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PRENATAL NICOTINE EXPOSURE AND EFFECTS ON THE HEALTH OF THE NEWBORN

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Prenatal nicotine exposure and effects on the health of the newborn
THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Den mätta dagen, den är aldrig störst.
Den bästa dagen är en dag av törst.

Nog finns det mål och mening i vår färd –
men det är vägen som är mödan värd.

Karin Boye (1900-1940). Från *I rörelse/On the Move* (Ur Härdarna, 1927).

The day of plenty, never is the greatest.
The best day is a day of craving thirst.

Yes, there is a meaning in our journey –
but 'tis the pathway, which is worth our while.

The excerpt from the poem by Karin Boye is published with kind permission from Mats Boye and from Hans Corell, who made the translation.

To my family

ABSTRACT

Maternal smoking is one of the most important preventable risk factors in pregnancy. Whether it is nicotine or combustion products, or possibly both, that cause the adverse effects is not clear. The common substance in snuff and cigarette smoke is nicotine.The main objective was to study whether it is safe to use snuff in pregnancy, and if cessation of tobacco use influences the risk of adverse effects on the health of the newborn.

Methods: Studies I-III were all based on data from the Swedish Medical Birth Register. Information on tobacco use three months before pregnancy and in early pregnancy was obtained from the register and categorized as nonuser, smoker 1-9 cigarettes/day, smoker≥10 cigarettes/day or snuff user. Most epidemiological studies on tobacco use are based on self-reported information. Snuff use in pregnancy has not been validated previously. In study IV, self-reported snuff use in a cohort of pregnant women was validated by the use of a biomarker of nicotine exposure, cotinine.

Results: In study I, the association between smoking or snuff use in pregnancy and risk of neonatal apnea was investigated. Maternal snuff use was associated with an almost twofold risk of neonatal apnea. In contrast, the increased risk of apnea in smokers was not significant after adjustment for gestational age, indicating different mechanisms for nicotine and tobacco smoke.

Study II investigated the association between tobacco use and risk of oral cleft malformations. Both maternal smoking and snuff use were associated with increased risk of oral cleft malformations. However, infants of women who had stopped smoking or using snuff before the antenatal booking were not at an increased risk of oral cleft malformations compared to that of nonusers.

In study III, the association between maternal smoking, snuff use and preterm birth was studied. Maternal smoking was associated with extremely (<28 weeks), very (28-<32 weeks) and moderately (32-<37 weeks) preterm birth. Snuff use in pregnancy was associated with extremely preterm birth and moderately preterm birth, whereas the association with very preterm birth was of borderline significance. Importantly, with cessation of tobacco use in early pregnancy, there was no increased risk.

In Study IV, self-reported snuff use was validated by measuring cotinine in maternal urine and meconium of the newborn. Self-reported use of snuff was found valid in late pregnancy. However, there was a large proportion of misclassification of snuff use in the Medical Birth Register in late pregnancy.

Conclusion: Snuff use and smoking in pregnancy are associated with increased risk of adverse effects such as neonatal apnea, oral cleft malformation and extremely preterm birth. Importantly, infants of women who stopped using tobacco were not at an increased risk. This thesis indicates that no forms of nicotine or tobacco are to be regarded as safe to use in pregnancy.

SVENSK SAMMANFATTNING

Globalt är rökning en av de främsta förebyggbara riskfaktorerna under graviditet. Rökning under graviditet är associerad med en ökad risk för placentablödning, prematur födsel, intrauterin dödföddhet, tillväxthämning hos fostret och ökad morbiditet och mortalitet i nyföddhetsperioden. Rökning under graviditet ökar också risken för plötslig spädbarnsdöd. Huruvida det är nikotin eller förbränningsprodukter i cigarettrök som orsakar de skadliga effekter som ses hos barn till rökare är inte helt klarlagt. Det är dock en viktig fråga, eftersom det i Sverige finns en utbredd användning av snus. Till skillnad från cigaretter innehåller snus inga förbränningsprodukter men däremot nikotin.

Syftet med denna avhandling var att undersöka effekterna på fostret av prenatal nikotinexponering i form av snus för att bedöma huruvida snus är säkert att använda under gravididitet, samt om snusstopp i tidig graviditet påverkar eventuella risker.

Studie I visade att rökning och snusanvändning ökade risken för andningsuppehåll (apné) i nyföddhetsperioden. Den ökade risken för neonatal apné hos barn till rökare förklarades av den ökade risken för prematur födsel. Hos barn till snusare kvarstod den fördubblade risken också efter justering för prematuritet.

Studie II visade att både rökning och snusanvändning ökade risken för läpp-käk-gomspalt (LKG) missbildning. Kvinnor som slutat röka eller snusa i tidig graviditet, före första besöket hos mödravården, hade ingen ökad risk för att föda ett barn med LKG-missbildning.

Studie III visade att både rökning och snusanvändning under graviditet ökade risken för prematur födsel. Rökning ökade risken för extremt, mycket och måttligt för tidig födsel, medan snusanvändning i graviditet var associerad med ökad risk för extremt prematur födsel och måttligt prematur födsel. Hos varken rökare och snusare sågs någon ökad risk för prematur födsel vid rök- eller snusstopp i mycket tidig graviditet.

Studie IV visade att självrapporterad snusanvändning under graviditet är valid i Sverige. Dock påvisades en omfattande missklassificering av kvinnor i sen graviditet i Medicinska födelseregistret. Kvinnor med kotininnivåer som indikerade tobaksanvändning samt som rapporterat snusanvändning under graviditet i studiens frågeformulär var registrerade som icke-tobaksanvändare i MFR.

Sammanfattningsvis var både rökning och snusanvändning under graviditet associerade med en ökad risk för andningsuppehåll i nyföddhetsperioden, LKG-missbildning samt extremt för tidig födsel. Ett viktigt folkhälsobudskap är att vid rök- och snusstopp i tidig graviditet ses inga förhöjda risker. Samtliga studier i denna avhandling pekar på att snus liksom rökning är förenade med allvarliga skadeverkningar för fostret och den nyföddes hälsa. Cigaretter, snus och andra nikotinprodukter bör inte användas under graviditet.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following original articles and manuscripts. The papers will be referred to in the text by their Roman numerals (I-IV).

I. **Gunnerbeck A**, Wikström A-K, Edstedt Bonamy A-K, Wickström R, Cnattingius S
Relationship of maternal snuff use and cigarette smoking with neonatal apnea
Pediatrics 2011; Vol 128, 503-509

II. **Gunnerbeck A,** Edstedt Bonamy A-K, Wikström A-K, Granath F, Wickström R, Cnattingius S
Maternal snuff use and smoking and risk of oral cleft malformations
- a population based cohort study
Plos One 2014; Vol 9, 1-8

III. Dahlin S**, Gunnerbeck A,** Wikström A-K, Cnattingius S, Edstedt Bonamy A-K
Maternal tobacco use and extremely premature birth
-a population based cohort study
BJOG 2016; Vol 123, 1938-1946

IV. **Gunnerbeck A,** Raaschaou P, Cnattingius S, Edstedt Bonamy A-K, Wickström R
Snuff use during pregnancy and biomarkers of exposure
- a validation study
Submitted

LIST OF ABBREVIATIONS

BMI	body mass index
CI	confidence interval
CL/P	cleft lip with or without cleft palate
CP	isolated cleft palate
CO	carbon monoxide
COPD	chronic obstructive pulmonary disease
DNA	deoxyribonucleic acid
FSH	follicle stimulating hormone
HPG	hypothalamic-pituitary-gonadal
HPLC-MS/MS	high-performance liquid chromatography tandem mass spectrometry
ICD-10	international classification of diseases
IVF	in vitro fertilization
LOQ	lowest limit of quantification
MBR	the Medical Birth Register
nAChR	nicotine-acetylcholine receptor
NPV	negative predictive value
NRT	nicotine replacement therapy
OR	odds ratio
PAH	polycyclic aldehydes
PIN	personal identification number
PPV	positive predictive value
PPROM	preterm premature rupture of membranes
ROC	receiver operator characteristics
SAB	spontaneous abortion
SGA	small for gestational age
SHS	second hand smoking
SIDS	sudden infant death syndrome
ST	smokeless tobacco
TSNA	tobacco specific nitrosamines

CONTENTS

1 INTRODUCTION

Smoking is one of the most important avoidable risk factors in pregnancy worldwide. Maternal smoking is causally related to preterm birth and fetal growth restriction and associated with adverse pregnancy complications, like ectopic pregnancy, placenta abruption, stillbirth, preterm birth and intrauterine growth restriction (IUGR).[1] Smoking in pregnancy is also associated with detrimental effects on the health of the newborn, such as an increased risk of sudden infant death syndrome (SIDS). Furthermore, maternal smoking is associated with long-term effects such as asthma and respiratory disease in childhood and morbidity caused by prematurity and intrauterine growth restriction.[2, 3]

The prevalence of smoking during pregnancy ranges from 5-20% in the Unites States and the European countries.[2, 4, 5] In Sweden, the prevalence of smoking during pregnancy is only 5%.[6] In Sweden, smokeless tobacco in the form of moist oral snuff, or snus, is widely used, also among women. Snuff use is most common among young women in reproductive age and the prevalence of snuff use in pregnancy is 1.3%.[6, 7]

Cigarette smoke contains nicotine and numerous combustion products. Despite the common perception that nicotine is responsible for many of the adverse effects of smoking in pregnancy, there is an ongoing discussion of whether it is safe to use nicotine-replacement therapy, NRT, in pregnancy.[8-10] Randomized controlled trials on NRT have been difficult to interpret from because of lack of compliance.[11]

Swedish snuff contains nicotine but no combustion products.[12, 13] Thus, studying snuff use in pregnancy offers an opportunity to differentiate between effects caused by combustion products in cigarette smoke and those caused by nicotine in snuff.

At the end of the 20th century, snuff use among women aged 16-24 almost tripled.[14] International researchers and policy makers, including the tobacco companies, proclaimed Swedish Snuff, or snus, as a harm reduction product in the struggle against smoke-related morbidity and mortality in high income countries.[13, 15-17] However, there was very little knowledge about the effects of using snuff in pregnancy.

Even though it is probably better for the health of the pregnant woman to switch from cigarettes to snuff, it does not necessarily mean that it is also better for the health of the newborn. The main aim of this thesis was to investigate if nicotine, in the form of snuff, is safe to use in pregnancy.

Figure 1. Cigarette advertising

Source: From the collection of Stanford Research Into the Impact of Tobacco Advertising (tobacco.stanford.edu).

2 BACKGROUND

"I say, if you can´t send money, send tobacco."
US President George Washington´s request to help finance the American Civil War (1776)

The earliest cultivation of the tobacco plant is believed to have occurred in America in around 6000 B.C. Christopher Columbus discovered the plant in 1492 and brought it to Europe, and from Europe it spread to the European colonies in Africa and Asia. It was originally believed to have healing properties and was used in medicine.[5]

Throughout history, the mode of nicotine use has changed from mainly chewing tobacco or inhaling dry tobacco powder as snuff to cigar and cigarette smoking.

World War I (1914-1918) effectively spread the habit of smoking, and with the rise of the manufactured cigarette in the 20th century the use of cigarettes exploded. Tobacco companies marketed cigarettes for women, and smoking rates among female teenagers tripled in the years 1925-1935. During World War II (1939-1945), cigarette sales increased to all time high levels.[2]

In the 50´s and 60´s, smoking was associated with success and fame. Manufactured cigarettes were very common among both men and women in the Unites States (US) and Europe.[2, 18] At the same time the first major reports on hazardous effects of smoking were published. In 1950, Richard Doll stated that smoking was causally related to lung cancer,[19] and in1964 the Surgeon General's report on "Smoking and Health" showed hazardous effects of smoking on health.[2]

2.1 THE PREVALENCE OF SMOKING

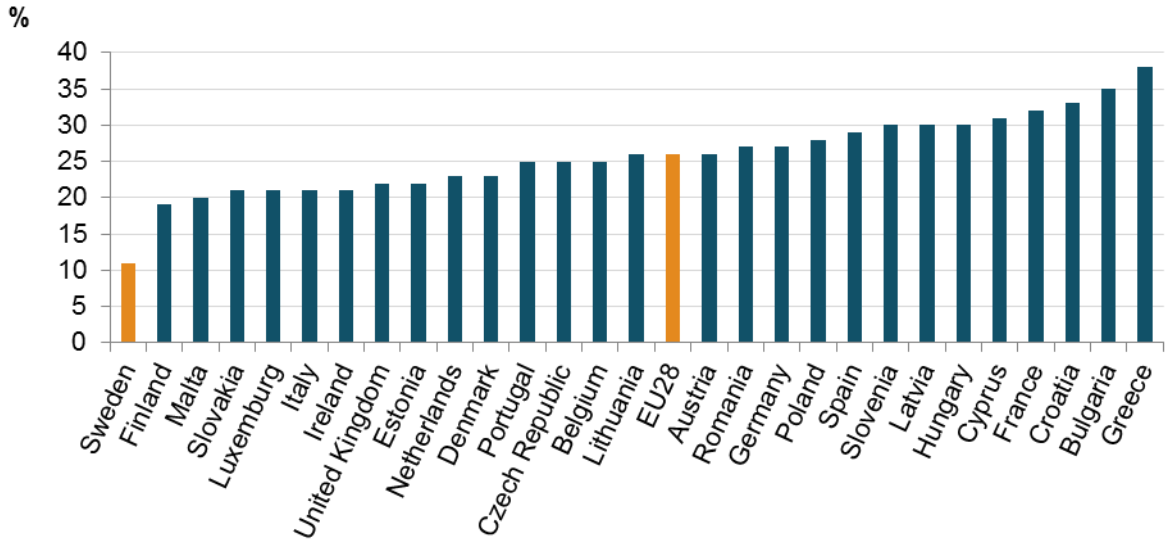
"…the current lower level of tobacco use among women in the world…does not reflect health awareness, but rather social traditions and women's low economic resources."
Dr. Gro Harlem Brundtland, Director General, WHO, 1998

Globally, approximately 967 million men and women smoke daily.[20] The top five high consumption countries of cigarettes in the world are China, Japan, the US, Russia and Indonesia.[5] China is the largest tobacco consuming nation in the world, and one third of all smoking men in the world live in China. According to the World Health Organization (WHO) Global Adult Survey data, 2010, the prevalence of smoking among men in China was 53%.[5, 20, 21]

In the last 30 years, the estimated prevalence of smoking in the world has decreased, but because of population growth the total number of smokers has increased.[20] There has been a shift in smoking prevalence from high-income countries, where smoking is declining, to low-income countries and developing countries where the smoking prevalence is continuously high. In some countries, such as Africa and Eastern Europe, the prevalence is actually rising.[20, 21]

In the US, the prevalence of cigarette smoking has declined from 42% in 1965 to 18% in 2012.[2] The decline of smoking is seen in both men and women and in all age-groups. In the US, there are today more former smokers than smokers and it is estimated that more than 50% of ever smokers have quit smoking.[2]

Figure 2. The prevalence of smoking in Europe, 201



Source 'Special Eurobarometer 429', European Commission, 2015

The average smoking prevalence in Europe is approximately 26%, with great diversity between countries.[4] Smoking is more common in southern and eastern Europe with the highest prevalence in Greece (40%), Bulgaria (36%) and Latvia (36%), in comparison to Sweden, where the smoking prevalence is around 10%.[4, 7] In the United Kingdom (UK), Sweden and many other European countries, smoking has decreased considerably among both men and women since the 1970's and 1980's.

2.2 DEFINITION AND PREVALENCE OF SMOKELESS TOBACCO

Smokeless tobacco (ST) is an umbrella term for a wide range of tobacco products that are not smoked. When used orally, ST is often used in the form of oral moist snuff, such as Swedish snus, and as chewing tobacco. It can also be sucked, applied to the gums as a paste, dissolved in the mouth, gargled or inserted in betel quid. ST can also be inhaled as a powder, called dry snuff.[22]

Over 300 million people around the world use smokeless tobacco. The vast majority, 89%, live in South Asia, especially India and Bangladesh, where the variety of products is large. In these countries, the most commonly used ST products are betel quid with tobacco, khanini and products applied to teeth and gum, like gul and mishri. The prevalence of ST use in India and Bangladesh is 25.9 % and 27.2%, respectively.[23]

In Europe and North America, the most common ST products are chewing tobacco and oral moist snuff. In the US, 7.1% of males use oral moist snuff. [23] In Scandinavia, and in Sweden in particular, oral moist snuff, or Swedish snus, is most widely used, with a prevalence of 20% among males and 3-4% among females.[7]

The diversity of ST products, combined with differences in characteristics between ST users in different regions of the world, makes health implications of ST use difficult to generalize. In this thesis, snuff use will be defined as the use of Swedish snus. Other forms of ST will not be discussed further.

Figure 3. Smokeless tobacco products

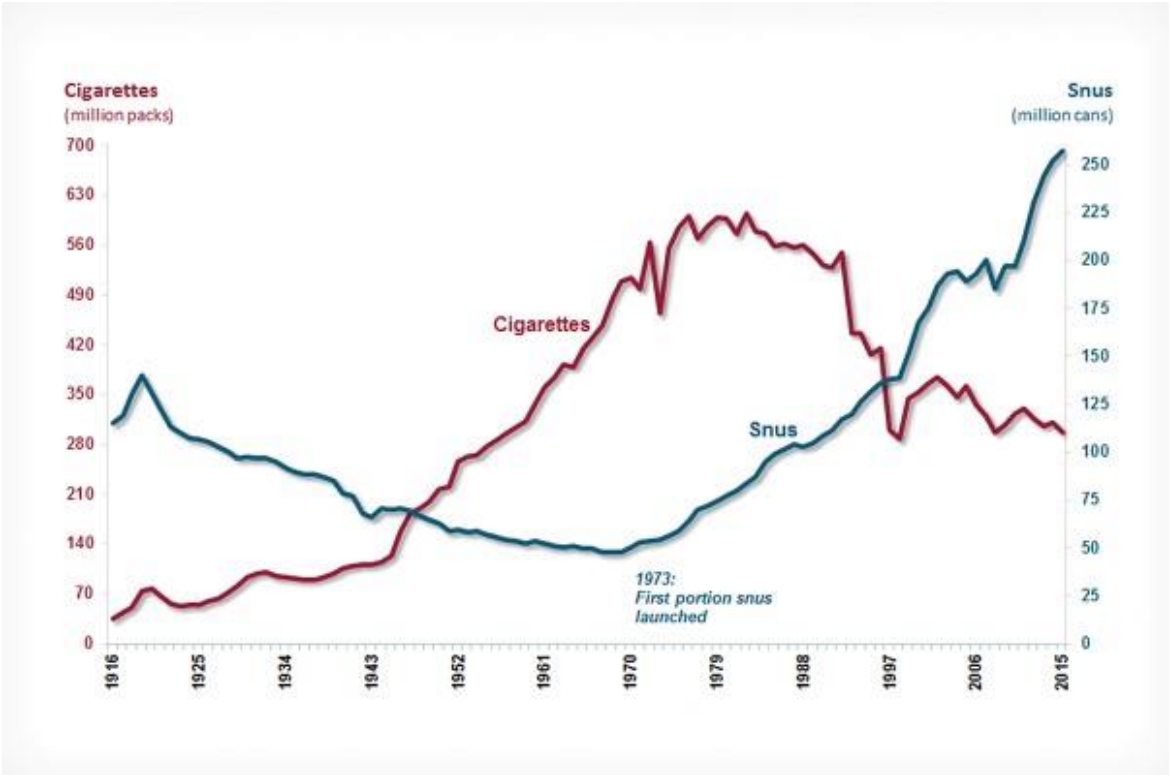
Source: Smokeless tobacco and public health: A global perspective, National Cancer Institute Centers for Disease Control and Prevention U.S. Department of Health and Human Services

2.3 SMOKING AND SNUFF USE IN SWEDEN

"EG? Inte utan min prilla!"
"The EU? Not without my pouch of snuff." (Bumper sticker attributed to Anders Andersö, "Tecknar-Anders", in the campaign for Swedish snuff 1994)

At the beginning of the19th century, snuff, in the form of oral moist snuff, became increasingly common and was used mainly by working class men in Sweden. In 1915, AB Svenska Tobaksmonopolet, today known as Swedish Match, was founded, and after World War I the use of snuff use peaked in Sweden with a consumption of 1.2 kg/per capita.[18] After World War II, though, cigarette smoking grew popular and was associated with fame and high social class. As a consequence, snuff use in Sweden declined substantially. In the 1960's, with 50% of all Swedish men being smokers, it was even discussed to stop the production of snuff altogether in Sweden. However, with increasing knowledge of the hazardous effects of smoking, the number of smokers has declined since the 1970's, and as a consequence the use of snuff, which is regarded as less harmful, has increased. In 1973, portions of snuff were launched as a popular alternative to loose snuff.[18, 24]

Figure 4. Tobacco consumption, cigarettes and snuff from 1916-2015.



