Maternal smoking during pregnancy - Long-term health effects in the offspring

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Long-term health effects in the offspring

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Abstract:

Globally, around 10 % of women smoke during pregnancy today. It is known that pregnancy smoking increases the risk of adverse short-term health effects in the offspring, such as preterm birth, low birthweight and spontaneous abortion. Less is known about whether any adverse health effects persist until adulthood.

In Sweden, there are nationwide population-based health registers that are becoming intergenerational, which lend themselves well for the study of such associations. This thesis is primarily based on the Swedish Medical Birth Register, which covers almost all births in Sweden since 1973. Additional data sources include a perinatal quality register (Perinatal Revision South Register), a regional biobank (Malmö Maternity Unit Serum Biobank), and prospective clinical study cohorts including all children who developed type 1 diabetes in Skåne between 1999 and 2005 (The Diabetes Prediction in Skåne Cohort, The Skåne Study and Better Diabetes Diagnosis Study).

The aim of this thesis was to investigate if maternal smoking during pregnancy was associated with long-term health effects in her offspring, by specifically looking at the risk of childhood type 1 diabetes, as well as the risk of obesity, gestational diabetes and preeclampsia in adult women. We also investigated the validity of the self-reported smoking data in the Medical Birth Register, by the use of biomarker measurements.

We found a higher risk of both type 1 diabetes, obesity and gestational diabetes in those exposed to tobacco smoking prenatally, but less consistent associations with preeclampsia. The validity of the self-reported smoking data in the Medical Birth Register was found to be high.

In conclusion, our studies suggest that maternal pregnancy smoking could have long-term health effects for her children, so there is reason to continue to make efforts to help women quit smoking when they are pregnant. When performing research on pregnancy smoking, Swedish register-data are of good quality and can be used.

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Maternal smoking during pregnancy, long-term health effects, Medical Birth Register, register validation, biomarker

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Possession of knowledge does not kill the sense of wonder and mystery. There is always more mystery.

- Anaïs Nin

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List of papers

This doctoral dissertation is based on the following four original publications:

I. **Mattsson K**, Jönsson I, Malmqvist E, Larsson HE, Rylander L. Maternal smoking during pregnancy and offspring type 1 diabetes mellitus risk: accounting for HLA haplotype. *Eur J Epidemiol*. 2015; 30(3);231-238.

II. **Mattsson K**, Källén K, Longnecker MP, Rignell-Hydbom A, Rylander L. 2013 Maternal smoking during pregnancy and daughters' risk of gestational diabetes and obesity. *Diabetologia*. 2013; 56(8): 1689-1695.

III. **Mattsson K**, Källén K, Rignell-Hydbom A, Hansson SR, McElrath T, Cantonwine D, Rylander L. Maternal smoking and daughters' risk of preeclampsia. *(Invited to revise; revision submitted)*

IV. **Mattsson K**, Källén K, Rignell-Hydbom A, Lindh CH, Jönsson BAG, Gustafsson P, Olofsson P, Ivarsson SA, Rylander L. Cotinine validation of self-reported smoking during pregnancy in the Swedish Medical Birth Register. *Nicotine Tob Res*. [Epub 20 April 2015]

Abbreviations

BMI	Body Mass Index
CI	Confidence Interval
DiPiS	Diabetes Prediction in Skåne
DOHaD	Developmental Origins of Health and Disease
ETS	Environmental Tobacco Smoke
G1	First Generation
G2	Second Generation
GDM	Gestational Diabetes Mellitus
HLA	Human Leukocyte Antigen
ICD-8-10	International Classification of Diseases, 8th-10th revision
MBR	Medical Birth Register
OR	Odds Ratio
PRSR	Perinatal Revision South Register
SGA	Small for Gestational Age
T1D	Type 1 Diabetes
T2D	Type 2 Diabetes
WHO	World Health Organization

Note: Abbreviations are mainly used in the published articles, attached at the end of this thesis, and only sparingly in the main text. Sometimes an abbreviation has been added if the concept is commonly known in this form (such as ADHD, DNA, IVF).

Svensk sammanfattning

Idag röker cirka 6% av alla gravida kvinnor i Sverige. I vissa regioner är andelen så hög som en av tio gravida kvinnor. Trots att det betyder att det har skett en kraftig nedgång sedan man började mäta utbredningen av graviditetsrökning i början på 80-talet, visar siffror att denna nedgång verkar ha avstannat.

