abdominal/head circumference	2102	0.94 (0.93–0.94)	0.92 (0.92–0.94)	0.93 (0.91–0.94)	0.45
Thoracic/abdominal circumference	2151	1.04 (1.037–1.04)	1.04 (1.04–1.05)	1.05 (1.04–1.06)	0.14
Thoracic/head circumference	2103	0.97 (0.965–0.97)	0.96 (0.96–0.97)	0.97 (0.96–0.98)	0.68
Birthweight/placenta weight	1729	5.6 (5.5–5.7)	5.5 (5.4–5.7)	5.7 (5.5–6.0)	0.54

Data are presented as n (%), n or mean (95% CI), unless otherwise stated. The reference group "never" includes all females who did not report use of tobacco or nicotine during pregnancy. The "smoke/dual" group includes dual smokers and snus users during pregnancy. Most of these subjects (>90%) quit snus use or smoking by 6 weeks of pregnancy. [#]: includes four females who answered "yes" to ever-use of other nicotine products (nicotine replacement therapy or electronic cigarettes); one was a daily user during pregnancy at 18 weeks and three quit when recognising pregnancy.

TABLE 3 Linear regression analyses: effect of tobacco exposure during pregnancy on birthweight (grams)

	Univariable			Multivariable[#] (1–5)			Univariable sensitivity analyses			Multivariable sensitivity analyses (1–6)		
	Subjects n	β (95% CI)	p-value	Subjects n	β (95% CI)	p-value	Subjects n	β (95% CI)	p-value	Subjects n	β (95% CI)	p-value
Tobacco exposure			0.085			0.113			0.110			0.550
Never	1772	Ref.		1772	Ref.		1694	Ref.		1694	Ref.	
Snus only	143	91.3 (10.1–172.5)		143	78.1 (4.7–151.5)		137	88.8 (5.6–172.0)		137	36.3 (−37.4–110.0)	
Smoke/dual	97	−6.5 (−103.9–90.9)		97	11.0 (−76.6–98.6)		90	−5.0 (−106.4–96.4)		90	−20.1 (−109.1–68.9)	
Tobacco exposure			0.120			0.032			0.180			0.250
Never	1772	Ref.		1772	Ref.		1694	Ref.		1694	Ref.	
Snus only up to 18 weeks	132	102.0 (17.7–186.3)		132	100.0 (23.9–176.1)		127	96.8 (10.6–183.0)		127	53.8 (−22.6–130.1)	
Snus only up to 34 weeks	11	−36.4 (−318.9–246.1)		11	−183.1 (−436.5–70.3)		10	−13.1 (−310.3–284.1)		10	−180.6 (−440.6–79.5)	
Smoke/dual	97	−6.5 (−103.9–90.9)		97	10.9 (−76.7–98.4)		90	−5.0 (−106.4–96.4)		90	−20.0 (−108.9–69.0)	

The reference group "never" includes all females who did not report use of tobacco or nicotine during pregnancy. The "smoke/dual" group includes dual smokers and snus users during pregnancy, of whom most quit before 6 weeks of pregnancy. The nonsignificant global p-values for snus-only and smoke/dual indicate that no significant associations were observed with birthweight. Covariates used in multivariable analyses: 1=parity, 2=gestational age at birth, 3=fetal sex, 4=pre-pregnancy body mass index, 5=maternal age, 6=gestational weight gain up to 18 weeks of pregnancy. Ref.: reference value. [#]: the results of the multivariable analyses restricted by the same study population as in the sensitivity analyses without adjusting for gestational weight gain, were similar in both populations (data not shown).

To our knowledge, this is the first study to investigate the potential effect of snus exposure in pregnancy on anthropometric and proportional size measures at birth. Birth size is determined by genetic predisposition and by the intrauterine environment, including potential unfavourable *in utero* exposures affecting fetal growth [34]. While birthweight and length are predictors of lean mass [35], abdominal circumference may indicate level of fat and/or size of the liver [34], and upper mid arm circumference predicts muscle mass [36]. Our study with predominantly early transient exposure to snus was not able to replicate the adverse effects on differential fetal growth by exposure to cigarette smoke [2, 3, 10].