Source: Published with permission from Swedish Match

Smoking has continued to decline in both sexes, but Sweden is one of the few countries in the world where more women than men smoke. The prevalence of smoking among women was 11% and among men 9% in 2016.[7] In contrast, most snuff users are men. Approximately, 20% of Swedish men and 3-4% of Swedish women use snuff. It is primarily young women in fertile age, aged 16-29 , who use snuff daily (4%) and sporadically (3%).[7]

Important dates for the tobacco policy in Sweden

Tobacco Act 2016 (SFS 2016:353)

- EU countries are required to have spacious health warnings with text and images on cigarette packs, on more than 65% of the size of the package.
- The text on tobacco products is not allowed to propose that the product is a healthier product in comparison to other tobacco products.
- No information about proportions of nicotine, tar and carbon monoxide is allowed.
- Information on taste or smell additives is not allowed on tobacco products, with the exception of snus.

EU membership 1995

- At the time of Sweden's entry into the EU 1995, tobacco products for oral use, except smoking or chewing tobacco, were been banned. As Swedish snus is neither smoked nor chewed it is prohibited for sale in EU. However, Sweden was granted a permanent exemption from the sales ban on snus.

Tobacco Act 1993 (1993:581)

- Ban of commercial advertising of tobacco products.
- Ban of smoking in public places, with the exception of outdoor seating at cafés and restaurants.

Source: Photo by Henrik Dahl, 2017

2.4 CHARACTERISTICS OF TOBACCO USERS

"It is important to know as much as possible about teenage smoking patterns and attitudes. Today´s teenager is tomorrow´s potential regular customer, and the overwhelming majority of smokers first begin to smoke while still in their teens. The smoking patterns of teenagers are particularly important to Philip Morris." Philip Morris Companies Inc. 1981

Tobacco use is correlated with social inequalities. Globally, high-income countries have lower prevalence of smoking than developing countries.[20] Both smoking and use of ST is more common in rural than in urban areas. Use of tobacco is correlated with lower education, unemployment, poverty, consumption of alcohol and substance use, as well as with psychiatric illness.[2, 4, 23, 25] More than 80% of smokers begin before 18 years of age, and smokers who started smoking early are less likely to quit.[1]

Worldwide, more men than women use tobacco. However, the gender differences in tobacco use vary between countries and cultural contexts. In many of the countries with the highest prevalence of smoking, like China, Eastern Europe, Russia and Asia, men smoke 10 times more than women.[5] In contrast, in high income industrialized countries, the gap between women and men is declining. According to the WHO report on the global tobacco epidemic 2015, 19% of females in Europe smoked tobacco, in comparison to 2-3% in Africa, Southeast Asia and the Eastern Mediterranean and Western Pacific Regions.[21] Sweden and Norway are exceptions with higher smoking prevalence among women than men.[7]

Also the use of smokeless tobacco shows gender differences. The majority of ST users worldwide are men, but in some countries, as for example India (18.4%), Bangladesh (26.4%), and South Africa (10.9), the prevalence among women is very high.[23]

2.5 SUBSTITUENTS OF TOBACCO

Smoked tobacco contains more than 4000 different chemicals, including nicotine, carbon monoxide, CO, tobacco specific nitrosamines (TSNA), polycyclic aldehydes (PAH), nitrate and aromatic amines. It also contains heavy metals like cadmium, arsenic, lead, nickel, chrome, mercury and polonium.[3] The concentration of heavy metals in tobacco is much dependent on the concentration in the soil where tobacco is grown.[23] The levels of TSNA are especially frequent in fire-cured tobacco, but also dependent on the level of nitrate producing bacteria. PAH and formaldehydes are carcinogenic and formed when tobacco is burned and are always present in tobacco smoke.[3]

Figure 5. Nicotiana tabacum

In addition to nicotine, smokeless tobacco also contains numerous toxic substances found in tobacco smoke, including TSNA and PAH. The levels of these substances differ among products, depending on which type of nicotine plant that is used, the soil where it grows, the production and storage process and the presence of nitrate producing bacteria. In addition, the tobacco is sometimes flavored or used together with other products which also affect the levels of nicotine content and carcinogens.[23]

Swedish snuff, or snus, contains powered tobacco, water, sodium carbonate, sodium chloride, moisturizer and flavoring.[13] Snus differs from other forms of smokeless tobacco, containing considerably lower levels of toxic substances than smoked tobacco and any other ST product.

Since 2000, Swedish Match has its own quality standard, Gothiatek,[26] which controls every step of the production process, including packaging and storage of snuff.[12] The tobacco is air-dried and pasteurized, which decreases the level of TSNA. Gothiatek controls that levels of nitrate, TSNA, NMDA (volatile nitrosamine), PAH and heavy metals (cadmium, lead, arsenic, nickel and chromium) in Swedish snuff are below WHO recommendations for smokeless tobacco.[27] Swedish snuff is also regulated by the Swedish National Food Agency, and production and additive standards are therefore the same as for those for food products.[12, 28, 29] American oral moist snuff is different from Swedish snuff regarding toxicity and level of harmful substances. Because of different production processes, the levels of TSNA, PAH, formaldehydes and metals are considerably higher in American snuff. Mean TSNA in Swedish snuff is 1.1ug/g[29], in comparison to that of American moist snuff with TSNA levels ranging from 4.87ug/g to 90.02ug/g tobacco.[30]

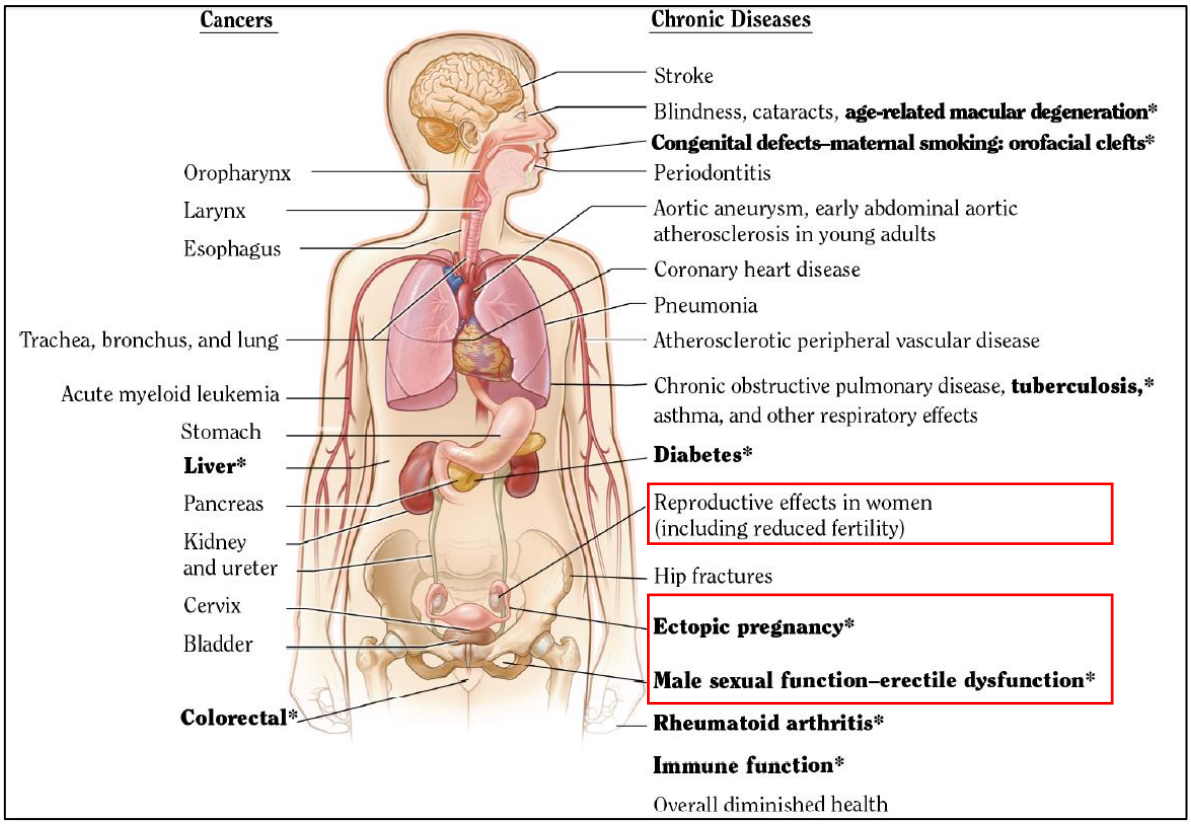
2.6 HEALTH EFFECTS OF SMOKING

"Smoking is a custom loathsome to the eye, hateful to the nose, harmful to the brain, dangerous to the lungs, and in the black, stinking fume thereof nearest resembling the horrible Stygian smoke of the pit that is bottomless." James I of England, A counterblast to tobacco, 1604

Smoking is predicted to account for 1 billion deaths in the 21th century. [31] Europe and North and South America have the highest proportion of smoke related mortality (25 and 17%, respectively) because of their long history of smoking.[31]

There is a causal relationship between smoking and diminished overall health as well as an increased risk of all-cause mortality in men and women.[2] Tobacco smoke has adverse effects on almost all organs. Smoking is a risk factor for non-communicable diseases like cancer, respiratory problems, cardiovascular disease, periodontitis, cataract and reproductive problems. Smoking also increases the risk of attracting communicable diseases like pneumonia and tuberculosis.[2]

Figure 6. Health effects related to smoking



Source: Adapted from USDHHS 2004, 2006, 2012, 2014
Note: Each condition presented in bold text and followed by an asterisk (*) is a new disease that has been causally linked to smoking in the USDHHS 2014 report.

Approximately 70% of all lung cancer is attributable to tobacco smoking. Tobacco smoke is also associated with oropharyngeal cancer, hepatocellular cancer, cancer in esophagus, pancreas, kidney, bladder, colorectal cancer and cervix cancer. Furthermore, the results of cancer treatment is poorer in smokers with cancer.[2]

Both active smoking and exposure to second-hand smoking increase the risk of adverse cardiovascular effects like atherosclerosis, acute myocardial infarct (MI), stroke, aortic aneurysm and sudden cardiovascular death.[2]

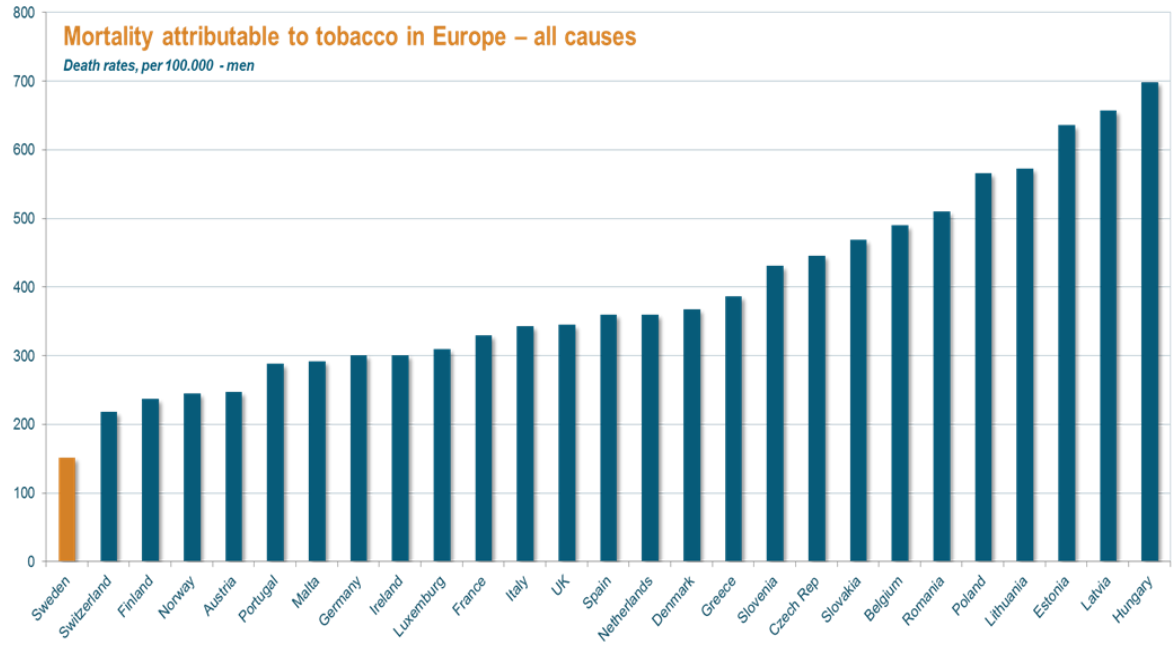
Combustion products in tobacco smoke increase the risk of developing chronic obstructive pulmonary disease, COPD, and more than 42% of all COPD is attributable to smoking. There is also an increasing risk of pneumonia and exacerbation of bronchitis in smokers with COPD. In addition, smoking is also associated with poor asthma control and exacerbation of asthma.[2]

2.7 HEALTH EFFECTS OF SWEDISH SNUFF

"The Swedish Experience." Swedish Match

Sweden has the lowest smoke–related mortality and morbidity in Europe, although the total level of tobacco consumption is comparable to the average consumption in Europe.[4] This is explained by the extensive use of snuff among men in Sweden.

Figure 7. The proportion of mortality attributable to tobacco in European males



Source: Swedish match. Adapted from WHO Mortality Attributable to tobacco, 2012

Internationally, "the Swedish Experience" is debated by researchers, politicians and public health experts, discussing if snuff is to be looked upon as a harm reduction product[32] in the struggle against the smoking related health problems encountered worldwide or as yet another tobacco product causing addiction[32, 33] and contributing to poorer health.[13, 16, 34, 35]

A few studies have found associations between snuff use and increased risk of pancreatic [36, 37] and esophageal cancer,[38] but the risk is significantly lower than with smoking.[39] Compared with non-tobacco users, snuff users generally have a slightly poorer cancer outcome, but if that is attributable to Swedish snuff or to confounding factors is not clear.[40]

Oral mucosal lesions from snuff use have not been associated with cancer.[41] In a review, Lee et al. argue that the evidence for snuff and risk of cancer is weak.[41]

There is little evidence that snuff use is associated with increased risk of acute myocardial infarct or stroke.[41] No association has been found between snuff use and hypertension.[41] Likewise, safety studies of nicotine replacement therapy have not been associated with increased cardiovascular risk.[3] However, there is growing evidence that snuff use, like smoking, is associated with increased insulin resistance and with increased risk of type 2-diabetes, and that the risk increases with increasing snuff use.[42, 43]

2.8 TOBACCO USE AND REPRODUCTIVE HEALTH

Smoking is associated with a poorer reproductive health in both men and women. The Practice Committee of the American Society has stated that 13% of infertility in the US may be attributable to smoking.[44] Studies of snuff use and fertility are lacking.

Smoking is associated with erectile dysfunction,[2] decreased production of testosterone in the Leydig cells[2] and an impaired spermatogenesis with DNA damage.[3] In females, smoking is associated with a shortened menstrual cycle, an earlier menopause and subfertility.[2, 3, 44] Smoking is also associated with hormonal disturbances, such as elevated levels of follicle stimulating hormone (FSH) and decreased progesterone levels.[2, 3]

Prenatal exposure to smoke has been associated with impaired fertility in adulthood in both men and women.[3, 45]

There is evidence in animal studies that nicotine is involved in the mechanisms behind reproductive dysfunction.[46] Nicotine seems to affect the hypothalamic-pituitary-gonadal (HPG) axis, controlling the hormonal balance and the sexual development and function.[3]

2.9 TOBACCO USE AND NICOTINE EXPOSURE IN PREGNANCY

”Smoking behavoiur of women differs from that of men….more highly motivated to smoke….they find it harder to stop smoking…. women are more neurotic than men… there may be a case for launching a female oriented cigarette with relatively high deliveries of nicotine…” 1976 research report, British American Tobacco

Maternal smoking is still one of the most important preventable risk factors for maternal and neonatal health globally. It is associated with adverse fetal and neonatal outcomes with increased risk of neonatal morbidity and mortality but also with long-term effects of the health of the child.[1]

The common substance in tobacco smoke and snuff is nicotine, and effects on the health caused by nicotine should theoretically be similar between smokers and snuff users. However, in the debate about Swedish snuff as a means of smoking cessation and a possibility to reduce the smoke related morbidity and mortality, the adverse effects of nicotine and use of snuff in pregnancy are rarely discussed.

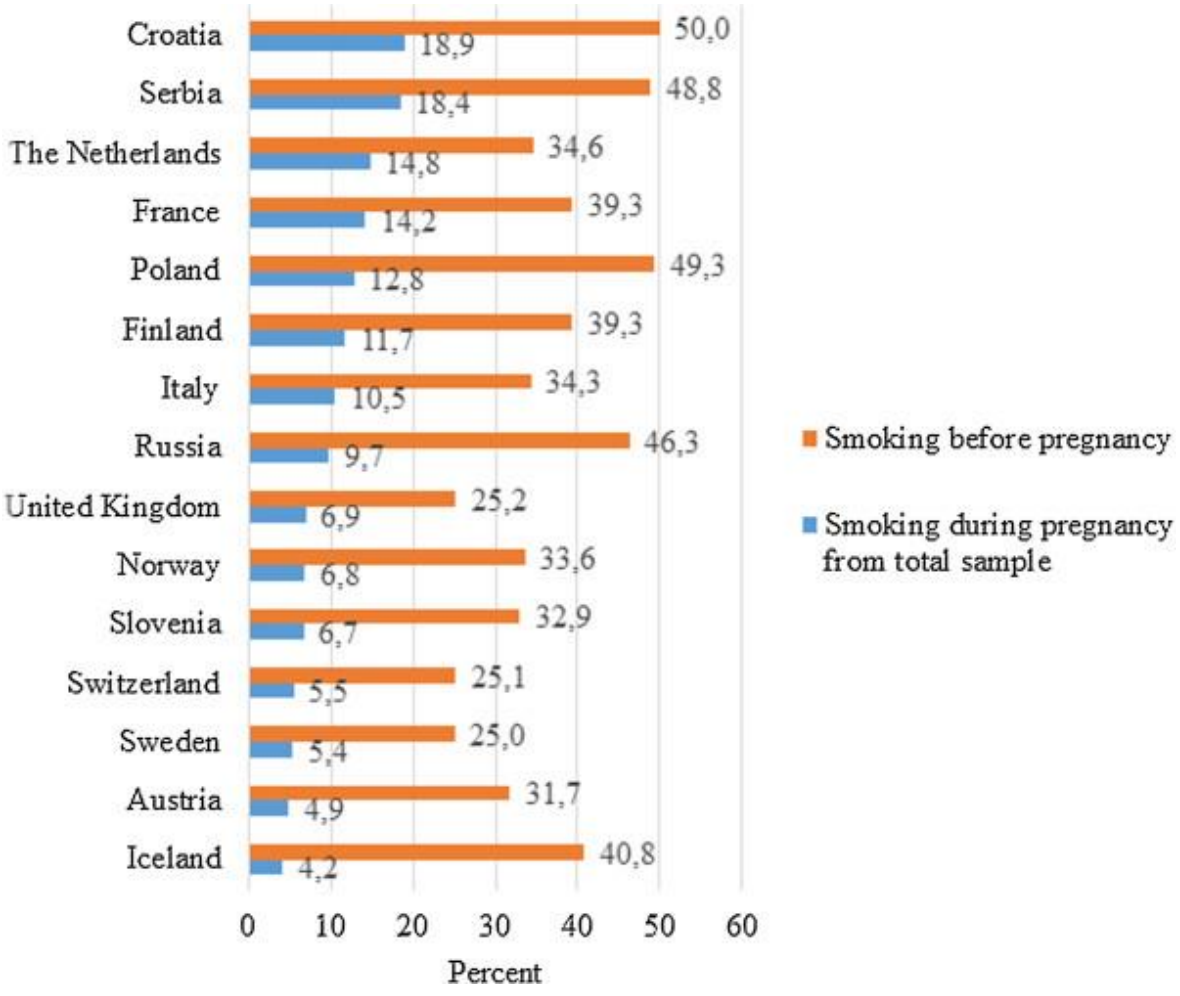
Prevalence of smoking and snuff use in pregnancy

Comparing data on the prevalence of maternal smoking on a global level is not unproblematic, because of the differences in maternal care systems, lack of national statistics in many countries, poor coverage of data and different periods in pregnancy when data is collected. Most data on tobacco use in pregnancy is based on self-reported information. Because of the social stigma related to smoking in pregnancy, there is a risk of underreporting of use, leading to misclassification of tobacco use.[47, 48]

The prevalence of maternal smoking in the US is approximately 15%, with great variation between states.[49] In the US, smoking in pregnancy is most frequent among American Indian/ Alaska Natives (26%), followed by non-Hispanics Whites.[49]

In the European countries, maternal smoking in early pregnancy varies from 5% to 20%.[50, 51] As many as 17% smoke in late pregnancy in France and 12% in the UK and Germany.[51, 52] In Eastern Europe, e.g. Poland, Greece and Croatia, the smoking prevalence in pregnancy ranges from 12% to 19%.[51]

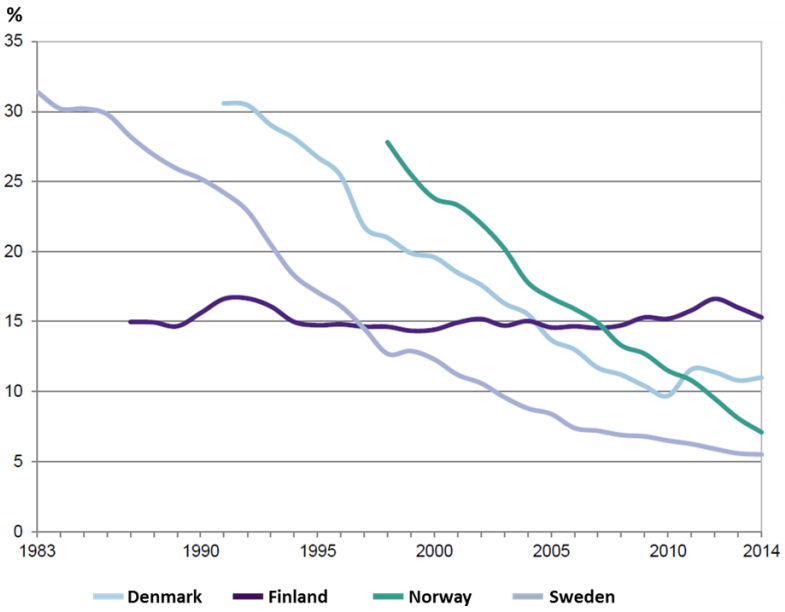
Figure 8. The prevalence of smoking during pregnancy in European countries



Source: Smedberg et al., 2014. Published from BMC Pregnancy Childbirth.

The prevalence of smoking before pregnancy in Sweden is 13.6%. However, the prevalence of smoking in early pregnancy has decreased from almost 30% in the mid-1980's to 5.5% in 2014.[6] The same is true for most Nordic countries except Finland, where the prevalence of maternal smoking in early pregnancy is still around 15%.[53]

Figure 9. The prevalence of maternal smoking in early pregnancy in the Nordic countries, 1983–2014



Source: Adapted from Perinatal statistics in the Nordic countries, THL, 2014

In Sweden, 4.6% use snuff three months before pregnancy, and 1.3% of women use snuff in early pregnancy. In late pregnancy, according to the Swedish Medical Birth Register, only 4% smoke and 0.7% use snuff.[6]

There are also large regional differences in tobacco habits within Sweden. In the northern counties, like Jämtland and Västernorrland, the proportions of smokers and snuff users in early pregnancy are similar. In contrast, in the southern part of Sweden, smoking in early pregnancy is 8-9%, whereas the prevalence of snuff use is less than 1%. The county of Stockholm has the lowest tobacco consumption in the country with only 3.3% smokers and 1.1% snuff users in early pregnancy.[6]

Maternal characteristics of tobacco use

Maternal smoking is associated with low socioeconomic status, low education level, not living with the father-to-be, having large number of children and inadequate prenatal care.[6, 49, 51, 54] A smoking partner increases the risk of continued smoking in pregnancy, as does a high level of pre-pregnancy smoking.[55]

According to the National Board of Healthcare in Sweden, as many as 16% of women with an education of 9 years or less smoked in early pregnancy, in comparison to 1.3% among women with more than 12 years of education. Approximately 20% of women below 20 years of age smoked in early pregnancy.[6]

Tobacco use and effects on pregnancy and the health of the newborn

Maternal smoking is associated with pregnancy complications like increased risk of spontaneous abortion,[56, 57] ectopic pregnancies,[3, 45] placenta dysfunction, placenta previa and placenta abruption.[1, 58, 59] Maternal smoking is also associated with increased risk of spontaneous and induced preterm birth, fetal growth restriction, stillbirth and oral cleft malformations.[2]

There is an increased risk of neonatal and infant mortality and morbidity in infants of smokers.[1, 2] Maternal smoking is also associated with increased risk of sudden infant death syndrome (SIDS).[1, 2]

Furthermore, prenatal exposure to smoke has also been associated with long term effects, such as respiratory problems and otitis in childhood.[2] Many studies have also shown associations between maternal smoking and behavioral problems in the offspring,[2, 3] but because of residual confounding it is difficult to infer causality.

In contrast to maternal smoking, snuff use during pregnancy has not been shown to be associated with placental dysfunction, such as placenta previa or placental abruption. [60] However, maternal snuff use has been associated with preterm birth[61, 62] and stillbirth.[60, 63] Baba et al. have also shown an association between snuff use in pregnancy and increased risk of being born small for gestational age (SGA).[64]

Spontaneous abortion and ectopic pregnancies

Spontaneous abortion (SAB) occurs in approximately 12% of all pregnancies and is defined as an involuntary termination of pregnancy before 20 weeks of gestation. Most SAB occur before the 12th week of gestation.[3] Maternal smoking and exposure to second-hand smoke is associated with increased risk of SAB.[56, 57] The mechanisms behind the effects of smoking are not clear, but placental insufficiency and fetal hypoxia from CO exposure and toxic effects of cadmium and cyanide in tobacco smoke have been suggested.[2, 44]

In ectopic pregnancy a fertilized egg is implanted outside the uterus, usually in the fallopian tube. It occurs in 1-2% of pregnancies.[65] Ectopic pregnancy is a risk factor for maternal mortality, subsequent infertility and/or recurrent ectopic pregnancies.[65]

The etiology of ectopic pregnancies is not fully understood but seems to involve motility of the fallopian tubes. The embryo remains within the fallopian tube because of structural or functional impairment of the tube, preventing implantation to occur.[2, 65] Suggested risk factors are history of previous ectopic pregnancy or SAB, sexually transmitted disease or surgical procedures affecting the fallopian tubes.[2, 65, 66]

The Surgeon General Report, 2014 concludes a causal relationship between smoking and ectopic pregnancy. There is evidence from animal studies that nicotine is playing a role in the mechanism behind ectopic pregnancy.[2, 66] There is a lack of knowledge about the association between snuff use and SAB or ectopic pregnancy.

Preeclampsia

Preeclampsia is a syndrome of reduced organ perfusion caused by vasospasm and endothelial dysfunction of the placenta, clinically defined as marked hypertonia and proteinuria, with an onset after 20 weeks of gestation.[2] Preeclampsia is a major cause of maternal and fetal mortality and morbidity globally and is associated with fetal growth restriction, stillbirth and placental abruption. It affects 3-7% of pregnant women.[67]

A two-stage model in the development of the disease is often discussed, where the first stage is characterized by defect implantation and formation of the placenta, leading to a hypoxic and dysfunctional placenta, inducing inflammation and endothelial damage. This results in the symptoms seen in the second stage with elevated blood pressure and proteinuria.[67]

Maternal smoking has a substantially protective effect and decreases the risk of developing preeclampsia.[68] However, if preeclampsia develops in smokers, the rates of adverse outcomes are increased substantially, which may occur as a result of synergistic effects of smoking and preeclampsia on the feto-placental circulation.[69] Snuff use does not protect against preeclampsia,[70, 71] implying that combustion products, such as CO with vasodilative properties, and not nicotine, are involved in the underlying protective mechanism. CO also reduces levels of anti-angiogenic protein soluble fms-like tyrosine kinas (sFlt-1), which in animal models initiate all symptoms of preeclampsia. Smokers have lower levels of sFlt-1 than nonsmokers.[2]

Placenta previa and placental abruption

Maternal smoking is associated with placenta dysfunction and antenatal bleeding, such as placenta previa and placental abruption, and the relationship is dose-dependent.[3, 58] The suggested mechanisms of placenta previa are related to chronic ischemia and hypoxia and consequent enlargement of placenta.[3]

The origin of placental abruption is multifactorial but related to placental dysfunction.[72] Potential risk factors are advanced maternal age, multiparity, previous placental abruption, hypertensive disorders, thrombophilia, previous cesarean section, trauma and uterine infections. Suggested underlying causes of abruption are vessel fragility and vascular malformation and impaired trophoblastic transformation.[3] Smoking has been proved to interfere with transformation of the spiral arteries and the thickening of the villous membrane in the placenta.[3]

Smoking-related placental abruption has been observed to be related to decidual necrosis at the periphery of the placenta, microinfarcts, atheromatous/fibrinoid changes in the placenta as well as with hypovascular and atrophic villi.[69, 73] Hypoxic changes, due to reductions in blood flow to the uteroplacental-fetal unit associated with cigarette smoking, may lead to placental infarcts and increased capillary fragility, in turn leading to arterial rupture and, subsequently, to placental abruption.[72, 73]

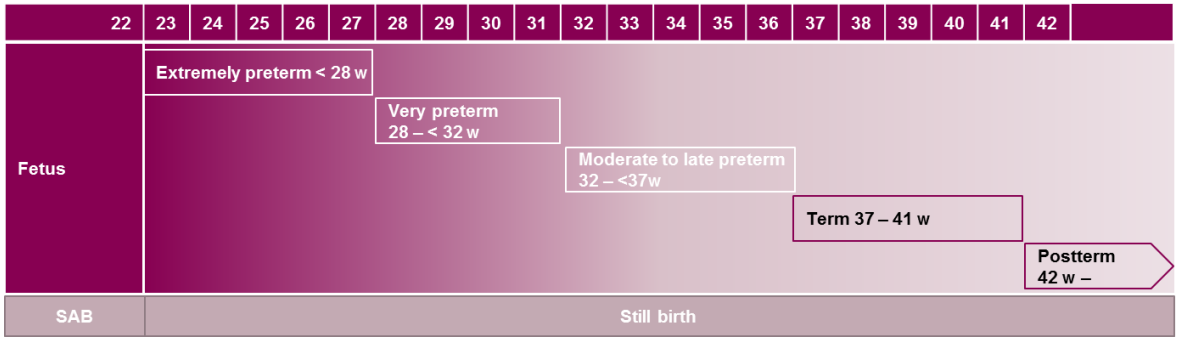
Smoking is supposed to impair collagen synthesis in placenta and induce inflammation in placental vessels.[1, 73] Studies of consecutive pregnancies show remaining but decreased risk when women stop smoking between pregnancies.[59]

Preterm birth

Preterm birth is defined as live birth before 37 weeks of gestation. Extremely preterm birth is defined as birth <28 weeks of gestation, very preterm birth 28 to <32 gestational week and moderately preterm birth 32 to <37 weeks of gestation. The highest morbidity and mortality is among the most preterm infants, born before 28 weeks of gestation.

More than 15 million babies are born preterm every year, and the incidence is increasing in most countries.[74] Preterm birth is the leading cause of perinatal morbidity and mortality in the developed countries, with an incidence of 11.1% globally.[75, 76] In the US, 12% of all babies are born preterm.[76] In Europe, preterm birth rates vary between 5-10%.[50] In Sweden, approximately 6% of all live born births are preterm.[6] Of live born singleton births in Sweden, 4.4% are born before 37 weeks; 0.2% are born extremely preterm, 0.6 % very preterm and 3.6% moderately preterm.[6]

Figure 10. Definition of preterm birth according to WHO



Preterm birth can be divided in spontaneous preterm birth (including spontaneous labor with intact membranes and spontaneous preterm premature rupture of membranes, PPROM) and induced preterm birth, i.e. induced labor or cesarean delivery for fetal or maternal indications.[77]

The cause of spontaneous preterm birth is multifactorial. Maternal history of preterm birth is a strong risk factor and suggested to be generated by the interaction of transgenic and environmental risk factors.[76] The role of ethnicity in preterm birth has been discussed and a variation in gestational length for different ethnic groups has been suggested; babies of African ancestry tend to be born earlier than babies of Caucasian ancestry.[76]

Previous preterm birth, multiple pregnancies, IVF pregnancies, intrauterine infection or inflammation and chronic diseases, such as hypertension, diabetes and thyroid disease are all associated with preterm birth.[76, 78] Preeclampsia, placental abruption, fetal growth restriction, maternal chronic disease and obesity are associated with medically indicated preterm birth.[77]

Maternal smoking has been casually related to preterm birth[1, 3, 45] and has been associated with both spontaneous and induced preterm birth.[79] The pathophysiological mechanisms are not clear, but smoking is associated with placental dysfunction, placenta previa and placental abruption as well as with fetal growth restriction, all associated with preterm birth.[2, 3]

Snuff use is also associated with preterm birth.[61, 62] However, snuff use is not associated with placental bleedings, placenta previa or placental abruption and the increased risk of impaired growth is moderate,[64] implying a different causal pathway for snuff and preterm birth than for that of tobacco smoke. The common substance in snuff and smoking is nicotine. In addition, tobacco smoke also contains numerous combustion products which may affect the risk of preterm birth, possibly through placenta dysfunction and subsequent fetal growth restriction.

Nicotine has vasoconstrictive properties, reducing the utero-placental blood flow[80] and may also affect the formation of the placenta in early pregnancy. Snuff use is a risk factor for early, but not late clinical stages of preeclampsia, suggesting that nicotine affects the formation of placenta,[71] which may play a role in the mechanisms behind preterm birth.

In addition, both combustion products of smoke and nicotine are supposed to affect the immune system,[81] and inflammation is a plausible explanation behind the increased risk of preterm birth. Inflammation and/or infection are known risk factors of preterm birth.[78, 82]

Fetal growth restriction

Both maternal smoking and maternal exposure to secondhand smoke are regarded causally related to fetal growth restriction.[2] A consistent finding is that infants of smoking mothers weigh 150-200 g less than infants of non-tobacco users.[3] Maternal smoking is also associated with SGA births, and the association is dose-dependent and correlated with maternal cotinine levels.[2, 3] How smoking affects fetal growth is not clear, but placenta dysfunction is believed to play a role. Maternal smoking is related to disturbances in the transformation of the spiral arteries and the thickening of the villous membrane forming the placenta, leading to placental dysfunction.[3] In addition, CO induces fetal hypoxia by binding to hemoglobin and inhibiting oxygen release. Nicotine is vasoconstrictive and has been supposed to decrease utero-placental blood flow with subsequent reduction in the delivery of oxygen and nutrients to the fetus.[80]

Animal studies of prenatal nicotine-exposure and effects on birth weight show conflicting results, but the majority show that nicotine impairs fetal and neonatal growth.[83] If nicotine has a major impact on fetal growth, smokeless tobacco and Swedish snuff should also be associated with low birth weight and SGA births, but most studies on snuff use or smokeless tobacco in pregnancy have shown very modest or no association with low birth weight.[70, 84, 85] However, Baba et al. found an association between snuff use and slightly increased risk of SGA birth, 1.38 OR (95% CI: 1.01-1.88), in comparison with that of nonusers.[64] The risk associated with smoking was higher, 3.21 OR (95% CI: 3.02-3.40).[64]

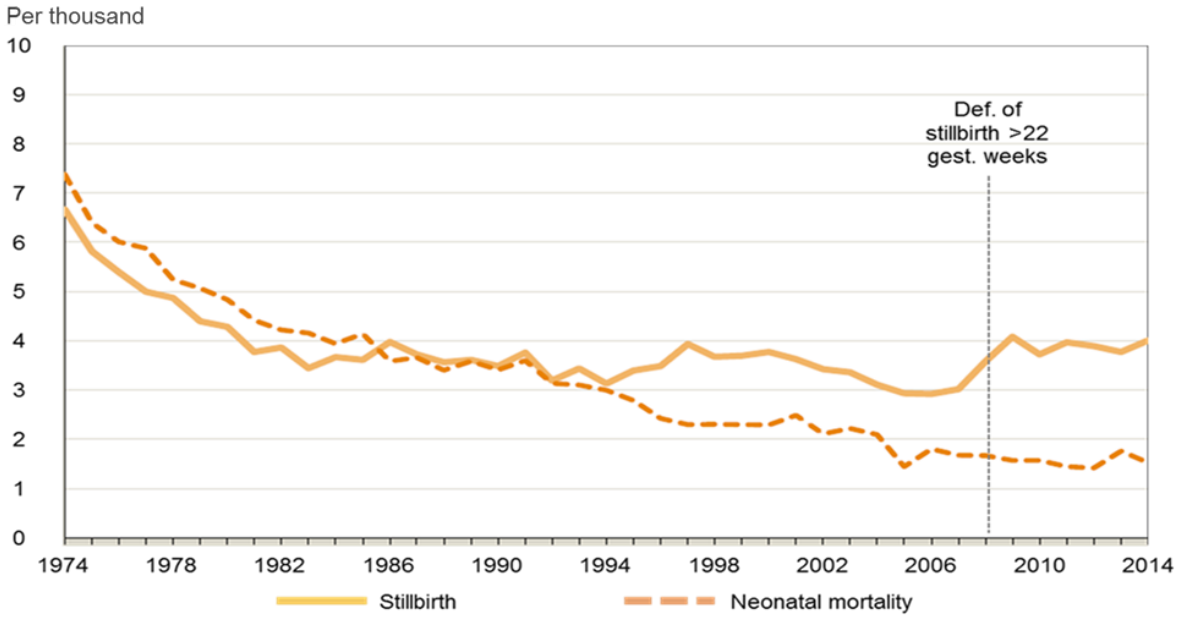
In addition, for women who had stopped using snuff before the antenatal booking, there was no increased risk of SGA birth, whereas women who stopped smoking before the antenatal booking had a lower, albeit increased risk of SGA birth compared to that of nonusers, OR 1.82(1.65-2.01).[64] Gupta et al. have shown decreased birth weight with use of mishri in pregnancy.[86] However, mishri and many other ST products contain numerous potentially toxic substances associated with low birth weight, such as PAH.[3, 23] The socio-demographic and cultural differences also make comparisons difficult to interpret.

To conclude, in humans the effect of nicotine on fetal growth seems to be modest in comparison to the effect of CO and other combustion products in smoke. Nicotine and CO and other combustion products may have synergistic effects on growth, explaining why smoking has a greater impact on growth than snuff use.

Stillbirth and neonatal and infant mortality

The estimated global stillbirth rate was 18.4/1000 live-born infants in 2015 (fetal age ≥28 weeks gestational weeks).[87] In Sweden, the incidence of stillbirth (fetal age ≥22 gestational weeks) is 4/1000 live born infants and the neonatal mortality is 1.5/ 1000 live-born babies.[6] As a consequence of a changed definition of stillbirth from fetal age ≥28 weeks to fetal age ≥22 weeks, the incidence of stillbirth in Sweden increased in 2008.