The study is strengthened by the prospective design, specifically designed questionnaires completed at 18 and 34 weeks of pregnancy with detailed information on the use of products containing nicotine during pregnancy and time of cessation specified by 2-week intervals. The study provides standardised detailed anthropometric measurements conducted by trained study personnel within the first 24 h after delivery.

The high early pregnancy cessation rates of both snus use and cigarette smoking in this study is clearly positive for maternal and infant health, but limited our ability to study the effects of persistent use during pregnancy. Nevertheless, it provides important information for pregnant women who have stopped using snus or are planning to quit, as well as for health professionals providing their prenatal care, that early exposure does not seem to affect the birth size of the baby. In addition, detailed information of frequency of use and number of snus portions and/or cigarettes smoked among those who stopped when recognising their pregnancies are lacking, thus limiting the possibilities to assess dose–response effects. Our data are based on self-reports with no objective validation of nicotine or cotinine levels during pregnancy. Nevertheless, studies have shown that self-reports represent valid markers for tobacco exposure [37, 38]. There is uncertainty regarding the exact pregnancy week of self-reported cessation, as we do not know if the subjects reported the ultrasound corrected gestational age or the calculated gestational age from the last menstrual period. If the reported gestational age was the latter, the first two pregnancy weeks correspond with the last 2 weeks before conception, thus nicotine exposure to the offspring might be limited. The gestational age was determined based upon the routine ultrasound examination, as described in the supplementary material, with a potential variation that could not be accounted for in the present analyses. Additionally, we only adjust for weight gain in the first 18 weeks of pregnancy, since we do not have weight of the mothers at delivery. However, since most women stopped using snus by pregnancy week six, one might assume the weight gain effect related to cessation might be in the period up to 18 weeks of pregnancy. Although the participants were recruited from a nonselected general population, the educational level in our study was higher than in the Norwegian general population [29]. However, this is unlikely to impact the prevalence of snus use, since we have recently found that educational level is not associated with snus use during pregnancy [16]. It might affect the choice of lifestyle and diet, potentially influencing fetal growth. As this is a prospective cohort study, nonparticipation cannot be associated with the outcome. Therefore, effect estimates of snus use on birth outcomes should not be biased [39]. Potential covariates such as pre-eclampsia, gestational diabetes or other relevant maternal diseases that possibly could explain birth size were unavailable at the time of analyses.

Conclusion

Maternal snus use in pregnancy, with most subjects stopping when knowing about their pregnancy, was not significantly associated with birthweight or anthropometric or proportional size measures of the newborn infants. Due to low prevalence of snus users up to 34 weeks of gestation, we could not conclude on potential effects of continued snus exposure in pregnancy on infant birth size.

Author contributions: All authors have contributed substantially to the design and/or clinical follow-up of the PreventADALL study, and have revised the work critically for important intellectual content and approved the final version before submission.

Acknowledgements: PreventADALL study group: Anna Asarnoj (Astrid Lindgren Children's Hospital, Karolinska University Hospital, and Dept of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden), Oda C. Lødrup Carlsen (Division of Paediatrics and Adolescent Medicine, Oslo University Hospital, Oslo, Norway), Åshild Wik Despriée (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, the Division of Paediatrics and Adolescent Medicine, Oslo University Hospital, and VID Specialized University, Oslo, Norway), Kim A. Endre (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, the Division of Paediatrics and Adolescent Medicine, and Dept of Dermatology, Oslo University Hospital, Oslo, Norway), Peder A. Granlund (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, and the Division of Paediatrics and Adolescent Medicine, Oslo University Hospital, Oslo, Norway), Hrefna K. Gudmundsdóttir (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, and the Division of Paediatrics and Adolescent Medicine, Oslo University Hospital, Oslo, Norway), Henrik Holmstrøm (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, and the Division of Paediatrics and Adolescent Medicine, Oslo University Hospital, Oslo, Norway), Geir Håland (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, and the Division of Paediatrics and Adolescent Medicine, Oslo University Hospital, Oslo, Norway), Caroline-Aleksi O. Mägi (Astrid Lindgren Children's Hospital, Karolinska University Hospital, and Dept of

Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden), Unni C. Nygaard (Dept of Toxicology and Risk Assessment, Norwegian Institute of Public Health, Oslo, Norway), Eva Maria Rehbinder (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, the Division of Paediatrics and Adolescent Medicine, and Dept of Dermatology, Oslo University Hospital, Oslo, Norway), Knut Rudi (Faculty of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway), Carina M. Saunders (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, and Division of Paediatrics and Adolescent Medicine, Oslo University Hospital, Oslo, Norway), Ingebjørg Skrindo (Dept of Ear, Nose and Throat, Akershus University Hospital, Lørenskog, Norway), Cilla Söderhäll (Astrid Lindgren Children's Hospital, Karolinska University Hospital, and Dept of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden), Sandra G. Tedner (Astrid Lindgren Children's Hospital, Karolinska University Hospital, and Dept of Women's and Children's Health, Karolinska Institutet, Stockholm, Sweden), Magdalena R. Værnesbranden (Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, Oslo, and Dept of Obstetrics and Gynaecology, Østfold Hospital Trust, Kalnes, Norway) and Johanna Wiik (Dept of Obstetrics and Gynaecology, Østfold Hospital Trust, Kalnes, Norway, and Dept of Obstetrics and Gynaecology, Institute of Clinical Sciences, Sahlgrenska Academy, Gothenburg, Sweden).

The study was performed within the ORAACLE group (the Oslo Research Group of Asthma and Allergy in Childhood; the Lung and Environment). We sincerely thank all the study participants and the individuals involved in facilitating and running the study, especially Ann Berglind, Malén Gudbrandsgard, Mari Rønning Kjendsli, Natasha Sedergren, Päivi Söderman and Ellen Tegnerud.

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

Support statement: The study was funded by the Regional Health Board South East, the Norwegian Research Council, Oslo University Hospital, the University of Oslo, Health and Rehabilitation Norway, and Østfold Hospital Trust; by unrestricted grants from the Norwegian Association of Asthma and Allergy, the Kloster Foundation, the Norwegian Society of Dermatology and Venerology, Arne Ingel's Legat, Fürst Medical Laboratory, the Foundation for Healthcare and Allergy Research in Sweden (Vårdalstiftelsen), Swedish Asthma and Allergy Association's Research Foundation, the Swedish Research Council Initiative for Clinical Therapy Research, the Swedish Heart–Lung Foundation, SFO-V Karolinska Institutet, and the Hesselman Research Foundation; and Thermo Fisher (Uppsala, Sweden) by supplying allergen reagents. Funding information for this article has been deposited with the Crossref Funder Registry.

Conflict of interest: I. Kreyberg has nothing to disclose. K. Hilde has nothing to disclose. K.E.S. Bains has nothing to disclose. K-H. Carlsen has nothing to disclose. B. Granum has nothing to disclose. G. Haugen has nothing to disclose. G. Hedlin has nothing to disclose. C.M. Jonassen has nothing to disclose. L.S. Nordhagen has nothing to disclose. B. Nordlund has nothing to disclose. C.S. Rueegg has nothing to disclose. K.D. Sjøborg has nothing to disclose. H.O. Skjerven has nothing to disclose. A.C. Staff has nothing to disclose. R. Vettukattil has nothing to disclose. K.C. Lødrup Carlsen has received grants as declared in the support statement and an honorarium from Thermo Fisher Scientific for a lecture given at the 2018 EAACI conference.

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