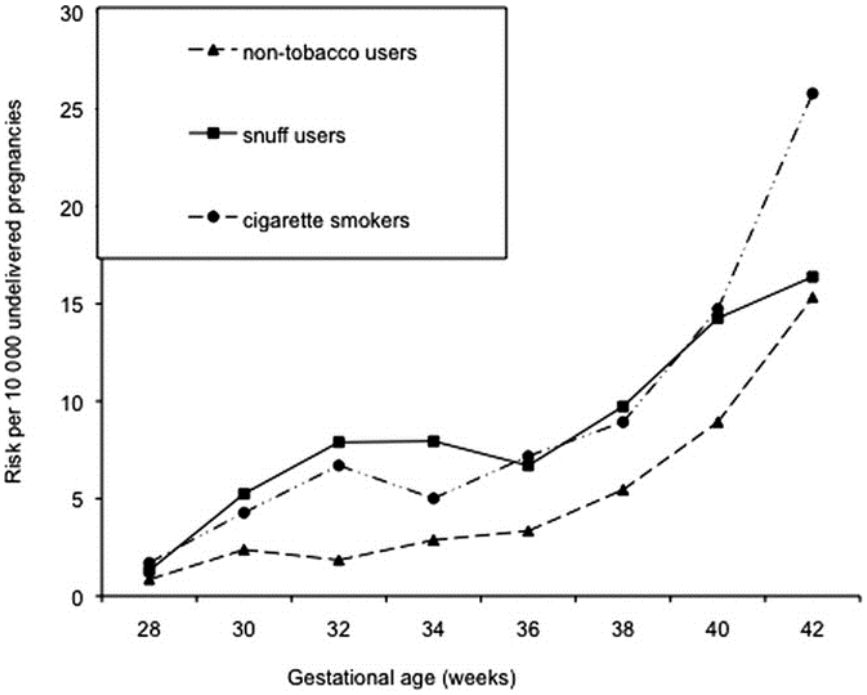
Figure 11. Incidence of stillbirth and neonatal mortality, 1974 -2014



Source: Adapted from National Board of Health: Pregnancies and Deliveries, 2015

The majority of stillbirths are preterm, but the risk of stillbirth increases with increasing gestational age. Risk factors for stillbirth are chromosomal and fetal abnormalities, high maternal age, obesity, intrauterine growth restriction, preeclampsia and placental bleedings.[88, 89] Both maternal smoking and use of snuff in pregnancy are associated with an increased risk of stillbirth.[63]

Figure 12. Risk of stillbirth per 10 000 pregnancies by gestational week



Source: Wikström et al. Epidemiology, 2010. Published with kind permission from Elsevier.

Maternal smoking is primarily associated with preterm stillbirth. Several studies have shown that the risk of stillbirth is mediated through increased risk of placental bleeding and intrauterine growth restriction.[88] Wikström et al. have shown that when women with preeclampsia, placental bleeding and/or small for gestational age deliveries were excluded from analysis, the smoking-related risk of stillbirth was attenuated, whereas it remained among snuff users.[60] Contrary to maternal smoking, snuff use in pregnancy is not associated with placental bleeding, and only a modest association between snuff use in pregnancy and fetal growth restriction has been found.[64] Importantly, women who stopped smoking or using snuff in early pregnancy were not at increased risk of stillbirth compared to that of non-tobacco users.[63]

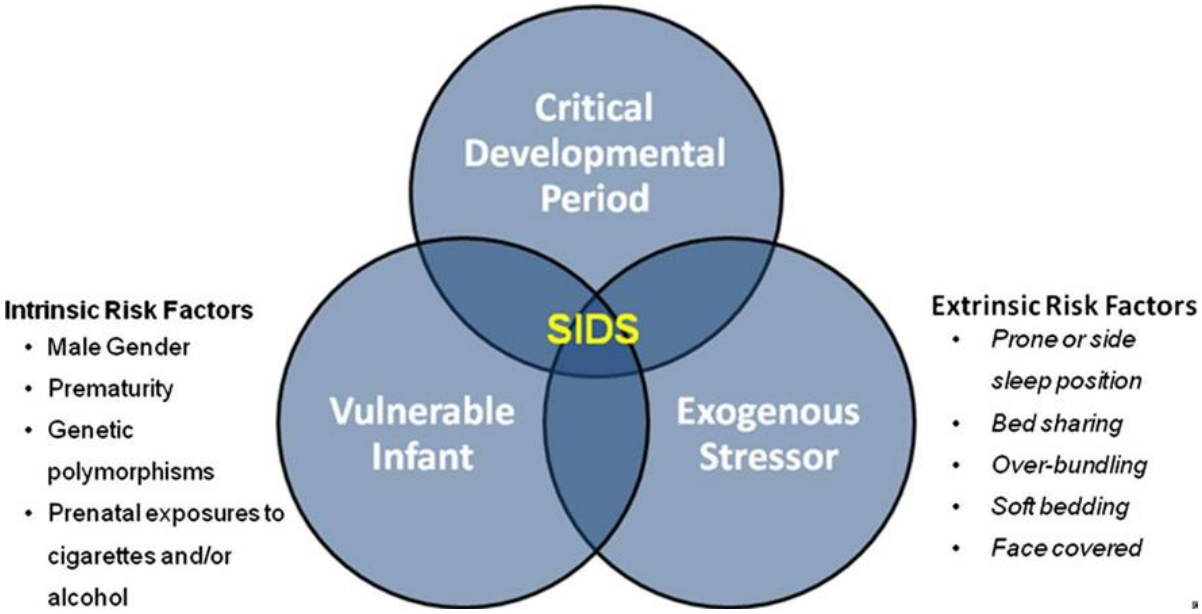
There is evidence that defect early placentation increases risk of stillbirth.[90] Nicotine possibly affects early placentation.[71] These findings suggest that nicotine is involved in the underlying mechanism of stillbirth, but in smokers combustion products may also play a role. In addition, Gupta et al. have shown increased risk of stillbirth after prenatal exposure to smokeless tobacco.[91]

In contrast to maternal smoking, snuff use is not associated with increased risk of early neonatal mortality.[63] However, Baba et al. showed that when adjusting for gestational age, the risk of early neonatal mortality decreased, suggesting that the early neonatal mortality is mediated through preterm birth. Maternal smoking is also associated with increased infant mortality, which is believed to be mediated by the increased risk of preterm birth and growth restriction[92] as well as with the increased risk of sudden infant death syndrome. [54]

Sudden Infant Death Syndrome

Sudden infant death syndrome, SIDS, is defined as a sudden unexplained death of a child below one year of age. A number of risk factors related to SIDS have been identified, such as prone position of sleep, bed sharing and overheating.[93] Since 1992, when the recommendation for non-prone sleeping position was launched, there has been a considerable decrease in SIDS cases.

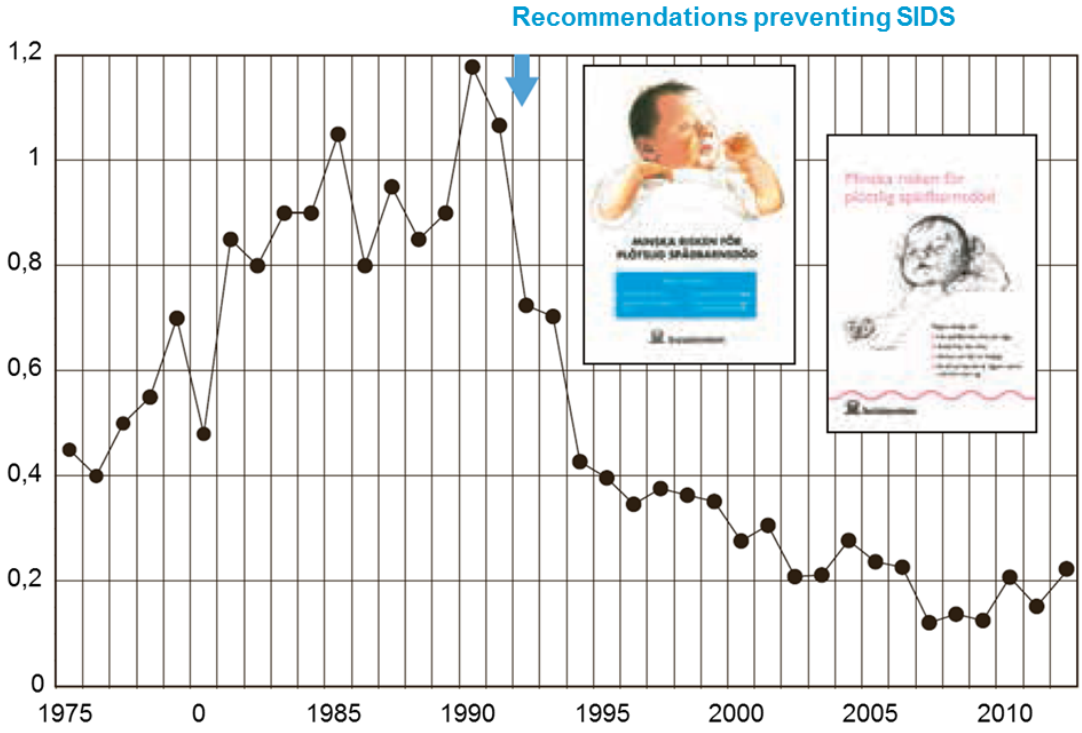
Figure 13. Factors associated with increased risk of Sudden Infant Death Syndrome



Source: Trachtenberg et al., Pediatrics. 2012 Apr; 129(4): 630–638.

A causal relation between maternal smoking, postnatal exposure to tobacco smoke and SIDS has been established.[2, 3] There is almost a threefold increased risk of SIDS among infants exposed to smoke prenatally, and the proportion of SIDS cases attributable to tobacco smoke is 23.2 according to Dietz et al., 2010.[54]

Figure 14.The incidence of SIDS and correlation with preventive recommendations



Source: Adapted from the National Board of Health and Welfare: Minska risken för plötslig spädbarnsdöd, 2014

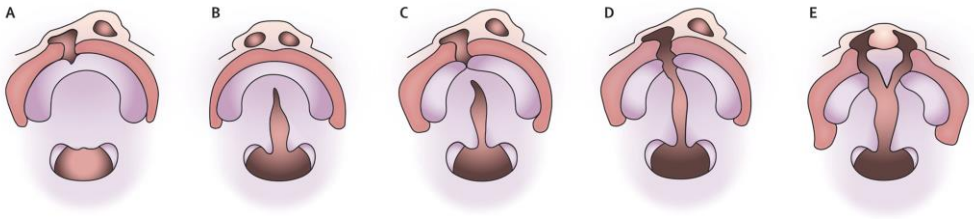
SIDS is associated with disturbed cardiorespiratory control[94] with blunt response to hypoxia and an impaired arousal.[95, 96] In the postnatal period, the cardiorespiratory system goes through maturation, which is a critical period for managing hypoxic stress, explaining why most cases of SIDS occur in this period.[94, 97]

Maternal smoking is associated with disturbed cardiorespiratory control, such as neonatal apneas and impaired arousal.[98-101] Several studies on prenatal nicotine exposure in animal studies have shown disturbances in the autonomic nervous system and disturbed cardiorespiratory control,[102, 103 104] which suggests that nicotine is involved in the mechanisms behind the smoking- related risks of SIDS. [3]

Oral cleft malformation

With an incidence of 1.7/1000 live-born, oral cleft malformations is one of the most common malformations.[105] Non-syndromic orofacial clefts include cleft lip, cleft lip and palate and isolated cleft palate. Oral cleft lip, with or without cleft palate (CL/P) and isolated cleft palate (CP) are embryological distinct entities with different epidemiological characteristics.[106]

Figure 15. Schematic presentation of different types of non-syndromic orofacial clefts



(A) Cleft lip and alveolus. (B) Cleft palate. (C) Incomplete unilateral cleft lip and palate. (D) Complete unilateral cleft lip and palate. (E) Complete bilateral cleft lip and palate.

Source: Mossey et al., Lancet 2009. Published with kind permission from Elsvier. Reprinted with permission from:Shaw WC. Orthodontics and occlusal management. Oxford: Butterworth-Heinemann, 1993

The etiology of oral cleft malformations is largely unknown and multifactorial, with large ethnic and genetic variation. Proposed environmental risk factors are maternal smoking, alcohol consumption and lack of vitamins, such as folic acids and zinc.[105-107]

CL/P and CP occur in the early trimester in the fourth to eighth gestational weeks as the result of disturbed fusion of the facial prominences. The earlier the interruption of fusion occurs, the greater the defect.[105] Moreover, CL/P has a different pathophysiological origin than CP. Cleft lip, with or without cleft palate is the result of interrupted fusion of the primary palate, while isolated oral cleft is the result of inhibited formation of the secondary palate.[105, 106]

Maternal smoking has been causally related to maternal smoking. There are also findings suggesting an interaction between maternal smoking and genetic polymorphism.[108-110] Maternal smoking is also associated with insufficient levels of vitamin C and zinc.[111-113] Vitamin deficiency is supposed to influence risk of oral cleft malformations,[105] and a few studies have found interactions between maternal smoking, vitamin supplements and risk of oral cleft malformations.[114]

Animal studies and in vitro studies suggest that nicotine inhibits the fusion of the facial prominences.[115-117] A Danish Medical Birth Register study found an association between NRT and oral cleft malformations and limb defects.[118] However, compliance with NRT in pregnancy is poor;[11] women continue to smoke using NRT. Thus, the results should be interpreted with caution.

Congenital malformations

Maternal smoking is not strongly associated with malformations, nor is nicotine in animal studies. Hackshaw et al., 2011, concluded in a meta-analysis that maternal smoking was associated with the following congenital abnormalities in addition to oral cleft malformations: clubfoot, gastroschisis and atrial septal heart defects, anal atresia, craniosynostosis, limb reduction defects and cryptorchidism.[2, 119] These findings were supported by a later Danish register based cohort study. [120]

Table 1. Summary of a systematic review of maternal smoking during pregnancy and its relationship with specific congenital malformations.

Outcome	No. of studies published, 1959-2010	OR (95% CI)
Orofacial clefts	38	1.28 (1.20-1.36)
Clubfoot	12	1.28 (1.10-1.47)
Gastroschisis	12	1.50 (1.28-1.76)
Congenital heart defects	25	1.09 (1.02-1.17)
Craniosynostosis	5	1.33 (1.03-1.73)
Anorectal atresia	7	1.20 (1.06-1.36)

Source: Adapted from Hackshaw et al., 2011

Long term effects of tobacco use in pregnancy

Maternal smoking is associated with increased risk of asthma and respiratory problems in children.[2, 3, 121, 122] Furthermore, smoking in pregnancy is associated with overweight, hypertension and type-2 diabetes in the offspring.[123, 124] Maternal smoking is related to growth restriction, which is a known risk factor for hypertension, type-2 diabetes and obesity later in life.[125-128] Many studies have found associations between maternal smoking and neurobehavioral disorders.[2, 129] However, this is difficult to study because of the socioeconomic and psychiatric confounding related to nicotine addiction. Findings in animal studies of nicotine and neurobehavioral disturbances support the findings in human epidemiological studies.[130, 131]

Tobacco cessation in pregnancy

"*To cease smoking is the easiest thing I ever did. I ought to know because I've done it a thousand times.*" Mark Twain

The majority of women stop smoking before the antenatal booking without any professional guidance.[132] However, almost 50% continue smoking in pregnancy.[49] A smoking partner, a high level of smoking prior pregnancy and multiparity are the most important predictive factors of continued smoking in pregnancy.[133] Although the proportion of smoking in pregnancy is higher in young women, the quitting rate during pregnancy is also higher among teenage mothers.[134]

Nicotine replacement therapy has not proved efficient in randomized control studies, because adherence to medication was low.[11, 135] Psychosocial clinic-based interventions in the prenatal care setting have also shown modest results on cessation rates.[132] According to the Surgeon General 2014, there is evidence of tobacco control policies having effect on maternal smoke cessation.[2]

2.10 NICOTINE

Nicotine binds to nicotine-acetylcholine receptors (nAChR), which are present at neuromuscular junctions, in the peripheral and central nervous system and also in many non-neural cells in muscle, skin, lung and pancreas. [136]

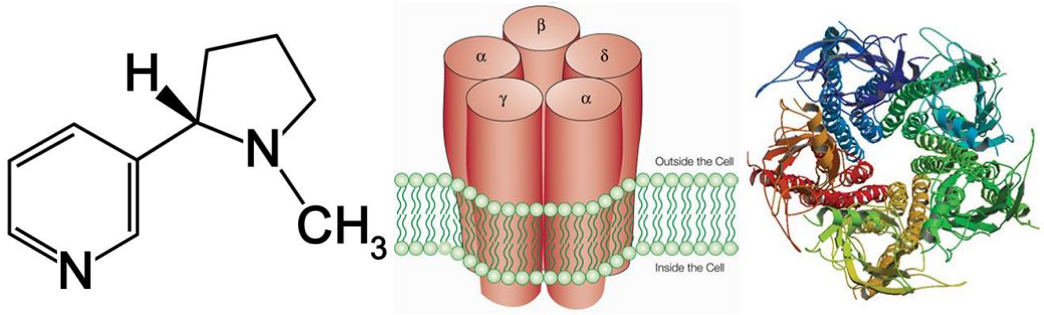


Figure16. Nicotine molecule and a schematic presentation of the nicotine-acetylcholine receptor.

The nicotine-acetylcholine receptor (nAChR) consists of five subunits forming a pore in the cell membrane and is a ligand-gated ion channel that mediates fast neurotransmission. The receptor consists of a combination of four related, but genetically and immunologically distinct, subunits forming a pentamer.

The variation of different types of subunits, combinations of subunits and the functional properties of different subunits explain the diversity of the physiological processes that the nAChR is involved in. The nAchR has metabolic and endocrine effects as well affecting the immune and nervous system. Furthermore, nAChR is also involved in control of endothelial function.[137]

Table 2.Physiological effects of nicotine

Cardiovascular	Endocrine and metabolic	Immune system	CNS-behavioral effects
Increased heart rate, cardiac contractility, and blood pressure	Adrenocorticotropic hormone, cortisol Catecholamine release	Immune suppressant	Relaxation or arousal
Cutaneous and systemic vasoconstriction	Release of growth hormone, vasopressin,	Affect T-cell signalling	Enhanced concentration
Increased muscle blood flow		Immune modulatory effects on the central nervous system by: 1) the HPA axis 2) direct effects on the autonomic nervous system.	Appetite suppression
Inhibition in prostacyclin synthesis, which affects endothelial cells and Platelet activation.			Alertness

Source:Adapted from Benowitz et.al.1988

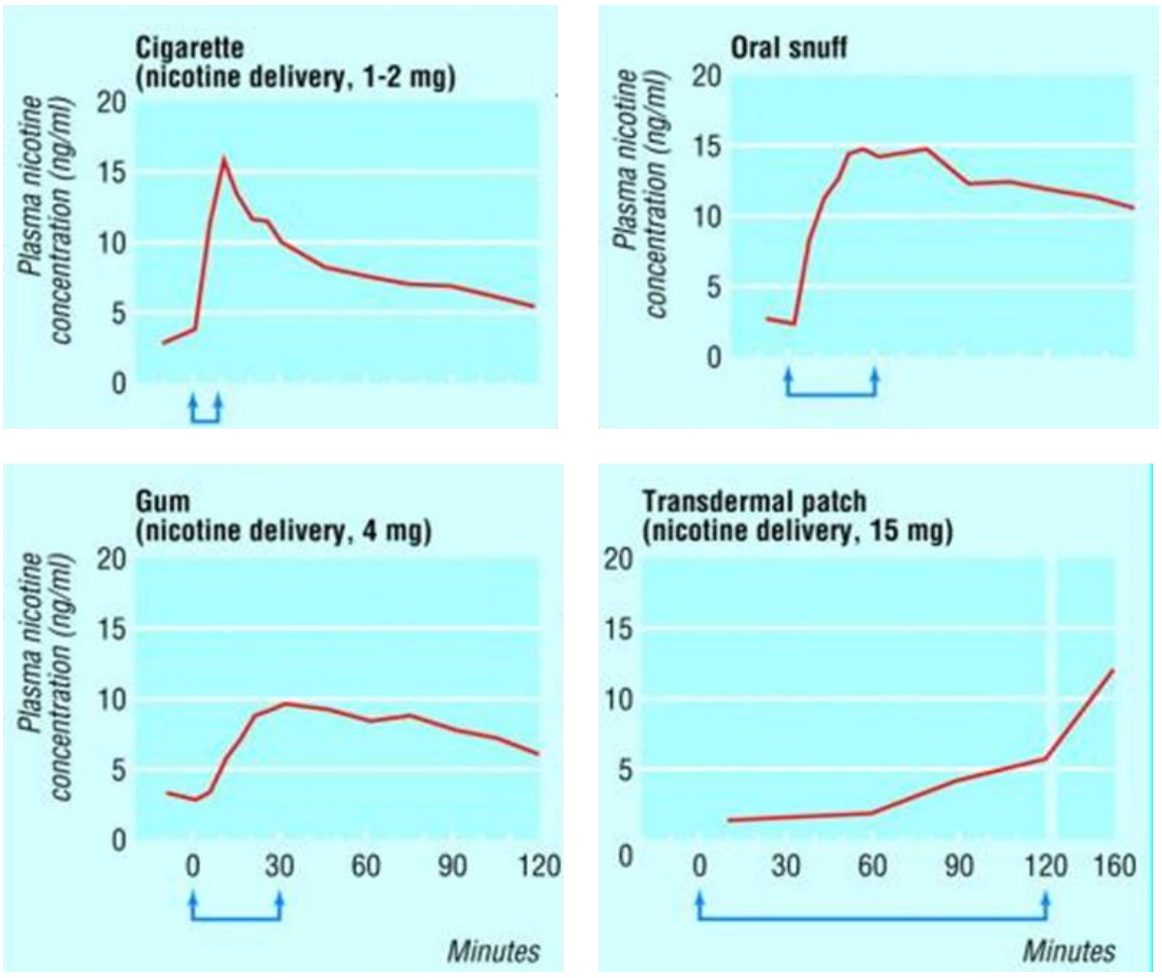
Pharmacokinetics of nicotine

Free nicotine, i.e. the non-ionized form of nicotine, easily passes cell membranes, including oral mucosa, pulmonary tissue, the blood-brain barrier, placenta and other tissues. It accumulates in breast milk[138-141] and amniotic fluid.[142] Nicotine is a weak base and the proportion of non-ionized nicotine increases with increasing pH-value. Oral moist snuff is therefore alkalized to facilitate the absorption of nicotine.

During cigarette smoking, nicotine reaches the brain within 10-20 seconds, with a peak plasma-concentration in 5 minutes.[136] During snuff use, nicotine is absorbed more slowly, but plasma-concentration peaks are similar to that of smoking. Whereas the plasma concentration of nicotine falls rapidly after smoking, the nicotine plasma-concentration of snuff users reaches a plateau after 30 minutes, and nicotine levels slowly decline after 1-2 hours. It has been speculated that the continued release of nicotine, also after use of oral snuff, comes from the mucus membranes.[143, 144]

NRT, such as gums and patches, has a substantially slower absorbance and lower peak plasma–concentrations, which is why the addictive potential is low.[145]

Figure 17. Plasma nicotine concentration from different sources of nicotine



Source: Adapted from Henningfield 1995 and Molneux, 2004.

Only a small amount of the nicotine in the cigarette or pouch of snuff is absorbed. Smoking behavior affects nicotine absorption through inhalation volume, number of puffs and number of covered ventilation holes in the filter of the cigarette.[146, 147] The amount of nicotine absorbed by the body during snuff use depends on how long the pouch is kept in the mouth and the properties of the snuff, such as the total nicotine content and the pH-value. The rate of absorbance and metabolism also varies substantially between individuals.[136]

As mentioned above, the pattern of tobacco use also affects nicotine plasma-levels. Whereas smoking gives high peaks of nicotine intermittently during the day, many snuff users have a pouch of snuff under the lip throughout the day which leads to a continuously high level of nicotine.

Nicotine metabolism

Nicotine is metabolized in the liver, primarily by cytochrome 2A6. It has a half-life of 2 hours and is subsequently eliminated by the kidneys. The primary metabolite of nicotine, cotinine, has a half-life of 18 hours and is the most frequently used biomarker of nicotine exposure.[136] There are major individual differences in nicotine and cotinine metabolism which are believed to affect the risk of addiction. Thus fast metabolizers appear to have a higher risk of addiction than slow metabolizers.[145]

Women have a higher metabolism of nicotine and cotinine than men. Women using oral contraceptives and pregnant women have a higher nicotine metabolism than non-pregnant women, not using oral contraceptives.[136] Nicotine metabolism is also elevated in pregnancy, which leads to a half-life of cotinine of 8-9 hours.[142, 148] In contrast, the nicotine metabolism in newborns is slower than in adults, with a half-life three times longer in infants. The cotinine metabolism seems to be the same as in adults, though.[136] This might explain why nicotine, which easily passes placenta, seems to accumulate in amniotic fluids.[80] Luck et al. found that fetal concentrations in cord blood were 15% higher than maternal levels at the time of delivery.[139]

Nicotine also easily passes into breastmilk, leading to a milk-to-maternal plasma ratio of 2.9.[141] Nicotine in breastmilk is swallowed by the infant, and there is insufficient knowledge about absorption of nicotine in the gastrointestinal tract of the infant.[141]

Effects of nicotine during development

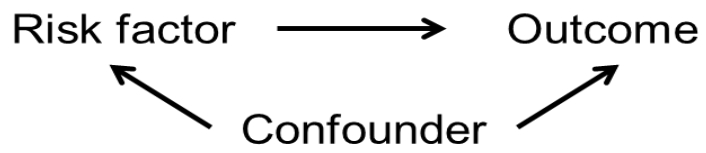
The effects of nicotine during development are very different from the effects in the adult. The nAChRs is present very early in the developing human fetus, as early as gestational week 4-5, and acetylcholine plays an important role in brain and lung maturation. Nicotine is a potent neuroteratogen and binds to nicotinic AChRs in the developing brain and lung, inducing apoptosis and affecting cell programming.[104 129, 131]

Prenatal nicotine exposure is also known to affect the cardiovascular, the endocrine and the immune system, inducing structural and even transgenetical changes that may have effect also in adulthood.[46, 149-153] In animal studies, prenatal nicotine-exposure induces lung hypoplasia, emphysema-like changes, such as decreased elastin and increased collagen synthesis in vascular endothelial cells and lung parenchyma.[102] The morphological changes are associated with increased airway resistance and hyper reactivity, observed in children exposed to maternal smoking, suggesting that nicotine is involved in the development of asthma and lung disease.[102] In addition, nicotine has effects on the nervous system and on the developing brain at doses that have no impact on fetal growth.[104] This is important in the discussion of NRT use in pregnancy.[131]

3 EPIDEMIOLOGICAL TERMINOLOGY

Bias is a systematic error in a study. **Selection bias** in a study stems from how study subjects are collected and/or from factors that influence study participation. **Information bias** or **misclassification** arises when there is a systematic error in the collecting of information. Misclassification of subjects of an exposure or outcome can be divided in *differential* and *non-differential* misclassification. The exposure bias is non-differential if the misclassification is unrelated to the outcome. If the exposure misclassification is related to the outcome, it is a *differential* misclassification. A *differential* misclassification is associated with under- or over -estimation of the effect on the outcome, whereas a *non-differential* misclassification tends to dilute the effect of the exposure on the outcome, resulting in a bias towards the null. **Recall bias** is a differential misclassification bias, where the exposure information is misclassified differentially for those with and without disease.

Confounding is a type of bias, that obscures or distorts the result of an association. A **confounding factor** is associated with both the exposure and the studied outcome. It may cause the outcome or be a proxy for the outcome but not be an effect of the outcome. A confounder is not an intermediate in the causal pathway between the exposure and the outcome.



Internal validity describes if the study or a test accurately measures what it is supposed to measure, which means lack of systematic errors such as bias, misclassification or confounding.

External validity or generalizability describes if the study is representative beyond the study population and if the result of the study is applicable not only to the study population, but also to the reference population.

Sensitivity is defined as the proportion classified by the test as having the disease among all in the population having the disease.

Specificity is defined as the proportion classified by the test as not having the disease among all in the population without the disease.

Positive predictive value is the probability of the disease among those with a positive test.

Negative predicted value is the probability of no disease among those with a negative test.

Receiver operating characteristics curve, ROC, describes the relationship between the sensitivity and specificity of a test and is used to decide the cut-off values for an optimal combination of sensitivity and specificity for a test.

4 AIM

The overall aim of this thesis was to study prenatal nicotine exposure, in the form of oral moist snuff and smoking, and its effects on the health of the newborn. The general research question was to investigate if it is safe to use snuff in pregnancy, and if cessation of snuff use or smoking during pregnancy influences the possibly adverse risks on the health of the newborn.

Specific research objectives:

Study I

- To investigate if maternal smoking or snuff use is associated with neonatal apnea.

Study II

- To study if smoking or snuff use in pregnancy is associated with increased risk of oral cleft malformations.
- To study if cessation of snuff use or smoking in early pregnancy influences the risk of oral cleft malformations in the newborn.

Study III

- To assess the relationship between maternal smoking or snuff use and extremely preterm birth.
- To study if cessation of snuff use or smoking influences the risk of extremely preterm birth.
- To investigate the association between tobacco use and spontaneous and medically indicated onset of preterm delivery.

Study IV

- To validate prospectively self-reported questionnaire data on snuff use in late pregnancy by quantifying cotinine in maternal urine and newborn meconium.
- To validate self-reported information on tobacco use in the Swedish Medical Birth Register in late pregnancy by cotinine in maternal urine and newborn meconium.
- To study if the levels of cotinine can be used to identify mothers with high nicotine intake.
- To investigate the relation between cotinine levels and neonatal birth characteristics, such as birth weight, length, head circumference and gestational age.

5 METHODOLOGICAL CONSIDERATIONS

The methods used in the individual papers are described in each paper and will not be explained in detail in this chapter.

5.1 DATA SOURCES

This thesis is predominately based on population-based studies in the Swedish Medical Birth Register (*paper I-III)* with linkage to other data sources from the National Board of Health, such as the Patient Register[154], the Education Register and the Total Population Register held by Statistics Sweden.[155, 156]

Study IV is a validation study of prospectively collected self-reported snuff use during pregnancy by the biomarker cotinine in maternal urine and in the meconium of the newborn. In addition, Medical Birth Register data for the cohort is linked to the cohort data.

The Medical Birth Register and other registers

The Swedish Medical Birth Register (MBR) was started in 1973 and is a population-based register that contains data on more than 98% of all births in Sweden, including maternal characteristics (demographic data, information on reproductive history and tobacco habits) and information on pregnancy, delivery and the neonatal period. Information on smoking in early pregnancy has been recorded since 1982, whereas tobacco use in late pregnancy was added in 1991. Snuff use was not reported to the MBR until 1999.

Data on tobacco use is based on prospectively collected self-reported information obtained by midwives at the antenatal booking in early pregnancy and in week 32. Tobacco use is categorized as no tobacco use, maternal smoking 1-9 cigarettes/day or >=10 cigarettes/day and snuff use (yes/no).[6]

Maternal diseases, complications during pregnancy or delivery and neonatal complications and diagnoses are classified according to the Swedish version of the International Classification of Diseases (ICD) system and reported at discharge from the delivery hospital.

Sweden has many other registers, such as the Register on Congenital Malformations, the Swedish Cancer Register, the National Cause of Death Register, the National Patient Register and the Prescribed Drug Register held by the National Board of Health.[154] Through the unique personal identification number (PIN), several registers and other data sources held by Statistics Sweden,[156] such as the Education Register, the Total Population Register and the Multi-Generation Register can be linked together.[155, 157]

The National Patient Register contains nationwide information on diagnoses and procedures and can be divided into the Inpatient Register, with coverage since 1987, and the Outpatient Register, with information on all hospital out-patient care since 2001.[154] Since 1997, both the MBR and the National Patient Register diagnoses are classified according to the 10th revision, ICD-10.

Validation of the Swedish Medical Birth Register

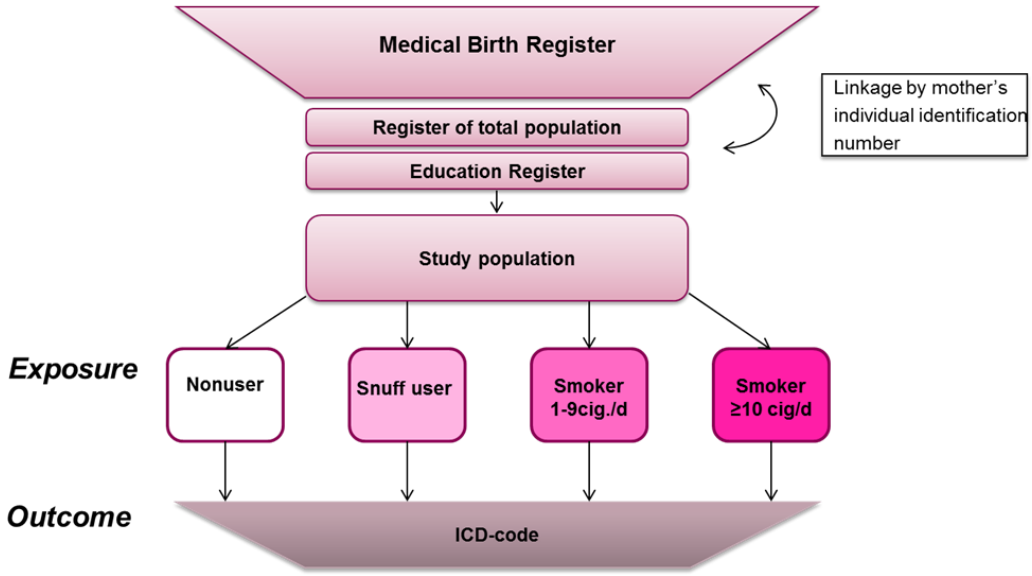
Since the start of the register in 1973, the MBR has been modified and variables added on several occasions, 1982, 1990, 1994 and 1998.[158] The MBR has been validated according to quality and coverage of data several times[159], the latest in 2002, and has been proved reliable with high coverage and data quality on most variables.[158] Smoking in early pregnancy has been missing in 4-9% throughout the years. However, smoking in late pregnancy had until 2000 a high percentage of missing information and was not useful in research.[158] According to the National Board of Health and Welfare, there was 4-5% missing information on smoking and snuff use prior pregnancy, in early pregnancy and in late pregnancy in the MBR 2014.[6] Information on drug and alcohol use in pregnancy is of poor quality.

Missing data on diagnoses of infants who have been transferred between hospitals and neonatal wards is still a problem.[158] Linkage to the Congenital Malformation Register, the Hospital Discharge Register or the National Patient Register can be necessary to ascertain certain diagnoses in epidemiological studies.[158]

5.2 STUDY DESIGN AND STUDY POPULATION, PAPER I-III

In *paper I-III*, tobacco exposure in the Medical Birth Register in early pregnancy was studied. *In paper I*, tobacco exposure was categorized as nonuser, snuff user, smoker 1-9 cigarettes/day and smoker ≥ 10 cigarettes/day.

Figure18. Schematic presentation of the study designs in *paper I-III*



What differed in the study design of *papers II* and *papers III* from *paper I* was the categorization of tobacco use. In *paper II* and *paper III*, tobacco use was categorized as: *no use* (i.e. no use three months prior pregnancy and in early pregnancy); *stopped using snuff* (i.e. snuff use three months before pregnancy but not in early pregnancy); *snuff use* (i.e. snuff use three months before pregnancy and in early pregnancy) *and stopped smoking* (i.e. smoking three months before pregnancy but not in early pregnancy); and *smoking* (i.e. smoking three months before pregnancy and in early pregnancy).

ICD-10 codes

For diagnoses of neonatal apnea and oral cleft malformations ICD-10 codes were used.

The American Association of Pediatrics[160] has defined infant apnea as "*an unexplained episode of cessation of breathing for 20 seconds or longer, or a shorter respiratory pause associated with bradycardia, cyanosis, pallor and/or marked hypotonia*." Neonatal apnea unspecified was defined by the following ICD-10 codes: P 28.2 ("cyanotic attack"), P28.3 ("sleep apnea") and P28.4 ("other apnea of the newborn"). Sleep apnea was also analyzed separately as ICD-10 code P 28.3.

Oral cleft malformation was defined by the ICD- 10codes Q35-37. The different subtypes of oral cleft malformations were analyzed separately, as cleft lip with or without cleft palate (ICD-10 codes Q36 and Q37) and isolated oral cleft (ICD-10 Q35).

In *paper II*, the Patient Register was linked to the Medical Birth Register to ensure adequate coverage on congenital malformation diagnoses.

Inclusion and exclusion criteria

Exclusion criteria for all papers were missing information on tobacco use and dual use (snuff use and smoking). In the adjusted models missing data on co-variates was also excluded.

Only mothers of live-born infants were included in the studies I and III. In *paper II* and *paper IV* both singleton and multiple births were included.

In *paper I*, only mothers born in the Nordic countries were included.

In *paper IV*, smokers and dual users were excluded from the study.

Confounders and intermediate variables

In *paper I-IV*, co-variates were chosen because of their association with the exposure and the outcome in previous literature. Possible confounders were adjusted for in the adjusted analysis.

BMI is defined as: body weight/height ^{2}. Body mass index, BMI, was not adjusted for in *paper I* and *paper II* despite being a possible confounder associated with both exposure and outcome. There is a large number of mothers with missing information on the BMI variable in the MBR. When BMI was adjusted for in the regression model without affecting the estimate, BMI was not considered a confounder and was left out of the final analysis. In *paper IV*, maternal education and mother's country of birth were not possible to obtain.

Table 3. Maternal and birth characteristics used as co-variates in the different studies

Maternal and birth characteristics	Study I Apnea	Study II Oral Cleft Malformation	Study III Preterm birth	Study IV Neonatal growth characteristics
Maternal age	x	x	x	x
Height	x			
BMI			x	x
Parity	x	x	x	x
Cohabitant with father-to-be	x	x	x	x
Education	x	x	x	
Pre-pregnancy diabetes		x		
Preeclampsia		x		
Mother's country of birth		x	x	
Cesarean Section	x			
Small-for-gestational age	x			
Gestational age	x			x
Gender	x	x		
Single or multiple pregnancies		x		

5.3 STUDY DESIGN AND STUDY POPULATION, PAPER IV

Self-reported snuff use in pregnancy has not been validated previously. The objective with this study was to study the accuracy of self-reported snuff use but also to validate the data on snuff use in the Medial Birth Register.

Between 2005 and 2011, women were asked to participate in a prospective cohort study of snuff use during pregnancy. Antenatal clinics in seven counties in Sweden (Jämtland, Västernorrland, Västerbotten, Stockholm, Gotland, Gävleborg and Dalarna), chosen by their high prevalence of snuff use, took part in the study.

Inclusion occurred at the antenatal booking, and tobacco use was categorized as no use, snuff use or smoking. Only nonusers and snuff users were analyzed further.

Figure 19. Schematic presentation of the study design, *paper IV*

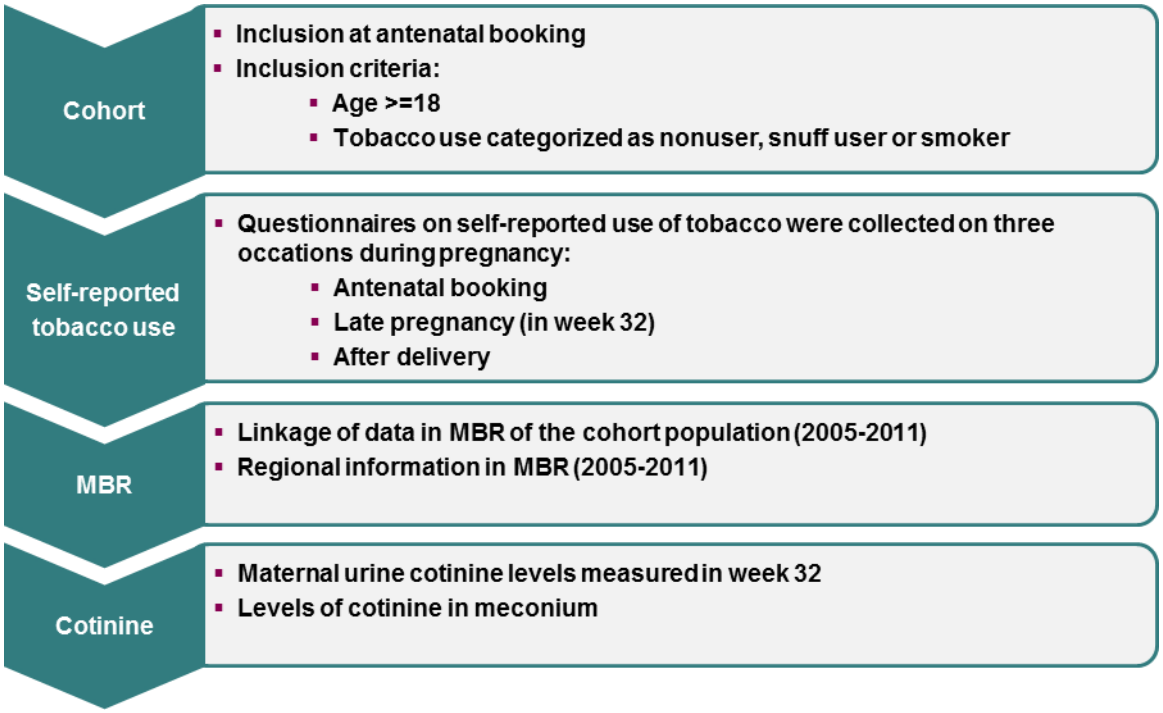
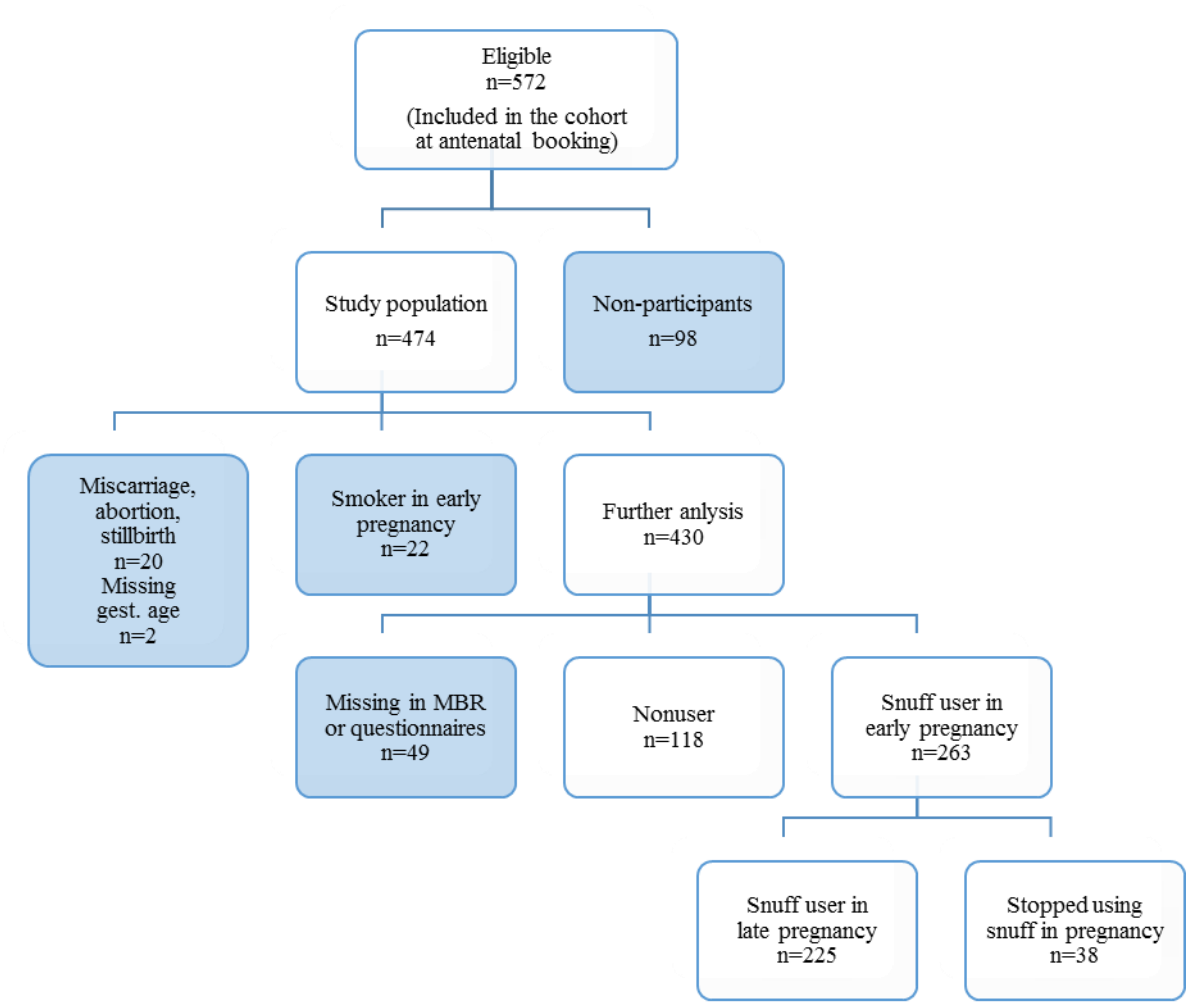


Figure 20. Schematic presentation of the study population



Self-reported snuff use through questionnaires

The participants were asked to report their tobacco use through questionnaires at antenatal booking, in late pregnancy and immediately after birth. The participants self-reported daily use of tobacco for every gestational week, including brand of tobacco used, use of nicotine replacement therapy (NRT) and exposure to second-hand smoke (SHS). Snuff use was reported as number of pouches/day each gestational week and categorized as follows: no use, 1-2, 3-4, 5-6 or ≥7 pouches/day. See Appendix A

Self-reported snuff use registered in the Medical Birth Register

We obtained information from medical records from the antenatal and delivery clinics on the participants and data from the Medical Birth Register (MBR). To assess if the study population was representative of the population, we also obtained data from the MBR for all births the corresponding years and geographic regions. As mentioned previously, tobacco use in the MBR was categorized as no use, snuff use, smoking 1-9 cigarettes/day and smoking >=10 cigarettes/day.

Analysis of cotinine

Maternal urine cotinine was collected in gestational week 32. In addition, cotinine in the meconium of the newborn was analyzed.

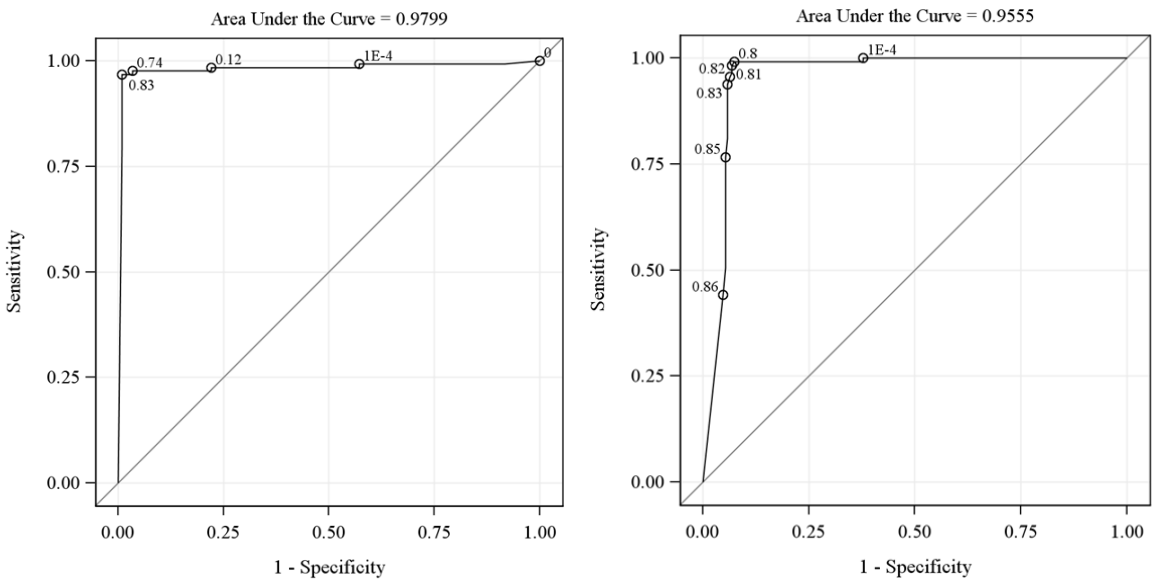
To determine cotinine in urine and meconium, a procedure based on high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) was used.[161] Meconium was first extracted with methanol. Further purification of the meconium methanol extracts and urine was done with solid phase extraction. Standard curves were created using seven calibrators at 0, 5, 15, 30, 50, 75 and 100 ng/mL, and a least square linear regression was generated with correlation coefficients > 0.99.

The lowest limit of quantification (LOQ) was 1 ng/ml for cotinine in urine and meconium.

The cut-off level of cotinine to distinguish tobacco exposure from no exposure was chosen by visually assessing the receiver operating characteristics (ROC) curve. Urine cotinine level ≥15ng/ml was defined as snuff use and urine cotinine <15 ng/ml was categorized as no use. In meconium, cotinine level ≥3 ng/ml was defined as snuff use and cotinine level <3ng/ml was categorized as no use.

Figure 21. ROC for snuff use and maternal urine cotinine (a) and meconium cotinine (b)

a) **b)**



5.4 STATISTICAL METHODS

The statistical methods used in this thesis are described in detail in each paper.

Paper I-III

Associations between tobacco exposure and outcomes were evaluated using multivariable adjusted logistic regression models. Results were presented as odds ratios, OR, with 95% confidence interval. In order to adjust for the dependence introduced by the fact that mothers may contribute with more than one child, the Generalized Estimation Equation method was applied.

Paper IV

Spearman's correlation coefficient was used to calculate the correlation between cotinine values in maternal urine and in meconium.

To discriminate snuff users from nonusers, optimal urine cotinine and meconium cotinine cut-off levels were determined using receiver operating characteristic curve analyses that maximize total probability of correct classification. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated and 95% confidence intervals were computed by the exact method, using binomial distribution. Moreover, the likelihood ratio (LR) for a positive test was calculated as the sensitivity/(1-specificity) and the LR for a negative test was calculated as the specificity/(1-sensitivity).[162]

The agreement between self-reported dose and cotinine levels was analyzed with non-parametric Wilcoxon signed rank test and Kruskal-Wallis test.

Linear regression was used to analyze the associations between cotinine levels and birth outcomes.

All data in *paper I-IV* was analyzed using SAS version 9.2 (SAS Institute, Inc.; Cary, NC).

5.5 INFORMED CONSENT AND ETHICS

Papers I-III are population-based register studies and individual consent was not obtained from the participants. The participants gave informed consent on *paper IV*. The studies were all approved by the Research Ethics Committee at the Karolinska Institutet, Stockholm, Sweden (No 2005/223-31/2, 2006-835-32, 2011/895-32, 2011/1148-32, 02-405, 2009/1725-32).

6 RESULTS

6.1 MATERNAL CHARACTERISTICS

Maternal characteristics and tobacco cessation in MBR (papers I-III)

Our studies demonstrated a high level of correlation between maternal smoking and a low socioeconomic status and a low level of education. This was also seen, albeit less pronounced, among women using snuff in pregnancy. Moreover, snuff using women were to a greater extent teenagers, less educated and multiparous. A higher proportion of snuff using women were obese and not living with the father-to-be in comparison to that of nonusers. That snuff use is a Swedish habit was evident; as many as 94% of snuff users were born in the Nordic countries. Both maternal smoking and snuff use was associated with increased risk of cesarean section and preterm birth. Maternal smoking was also associated with increased risk of small for gestational age births, whereas the risk for snuff users was in comparison with that of nonusers.

Approximately 60% of snuff users and more than 50% of smokers stopped smoking before the antenatal booking. Tobacco cessation was most common among women giving birth to their first child. Among mothers who continued to use tobacco, a higher proportion were teenage mothers, multiparous and had less than eleven years of education in comparison to both nonusers and quitters. Among women who continued to smoke in pregnancy, 58% had less than eleven years of education. (*Paper 1 and II)*

Table 3. Tobacco use before pregnancy and in early pregnancy in the MBR, 1999-2009

Tobacco Use		**Before pregnancy**				
		Total No. (%)	**Nonuser (n)**	**Snuff user (n)**	**Smoker (n)**	**Missing (n)**
Early pregnancy	**Total No. (%)**	1,086,213 (100)	773,625 (71.2)	21,994 (2.0)	185,248 (17.2)	102,451 (9.4)
	Nonuser	917,900 (<4.5)	765,145	12,834	97,776	40,669
	Snuff user	11,461 (1.0)	1,237	8,859	732	36
	Smoker	92,092 (8.5)	953	74	85,144	5,626
	Missing	64,014 (5.9)	6,279	211	1,389	56,118

Table 4. Maternal and birth characteristics and tobacco use in MFR

Maternal and birth Characteristics	*paper I*, 1999-2006				*paper III*, 1999-2012			
	Nonuser N=503,460 **Rate %**	**Snuff user** N=7,599 **Rate %**	**Smoker 1-9 cig./day** N=41,391 **Rate %**	**Smoker ≥10cig./day** N=16,928 **Rate %**	**Nonuser** N=1,777,464 **Rate %**	**Snuff user** N=14,671 **Rate %**	**Smoker 1-9 cig./day** N=79,783 **Rate %**	**Smoker ≥10 cig./day** N=28,459 **Rate %**
Age								
≤ 19 years	1.1	1.9	6.4	3.6	1.4	2.0	6.2	3.8
Height					-	-	-	-
≤159 cm	8.6	11.3	11.8	12.4	-	-	-	-
BMI								
≥30	10.1	12.3	14.8	18.8	11.0	14.0	16.0	20.0
Multiparity *								
	4.2	7.3	8.0	18.5	18.4	22.6	23.1	39.2
Education								
≤9years	5.6	11.3	27.0	34.0	8.0	11.3	26.1	33.0
Not cohabitant with father-to-be								
	3.4	6.6	14.3	18.5	4.8	7.6	15.8	19.7
Mother's country of birth								
Nordic	-	-	-	-	80.1	94.0	82.4	85.3
Cesarean section								
Yes	15.4	18.2	16.5	16.6	-	-	-	-
Small for gestational age**								
Yes	2.2	2.4	4.7	5.8	-	-	-	-

*In *paper I* multiparity is defined as ≥4 children and in *paper III* multiparity is defined as ≥3 children, why figures are not comparable between papers.
**Small for gestational age (SGA) was defined as > 2 standard deviations below the mean birth weight for gestational age according to the sex specific Swedish fetal growth curve, Marsal.

Maternal characteristics and tobacco cessation in cohort (Paper IV)

Of the prospectively studied women, only 38 (14%) stopped using snuff during pregnancy, and the majority of these stopped between the first and second trimester. All quitters had stopped before 28th weeks of gestation. In agreement with the register studies mentioned above, women who ceased using tobacco during pregnancy were to greater extent <20 years and primiparous.

Few participants reported exposure to second hand smoke (SHS) in pregnancy. None of the nonusers reported daily exposure to SHS, whereas 9% reported exposure to SHS occasionally. Two nonusers reported continuous weekly, but not daily, exposure throughout pregnancy. Of the snuff users, 17% reported occasional exposure to SHS in early pregnancy and 24% in late pregnancy. Twenty-six women were exposed weekly throughout pregnancy and 10 were exposed daily.

Few participants used NRT in pregnancy, and none of the women who stopped using snuff in pregnancy were NRT users. Only 4 participants reported use of NRT in early pregnancy and 7 reported use in the second trimester, two of whom reported use also in the third trimester. All participants who reported use of NRT were also reporting simultaneous use of high doses of snuff.

6.2 NEONATAL APNEA

The most important risk factor for neonatal apnea *(Paper I)* was preterm birth, and the risk increased with decreasing gestational age. For very preterm infants (<32 gestational weeks), the adjusted OR was 139 (95% CI: 115-167). There was also a U-shaped association between parity and apnea, with increased risk of apnea in infants of primiparous and multiparous (≥4) women.

Snuff use in pregnancy was associated with a twofold increased risk of neonatal apnea, adjusted OR 2.2 (95% CI: 1.4-3.2), in comparison to that of nonusers. Maternal smoking was associated with lower risk than that associated with snuff use, but the risk increased with number of cigarettes smoked, adjusted OR 1.3 (95% CI: 1.0-1.7) and OR 1.5 (95 % CI: 1.1-2.1), for moderate and heavy smoking, respectively. However, the risk of neonatal apnea was no longer significant among infants of smoking mothers when adjusting for gestational age, SGA-births and cesarean section but was only slightly attenuated for snuff users.

Next, sleep apnea (ICD-10 P28.3) was analyzed separately. In infants of snuff users the adjusted OR was 2.5(1.4-4.4) and for moderate and heavy smokers adjusted OR was 1.2 (95% CI: 0.9-1.7) and 1.4 (95% CI: 0.9-2.1), respectively.

Table 5. Tobacco use and neonatal apnea UNS, **(**ICD-10 P28.2-28.4)**,** n=931

Tobacco use	No.	Rate/ 1000	Odds ratio (95% Confidence Interval)		
			Crude	Adjusted model 1*	Adjusted model 2**
Nonuser	771	1.5	reference	reference	reference
Snuff user	26	3.4	2.2 (1.5-3.3)	2.2 (1.4-3.2)	2.0 (1.3-3.0)
Smoker					
1-9 cig./day	94	2.3	1.5 (1.2-1.8)	1.3 (1.0-1.7)	1.1 (0.9-1.4)
≥10 cig./day	40	2.4	1.6 (1.1-2.1)	1.5 (1.1-2.1)	1.1 (0.8-1.5)

**Adjusted model 1: adjustment for maternal characteristics, such as maternal age, height, parity, education, cohabitant with father-to-be* ***Adjusted model 2: adjustment for maternal characteristics as in model 1 and birth characteristics, such as cesarean section, SGA-births and gestational age.*

Table 6. Tobacco use and primary sleep apnea, (ICD 10 P28.3), n=416

Tobacco use	No.	Rate /1000	Odds ratios (95% Confidence Intervals)		
			Crude	Adjusted model 1*	Adjusted model 2**
Nonuser	320	0.6	reference	reference	reference
Snuff user	14	1.8	2.9 (1.7-5.0)	2.8 (1.7-4.9)	2.5 (1.4-4.4)
Smoker					
1-9 cig./day	56	1.4	2.1 (1.6-2.8)	1.7 (1.2-2.3)	1.2 (0.9-1.7)
≥10 cig./day	26	1.5	2.4 (1.6-3.6)	2.2 (1.5-3.4)	1.4 (0.9-2.1)

**Adjusted model 1: adjustment for maternal characteristics, such as maternal age, height, parity, education, cohabitant with father to be* ***Adjusted model 2: adjustment for maternal characteristics as in model 1 and birth characteristics as cesarean section, SGA-births and gestational age.*

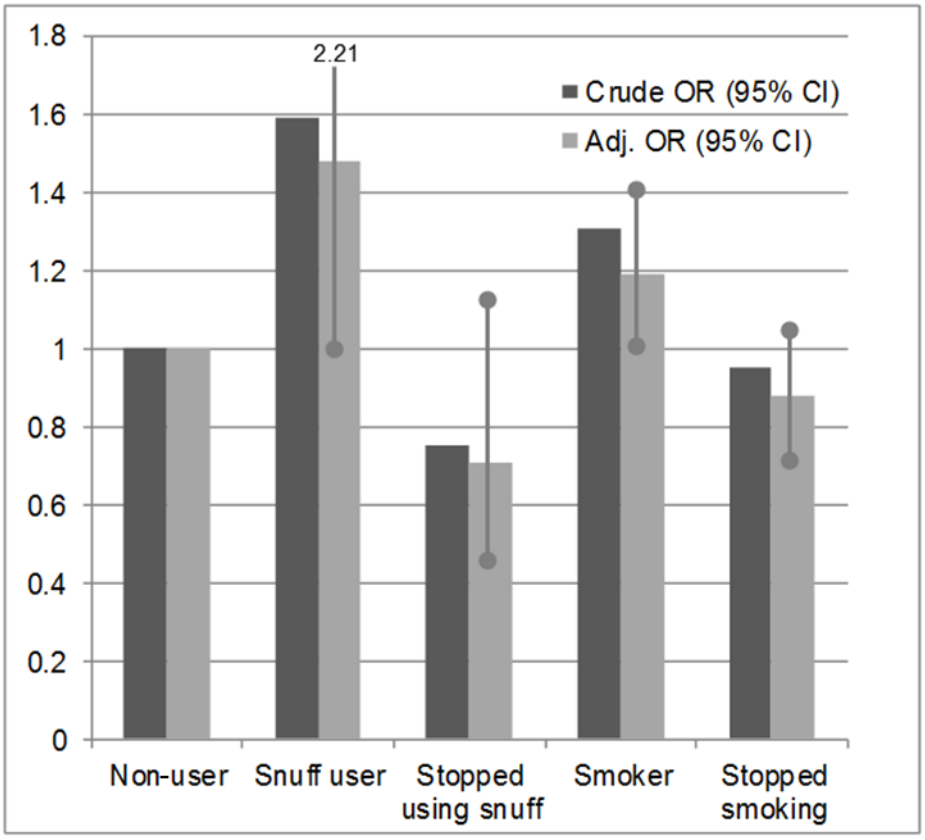
6.3 ORAL CLEFT MALFORMATION

The incidence of oral cleft malformations *(paper II)* was 1.8/1000 births. Maternal age ≥ 35 years, chronic hypertension, preeclampsia, male sex, multiple births and Nordic country of birth were all associated with an increased risk of oral cleft malformations.

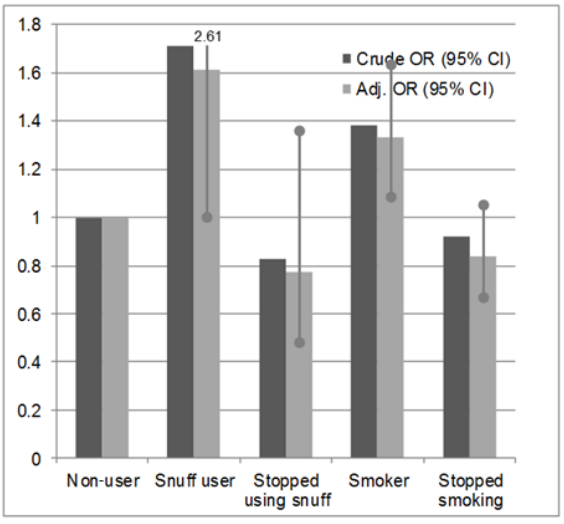
Both maternal snuff use and smoking were associated with an increased risk of oral cleft malformations, but varied between subtypes. Whereas risks of cleft lip with or without cleft palate were increased following maternal snuff use or smoking, no significant association was seen for isolated cleft palate. Infants of mothers who stopped using tobacco (snuff or cigarettes) before the antenatal booking were not at an increased risk of oral cleft malformations.

Figure 22. Maternal tobacco use in early pregnancy and risk of (a) oral cleft malformations, (b) cleft lip with or without cleft palate, (c) isolated cleft palate (CP). Odds ratios presented with 95% confidence interval

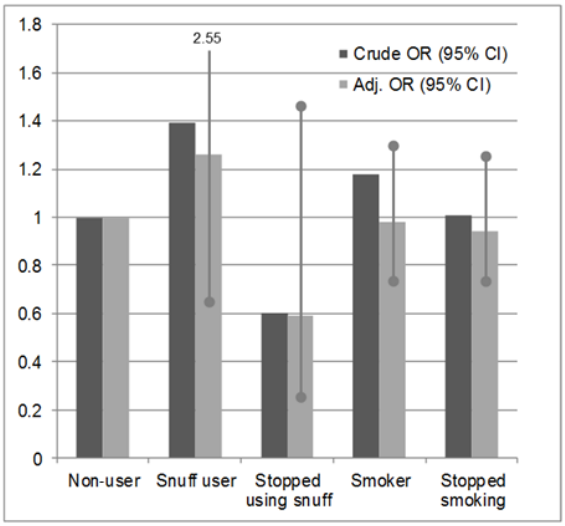
a) Oral cleft malformations



(b) Cleft lip with or without cleft palate



(c) Isolated cleft palate (CP)



6.4 EXTREMELY PRETERM BIRTH

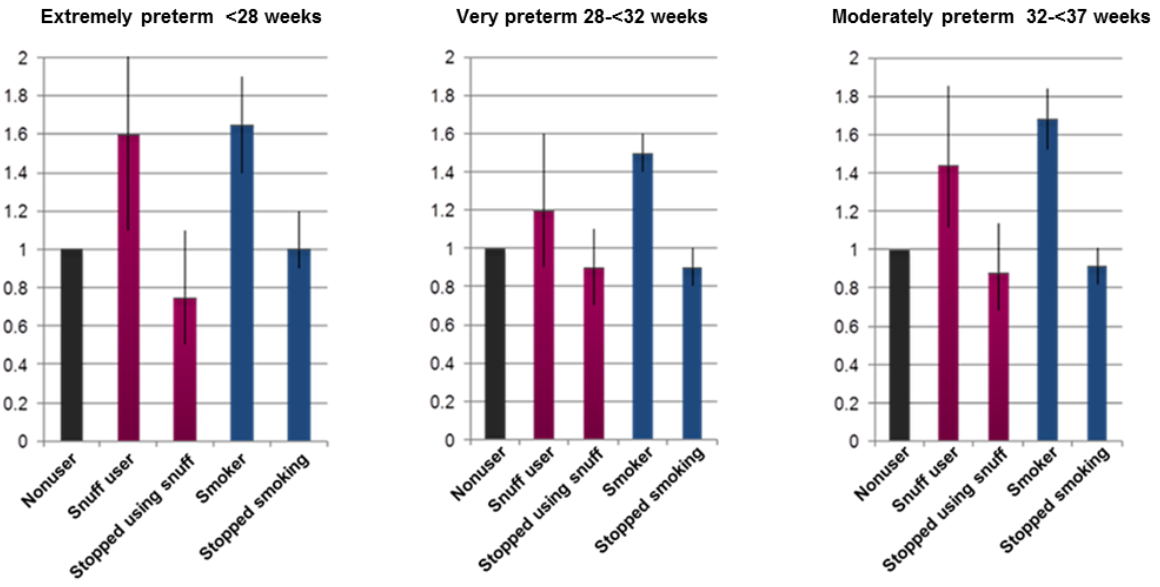
In the study population, 4.94% were born preterm (<37weeks), 4.26% of whom were moderately preterm, 0.45% very preterm (<32 weeks) and 0.23% extremely preterm (<28weeks). *(Paper III)*

Maternal age ≥ 35 years of age, primipara <12 years of education, BMI ≥30 and non-Nordic country of birth of the mother were all factors associated with an increased risk of preterm birth in the adjusted analysis.

Maternal smoking in early pregnancy was associated with all categories of preterm birth. The risk increased with the number of cigarettes smoked and with decreasing gestational age. An increased risk was seen both of spontaneous and medically induced preterm birth and across all gestational age strata. Previous smokers who had stopped smoking before the antenatal booking were not at an increased risk of preterm birth.

Maternal snuff use was associated with increased risk of extremely and moderately preterm birth, whereas the risk of very preterm birth was of borderline significance. Women using snuff three months before pregnancy but who had ceased using it at the antenatal booking were not at an increased risk. Snuff use was associated with medically induced extremely preterm birth and both spontaneous and medically indicated moderately preterm birth.

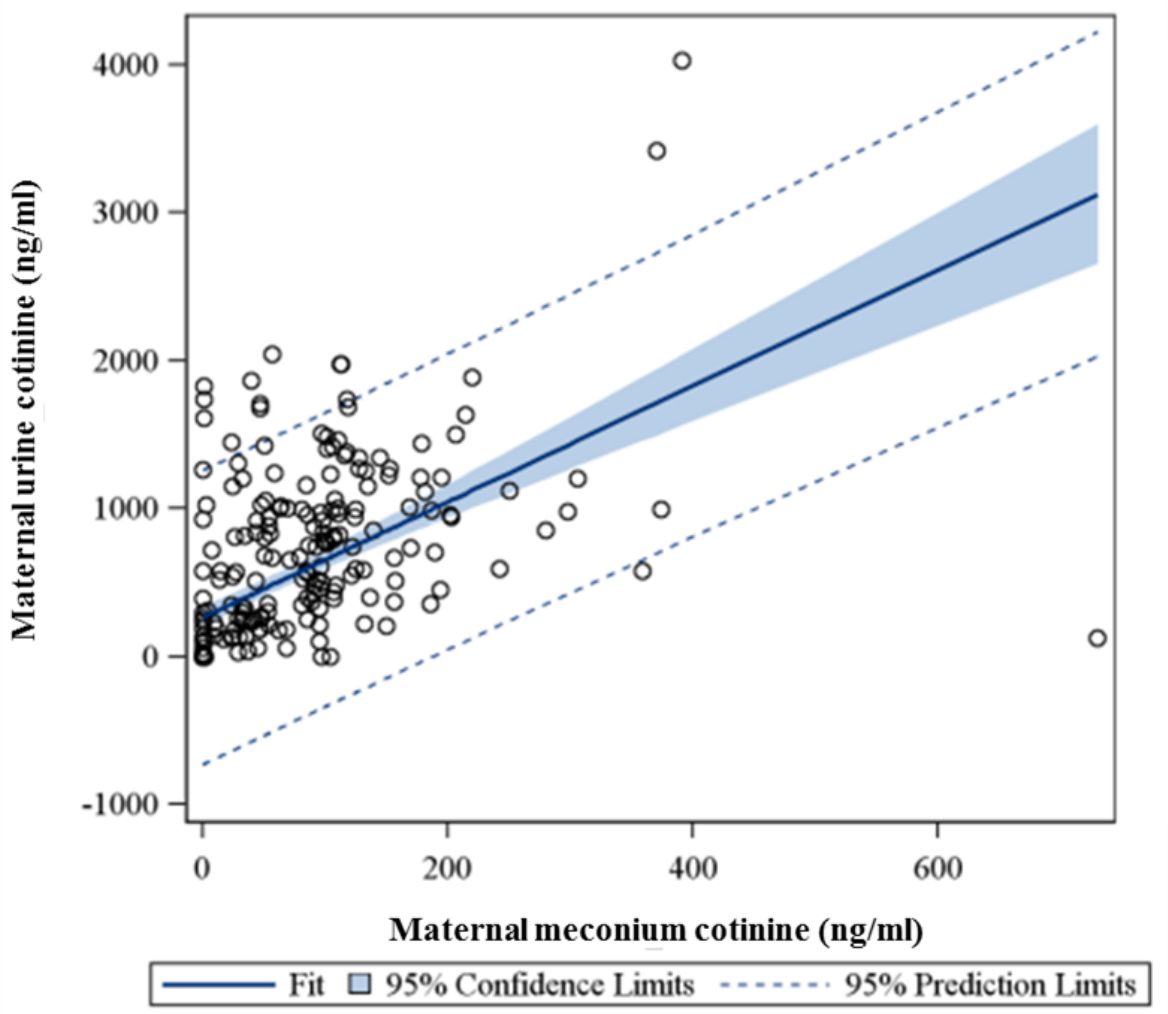
Figure 23. Maternal tobacco use in early pregnancy and risk of preterm birth. Odds ratios presented with 95% confidence interval.



6.5 ACCURACY OF SELF-REPORTED SNUFF USE

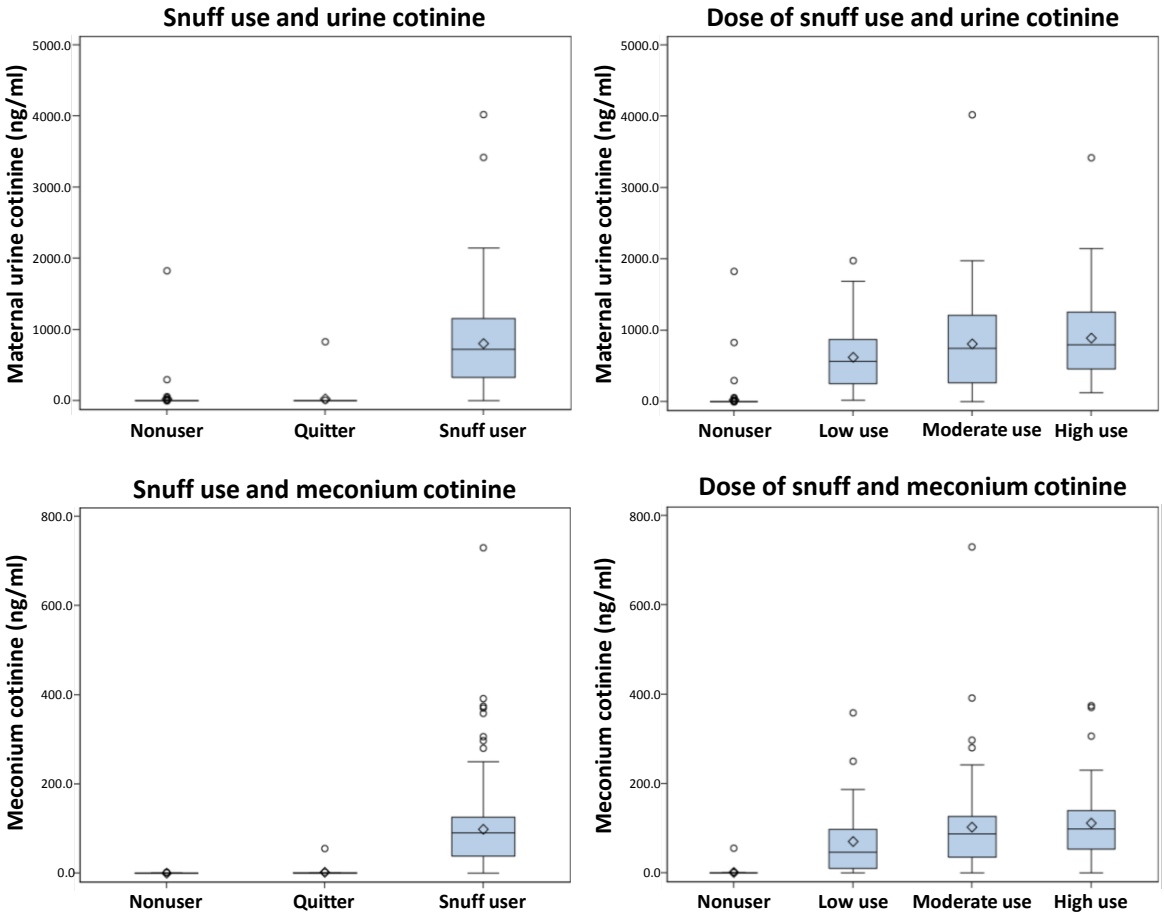
Self-reported snuff use in pregnancy was found to be valid (*paper IV*). There was a high agreement between self-reported use through questionnaires and maternal urine cotinine concentrations in late pregnancy. There was also a high agreement between self-reported snuff use in late pregnancy and cotinine in the meconium of the newborn. Furthermore, there was a correlation between maternal urine cotinine and cotinine in the meconium of the newborn, the correlation coefficient being 0.77 ($p<0.001$).

Figure 24. Correlation between the concentration of cotinine in maternal urine and in the meconium of the newborn.



There were also significant differences between low, moderate and high self-reported doses of nicotine and corresponding median cotinine levels in urine and meconium.

Figure. 25 Snuff use and cotinine levels in maternal urine and meconium



Misclassification in the Medical Birth Register

The information in the Medical Birth Register on snuff use in late pregnancy showed a high rate of misclassification. In the cohort, less than 15% stopped using snuff between the antenatal booking and week 32. However, according to the MBR register data, as many as 44% of the (same) cohort population were registered as nonusers in late pregnancy.

When validating the register data with cotinine levels in maternal urine, the sensitivity was only 50% (95% CI: 43-57) and the specificity was 98% (95% CI: 94-100). In late pregnancy, 45.5% of the MBR registered nonusers (i.e. registered non-users both in early and late pregnancy and registered quitters) had cotinine levels above cut-off level in maternal urine. As many as 77 % of participants who were registered as having ceased using snuff, i.e. registered as quitters, had maternal cotinine values above the cut-off level ≥15ng/ml.

Cotinine levels and neonatal growth outcomes

Of 381 participants in the study cohort, 15 (3.9%) women gave birth prematurely. Among snuff users, 11 (4.2%) infants were born preterm, and among nonusers 4 (3.4%) infants were born preterm. Among snuff users, two births were extremely preterm and two were very preterm, but they were all excluded because of missing information on cotinine as a consequence of the preterm birth. With them included, 4.9% were born preterm in the cohort, and the proportion of preterm birth among snuff users was 5.6%.

There was a significantly reduced gestational age of 3.3 days ($p<0.01$) for infants of mothers with cotinine levels in urine above cut- off, as compared to infants of mothers with cotinine levels below cut- off (Table 7a).

Table 7a. Urine cotinine levels and neonatal outcomes in paper IV, n= 307

Tobacco use	Crude			Adjusted*		Adjusted**	
	(95%CI)	mean difference	*p*	mean difference	*p*	mean difference	*p*
Gestational age							
Nonuser							
<3ng/ml	282(280-284)	ref		ref		-	-
Snuff user							
≥3ng/ml	278(277-280)	-4.13	<0.001	-3.32	<0.01	-	-
Birth weight							
Nonuser							
<3ng/ml	3,653(3,554-3,750)	ref		ref		ref	
Snuff user							
≥3ng/ml	3,588(3,513-3,662)	-66.1.	0.29	-99.2	0.11	-19.4	0.72
Length at birth							
Nonuser							
<3ng/ml	50.9(50.5-51.3)	ref		ref		Ref	
Snuff user							
≥3ng/ml	50.5(50.2-50.8)	-0.39	0.11	-0.44	0.08	-0.02	0.91
Head circumference							
Nonuser							
<3ng/ml	35.3(35.1-35.6)	ref		ref		ref	
Snuff user							
≥3ng/ml	35.2(35.0-35.4)	-0.06	0.67	-0.10	0.57	-0.11	0.49

Crude means calculated only for individuals with information on maternal characteristics and urine cotinine. Only participants who were categorized as nonusers or snuff users by both urine cotinine levels and self-reported information through questionnaires were included. Only singleton births were included.
**Adjusted for maternal characteristics, such as maternal age, bmi, co-habitant with father-to-be and parity.*
***Adjusted for maternal characteristics and gestational age.*

Infants with cotinine above cut-off level in their meconium also had a significantly reduced lower mean adjusted gestational age (-2.6 days, p<0.05) than that of infants with cotinine levels below cut-off in their meconium (Table 7b).

Table 7b. Meconium cotinine levels and neonatal outcomes, in *paper IV*, n= 283

Tobacco use	Crude			Adjusted*		Adjusted**	
	(95%CI)	mean difference	*P*	mean difference	*p*	mean difference	*p*
Gestational age							
Nonuser <3ng/ml	281.(280-284)	ref		ref		-	-
Snuff user ≥3ng/ml	278(277-280)	-3.61	<0.01	-2.63	<0.05	-	-
Birth weight							
Nonuser <3ng/ml	3650(3553-3748)	ref		ref		ref	
Snuff user ≥3ng/ml	3585(3502-3667)	-65.4	0.32	-98.8	0.12	-32.1	0.56
Length at birth							
Nonuser <3ng/ml	51.0(50.6-51.4)	ref		ref		ref	
Snuff user ≥3ng/ml	50.5(50.2-50.8)	-0.46	0.07	-0.47	0.07	-0.19	0.40
Head circumference							
Nonuser <3ng/ml	35.3(35.1-35.6)	ref		ref		ref	
Snuff user ≥3ng/ml	35.3(35.1-35.5)	-0.37	0.85	-0.05	0.77	-0.07	0.67

*Crude means calculated only for individuals with information on maternal characteristics and cotinine in meconium. Only participants who were categorized as nonusers or snuff users by both meconium cotinine levels and self-reported information through questionnaires were included. Only singleton births were included.*Adjusted for maternal characteristics, such as maternal age, bmi, co-habitant with father-to-be and parity.**Adjusted for maternal characteristics and gestational age.*

There were no significant differences in mean birth weight, birth length or head circumference adjusted for gestational age between newborns of women with maternal urine cotinine levels over and below cut-off , nor were there differences between newborn meconium cotinine levels over and below cut-off (cotinine level ≥ 3ng/ml) (Table 7 a and b).

7 DISCUSSION

"If younger adults turn away from smoking, the industry will decline, just as a population which does not give birth will eventually dwindle." RJ Reynolds researcher, 1984

The focus of this thesis is prenatal nicotine exposure in the form of oral moist tobacco, snuff and cigarette smoking and effects on the health of the newborn.

The rationale for the included studies was to study the differences between the effects of smoking and snuff on the health of the newborn. The adverse effects of smoking in pregnancy have been thoroughly investigated,[1, 45] and, likewise, animal studies have shown that prenatal nicotine is hazardous to the developing fetus.[83, 163] However, whether maternal tobacco use in the form of snuff is safe during pregnancy had not been elucidated.

Neonatal apnea

Maternal snuff use was associated with a twofold risk of neonatal apnea. This is the first study of snuff use and neonatal apnea, but previous studies on maternal smoking have found an association with neonatal apnea.[98] The risk of sleep apnea was increased almost threefold in infants of snuff users in comparison to that of nonusers.

The risk of apnea associated with maternal smoking was explained by the increased risk of preterm birth among smokers. In contrast, in infants of snuff users the risk was not only higher than in that of smokers, it also remained significant after adjustment for gestational age.

Neonatal apnea is a sign of disturbances in the cardiorespiratory system and is suggested to be associated with an increased risk of SIDS. Preterm birth is a strong risk factor for neonatal apnea, and the risk increases with decreasing gestational age.[164] Prematurity and maternal smoking are regarded as the most important risk factors for SIDS,[165] particularly since the back to sleep campaign was introduced.[93]

SIDS is a rare event, less than 0.2/1000 live born infants yearly in Sweden,[166] and the prevalence of snuff use in pregnancy is 1.1%. The association between snuff use and SIDS has not been possible to study because of lack of power.

SIDS is suggested to be related to a disturbed autonomous control with decreased arousal. In addition, a blunted response to hypoxia with a disturbed pattern of breathing, apnea and bradycardia is associated with the mechanisms behind SIDS.[94, 95, 165, 167]

Preterm infants and infants prenatally exposed to smoking show similar disturbances in the neural control of the cardiorespiratory system, with vascular, cardiac and blood pressure hyperactivity.[168] These similar findings thus represent an effect of immaturity in the premature infant and a developmental programming effect in the nicotine-exposed infant. As nAChRs are present as early as gestational week 4-5 in fetal development,[137] nicotine may carry out teratogenic effects by binding these receptors, induce apoptosis and affect cell programming.[104]

Nordenstam et al. found decreased heart rate variability among infants of smokers and snuff users compared to that of nonusers, also implying disturbances in autonomic control. A decreased heart rate variability was found also in infants of snuff users and smokers who had ceased using tobacco during pregnancy,[169] indicating early programming effects of nicotine on the development of the nervous system.

Furthermore, animal studies of prenatal nicotine exposure have shown similar disturbances in the cardiorespiratory system as those observed in infants of maternal tobacco use. Findings in animal studies of prenatal nicotine exposure include abnormalities in the sympathovagal balance, with an increased frequency of apneas and a blunted cardiorespiratory response to hypoxia. Changes in breathing patterns, such as a higher breathing frequency and a lower tidal volume in the neonatal, have also been observed.[102, 103, 151, 153] In addition, prenatal exposure to nicotine leads to a decreased arousal and ability to resuscitate, as well as to an impaired catecholamine release from the adrenal medulla, followed by a reduced cardiac response to adrenergic stimulation in rat pups.[104]

As mentioned, the plasma concentration of nicotine remains at a high level for hours after snuff use, in contrast to the rapid decline after smoking.[136] Nicotine also accumulates in the amniotic fluid[80] and the dose of nicotine to which the fetus is exposed may therefore be higher in infants of snuff users. This may be an underlying explanation for the higher risk of neonatal apneas in infants of snuff users than in infants of smokers.

Smoking during pregnancy is associated with both increased risk of preterm birth and SIDS.[97, 165] The increased risk of SIDS in smokers may therefore be an effect of both an immature nervous system caused by preterm birth and the toxic effect of nicotine on the autonomous nervous system.

Tobacco smoke and nicotine also affect the immune system,[81] which may influence both the susceptibility to infections and the subsequent inflammatory response.[170] Both preterm infants and infants of smokers are more prone to get respiratory infections,[121, 122 171] causing a mild hypoxia. Although such infections in most cases are benign, they may induce apnea and bradycardia. It is possible that this may cause a serious and even fatal event in the more vulnerable nicotine exposed infant. [170]

Oral cleft malformations

The association between snuff use in pregnancy and oral cleft malformations has not been studied previously. However, the finding that maternal smoking was associated with a moderately increased risk of oral cleft malformations is in agreement with previous studies on maternal smoking.[106, 172, 173] The Surgeon General states in their report from 2014 that there is sufficient evidence to conclude that a causal relationship between maternal smoking and oral cleft malformations exists.[2]

In the present study, smoking and snuff use in pregnancy were associated with increased risk of CL/P, but in contrast to previous studies on smoking in the MBR there was no increased risk of isolated CP.[172, 174] Previous literature is not consistent as to whether smoking increases the risk of both CL/P and CP. Most previous studies have found a relationship between smoking and CL/P,[120, 175-177] and others between both CL/P and isolated CP.[114, 178] In a meta-analysis Little et al. found a 34% risk increase of CL/P and a 22% increased risk of CP associated with maternal smoking.[106]

The lack of an association between tobacco use and isolated CP in *paper II* may be due to lack of power. Smoking in pregnancy and exposure to second hand smoke has decreased considerably in Sweden since the end of the 90's when the previous studies in the MBR were performed.[6] Furthermore, there is evidence of a dose-dependent association with oral cleft malformations.[178] It is possible that the different results between the present study and previous studies in the MBR may be that the dose of nicotine required to cause damage is higher for isolated CP than for CL/P.

The finding that both snuff use and smoking in pregnancy increase risk of oral cleft malformations implies that nicotine is involved in the mechanism. The risk was higher for snuff users than for smokers, which further strengthens this hypothesis. The dose of nicotine to which the fetus is exposed is likely to be higher in infants of snuff users than it is in infants of moderate, and possibly also heavy, smokers. Findings in vitro and from animal studies have shown disturbances in the fusion of the facial prominences, which further supports the theory that nicotine is involved in the mechanism.[115, 116, 179, 180]

Extremely preterm birth

Extremely preterm birth is rare, with an incidence of 0.3%-0.4% yearly in Sweden.[6] However, morbidity and mortality of preterm birth is increasing with decreasing gestational age, and extremely preterm birth is the major cause of neonatal death in most developed countries.[74] Both snuff use and smoking have previously been associated with preterm birth.[62] However, the association with extremely preterm birth (<28 gestational weeks) has not been studied previously in snuff users.

Maternal snuff use was associated with increased risk of extremely preterm birth and moderately preterm birth, but the risk of very preterm birth was only borderline significant. Maternal smoking was associated with increased risk of preterm birth in all gestational ages.

The risk increase was higher for smokers than for snuff users. The association with preterm birth and smoking was dose-dependent across all gestational ages.

In addition, smoking in pregnancy was associated with both spontaneous and medically indicated preterm birth in all gestational ages. Maternal snuff use was associated with a twofold increased risk of medically indicated extremely preterm birth and a moderately increased risk of both spontaneous and medically indicated moderate preterm birth.

The risk of preterm birth was higher for smokers than for snuff users but also dose dependent, which implies that both combustion products and nicotine are involved in the mechanism underlying preterm birth. It is possible that combustion products and nicotine have synergistic effects, leading to a high risk of preterm birth in smokers.

Several mechanisms through which smoking may contribute to preterm delivery have been suggested and include dysfunctional placentation, leading to placental abruption[58, 59, 72] and defect collagen synthesis, leading to premature rupture of the membranes.[3] Furthermore, smoking increases the risk of fetal growth restriction,[1, 45, 83] possibly via placental dysfunction, which is an important indication for medically indicated preterm birth.

Snuff use has not been shown to be associated with placenta previa or placental abruption. [60] However, there is a lack of studies exploring the associations between snuff use and placenta previa and placental abruption. The association between snuff use and risk of fetal growth is modest,[63] implying that although nicotine affects fetal growth, the fetal growth restriction caused by smoking is also due to placental dysfunction caused by combustion products.

Another important indication for medically indicated preterm birth is pre-eclampsia. Maternal smoking protects against preeclampsia, supposedly through the vasodilative properties of CO.[68, 71] In contrast, snuff use in pregnancy does not protect against preeclampsia.[70, 71] Maternal snuff use is associated with preterm but not term preeclampsia,[70, 71] suggesting that nicotine is involved in the early stages of the disease.[71] Furthermore, nAChRs are expressed in the preeclamptic placenta in nonsmokers, implying that nicotine is involved in the development of pre-eclampsia.[181] However, it was not possible to explore the association between preeclampsia and extremely preterm birth in snuff users *in paper III*, owing to lack of power.

Human labor is supposed to begin with functional progesterone withdrawal leading to decidual activation.[77, 182] At term, decidual activation seems to be mediated by the fetal-decidual paracrine system. In many cases of early preterm labor, decidual activation rather seems related to intrauterine bleeding or an intrauterine infection.[77] Smoking also affects the immune system, increasing inflammation and the susceptibility to infections.[81] It is thus possible that nicotine may influence preterm birth by an effect on the immune system,[81, 137] but also through the effect on the endocrine system inhibiting progesterone release.[183]

In conclusion, there is evidence suggesting that both nicotine and combustion products are responsible for increased risk of preterm birth, but the underlying mechanisms remain largely unexplained. Importantly, mothers who had stopped using tobacco (smoking or snuff use) very early in pregnancy, before the antenatal booking, had no increased risk of giving birth preterm.

Validation of self-reported snuff use

Most epidemiological studies on the effects of maternal smoking are based on self-reported information on tobacco use. The information in the MBR on maternal tobacco use is also based on self-reported information, with the advantage of prospectively collected data minimizing risk of recall bias. However, maternal smoking, and to some extent also snuff use in pregnancy, is a social stigma. With increasing knowledge of the hazardous effects of tobacco use in pregnancy there is a risk of underreporting use, which in epidemiological studies increase risk of misclassification, leading to both under- and overestimation of risks.[48]

Validation studies using cotinine as the golden standard have shown conflicting results. Most of them have concluded that self-reported use is valid,[184-187] but some have found a considerable proportion of underreporting of tobacco use.[47, 188] George et al. showed that self-reported maternal smoking is valid in Sweden.[184]

Most validity studies are based on women participating in a study cohort. Taking part in research may influence women to answer more truthfully than they would otherwise. Therefore, women willing to participate in research may not be representative of the general population smoking in pregnancy. Mattson et al. conducted a study on maternal smoking in the MBR, using cotinine in serum samples from the umbilical cord at delivery as well as maternal venous samples in early labor, showing that maternal self-reported smoking was valid in the MBR.[185] A methodological problem with the study was that self-reported smoking in early pregnancy at the antenatal booking was validated with cotinine in serum at delivery. Women who changed their smoking habits in pregnancy or ceased smoking were not taken into account.

Self-reported maternal snuff use has not previously been validated. Moreover, there is no information in the MBR on dose of snuff use in pregnancy, whereas smoking is categorized as moderate (1-9 cigarettes/day) and heavy (≥10 cigarettes/day) smoking.

Maternal self-reported snuff use in late pregnancy proved valid in our cohort. Interestingly, only14% reported cessation of tobacco use during pregnancy. According to the MBR data on the same individuals, 45% stopped using tobacco during pregnancy. Likewise, when validating the MBR information in late pregnancy with cotinine in maternal urine, the sensitivity was as low as 50%, whereas specificity was high, 98%. Of the registered quitters in the MBR in late pregnancy,77% had cotinine levels over the cut-off.

Surprisingly, none of the women who ceased using snuff in pregnancy used NRT. In addition, the few women actually using NRT were reporting high use of snuff simultaneously. This supports previous results of NRT not being effective for tobacco cessation in pregnancy.[135]

A possible explanation for the misclassification in late pregnancy in the MBR is that health care personnel fail to ask about snuff use, especially in late pregnancy. England et al. concluded that a large proportion of obstetrician/gynecologists never or inconsistently screen patients for smokeless tobacco products in the U.S. There was also lack of knowledge of potential adverse effects in pregnancy.[189] Another possible explanation may be difficulties in filling in the register form correctly.

Methodological strengths and limitations

Papers 1-III are all population-based cohort studies based on data from the Medical Birth Register. Self-reported tobacco use is prospectively collected, minimizing the risk of recall bias. Maternal socioeconomic characteristics are possible to obtain through linkage to the Education Register and the Register of Total Population.

However, register studies have a number of limitations. There is always a risk of differential misclassification of tobacco use with self-reported use not confirmed by biomarkers. Smoking in early pregnancy in the Medical Birth Register has been validated and proved reliable, but snuff use has not been validated previously. Register studies do not reflect patterns of tobacco use or dose of tobacco. Snuff use is merely categorized as yes/no. Use of NRT or electronic cigarettes are not registered, there is no information on the tobacco habits of the father-to-be and no information on exposure to second hand smoking.

There is also a problem of unmeasured confounding or residual confounding in register studies. Possible confounders, such as maternal substance use, alcohol consumption in pregnancy, maternal vitamin substitution and medications, and other life-style factors are not possible to obtain from the register. Smoking was associated with low socioeconomic status and low level of education. Snuff use was not to the same extent associated with low socioeconomic status and low education as was the case with smoking, a finding consistent with a study by Kvalvik et al. describing female smokers and snuff users in Norway.[186]

Paper IV is a cohort study with prospectively collected self-reported data where pattern of use and the dose of snuff use may be studied. Information on NRT and second-hand smoking was also possible to obtain. In addition, the self-reported tobacco use in late pregnancy was validated by cotinine in maternal urine and cotinine in the meconium of the newborn. The cohort was linked to the MBR register data, and validation of the MBR data in late pregnancy for snuff use was also possible. By comparing the cohort with a regional cohort in the MBR the same years, it was possible to conclude that the cohort was representative of the population, with the exception of the proportion of snuff users, smokers and nonusers.

However, there are limitations in the study design. The women included in the study were informed that self-reported snuff use would be confirmed by cotinine, which may influence the accuracy of the self-reported use There is also a risk of selection bias, i.e. women willing to participate in a validation study on snuff use may differ from the general population on basis of exposure. Another limitation is the categorization of snuff use. The high-use cut-off was defined as more than 7 pouches a day. This turned out to be too low, as the range of pouches used daily in the high-use category was very wide, 7- > 20 pouches a day. Therefore, a dose dependency may have been blunted.

Moreover, some possible confounding factors, such as consumption of alcohol, medication and use of vitamins were not obtained through the questionnaires. Information on tobacco habits of the father-to-be was not obtained either.

An important limitation is that the accuracy of self-reported snuff use in early pregnancy was not possible to obtain, because maternal urine cotinine was not analyzed in early pregnancy. Most register studies on maternal snuff use and smoking are based on self-reported use in early pregnancy, why validation in early pregnancy would have been very useful.

Conclusion and further perspectives

Nicotine is highly addictive, and smoking cessation in pregnancy has been found very difficult to achieve. Neither psychosocial interventions, nor NRT have proved successful.[2, 11, 132, 135] Use of nicotine is generally regarded as safer than smoking, and as a consequence snuff and new nicotine products, such as electronic cigarettes, are marketed as a safer alternative than smoking.[13, 34, 129] The adverse effects of nicotine on pregnancy and the developing fetus are often neglected in discussions of e-cigarettes or snuff as a means of smoke cessation and harm reduction.[32, 34, 129]

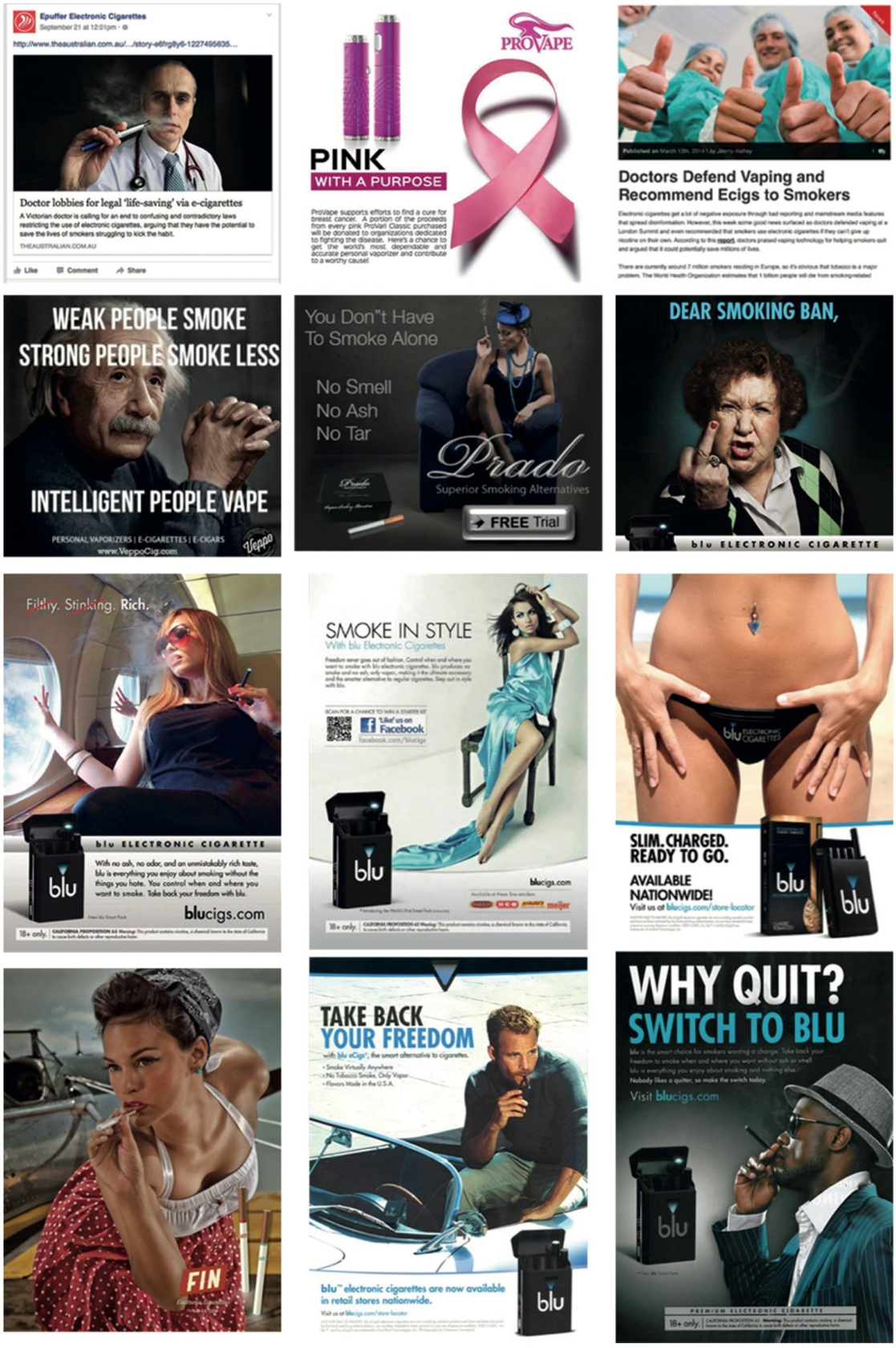
Because of population growth, the number of smokers is actually growing in the world despite decreasing prevalence in many countries. In some developing countries, smoking actually increases.[20] In developed countries a whole new market of nicotine and non-cigarette tobacco products, such as electronic cigarettes, has evolved, attracting young people in particular.[190] Among young people, aged 18 to 24, e-cigarettes are now more popular than conventional cigarettes.[191] E-cigarette use is strongly associated with the use of other tobacco products.[191] The use of new tobacco products and dual use of cigarettes and other nicotine sources place high demands on society and the health care professionals regarding preventive strategies. England et al. have shown that obstetricians seldom or never screen for nicotine products other than tobacco cigarettes and that the knowledge of adverse effects in pregnancy is limited.[189]

To conclude, snuff use in pregnancy has adverse effect on pregnancy and the health of the newborn. The mechanisms behind the harmful effects of smoking are not clear and for some conditions, such as fetal growth and possibly also preterm birth, both nicotine and combustion products seem to be involved. The risk increase of fetal growth restriction and preterm birth is higher for smokers than for snuff users, and there is evidence of combustion products affecting placental function.[2, 3] In contrast, the risks of adverse effects supposedly caused by nicotine, such as neonatal apnea and oral cleft malformation, seem to be higher in snuff users than in smokers.

Of those who cease smoking in pregnancy, the majority stop without counselling or use of NRT before the first visit to the prenatal clinic.[55, 133] However, among those who smoke at the antenatal booking, the majority continue to smoke throughout pregnancy. Pharmacological and psychological therapies have not proved effective for smoke cessation in pregnancy.[2, 132] Strategies for snuff cessation in pregnancy are lacking. In addition, because of lack of adherence to NRT, safety has been difficult to study.[135]

With growing knowledge about the detrimental effects of snuff, containing merely nicotine, caution is recommended regarding use of NRT and e-cigarettes in pregnancy. No safe level of prenatal nicotine exposure has been established.[192] Nicotine, in any form, is not to be considered safe and should be avoided in pregnancy.

Figure 26. Advertising for electronic cigarettes



Source: ***From the collection of Stanford Research Into the Impact of Tobacco Advertising (tobacco.stanford.edu).***

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10 APPENDICES

A. Questionnaires used in paper IV