Det är känt sedan länge att rökning under graviditeten är skadligt för barnet på kort sikt, med en ökad risk för bland annat för tidig födsel, låg födelsevikt och spontan abort. Däremot vet man fortfarande mycket lite om huruvida det kvarstår några negativa hälsoeffekter upp i vuxen ålder av att ha utsatts för tobaksrökning under fosterlivet. Detta är till stor del på grund av att det fram tills nu har saknats data som man kan använda för att undersöka samband som spänner över så lång tid.

I Sverige finns stora register som täcker hela befolkningen, som lämpar sig väl för att undersöka sådana frågeställningar, då många av dessa register är flera decennier gamla. Ett sådant register är det Medicinska Födelseregistret, som i princip täcker alla födslar i Sverige sedan 1973. Den huvudsakliga datan för detta projekt är hämtad från detta register.

Syftet med den här avhandlingen var att undersöka potentiella hälsoeffekter i det långa perspektivet av att ha utsatts för tobaksrökning under sitt fosterliv. Mer specifikt ville vi undersöka om en sådan exponering var relaterad till en högre risk för typ 1-diabetes hos barn, samt övervikt, graviditetsdiabetes och havandeskapsförgiftning hos vuxna kvinnor.

Eftersom denna forskning är baserad på självrapporterad registerdata om rökvanor, var ett ytterligare syfte även att undersöka kvaliteten på rökdatan i Medicinska Födelseregistret med hjälp av en biomarkör för nikotinexponering (kotinin).

Vi fann i våra studier att risken ökade för både övervikt, graviditetsdiabetes och typ 1-diabetes om man varit utsatt för tobaksrökning under fosterlivet. Möjligen kunde man också se en svag riskökning för havandeskapsförgiftning, men här var sambanden mindre robusta. Ett viktigt fynd ur metodologisk synvinkel var att kvaliteten på rökdatan i Medicinska Födelseregistret befanns vara hög, varför den går bra att använda i epidemiologisk forskning.

Sammanfattningsvis kan sägas att fosterexponering för tobaksrökning kan ha långtgående konsekvenser, så det finns goda skäl att fortsatt försöka hjälpa kvinnor att sluta röka när de blir gravida.

Abstract

Globally, around 10 % of women smoke during pregnancy today. It is known that pregnancy smoking increases the risk of adverse short-term health effects in the offspring, such as preterm birth, low birthweight and spontaneous abortion. Less is known about whether any adverse health effects persist until adulthood.

In Sweden, there are nationwide population-based health registers that are becoming intergenerational, which lend themselves well for the study of such associations. This thesis is primarily based on the Swedish Medical Birth Register, which covers almost all births in Sweden since 1973. Additional data sources include a perinatal quality register (Perinatal Revision South Register), a regional biobank (Malmö Maternity Unit Serum Biobank), and clinical study cohorts including all children who developed type 1 diabetes in Skåne between 1999 and 2005 (The Diabetes Prediction in Skåne Cohort, The Skåne Study and Better Diabetes Diagnosis Study).

The aim of this thesis was to investigate if maternal smoking during pregnancy was associated with long-term health effects in her offspring, by specifically looking at the risk of childhood type 1 diabetes, as well as the risk of obesity, gestational diabetes and preeclampsia in adult women. We also investigated the validity of the self-reported smoking data in the Medical Birth Register, by the use of biomarker measurements.

We found a higher risk of both type 1 diabetes, obesity and gestational diabetes in those exposed to tobacco smoking prenatally, but less consistent associations with preeclampsia. The validity of the self-reported smoking data in the Medical Birth Register was found to be high.

In conclusion, our studies suggest that maternal pregnancy smoking could have long-term health effects for her children, so there is reason to continue to make efforts to help women quit smoking when they are pregnant. When performing research on pregnancy smoking, Swedish register-data are of good quality and can be used.

Aims

The overall aim of this thesis was to examine long-term health effects associated with prenatal smoking exposure, by specifically investigating the risk of:

i. Childhood health effects, in the form of type 1 diabetes.

ii. Adult health effects, in the form of obesity.

iii. Pregnancy-related health effects, in the form of gestational diabetes and preeclampsia, when women exposed to smoking during their fetal life became pregnant themselves.

The second aim was to examine the validity of the self-reported data on maternal pregnancy smoking in the Swedish Medical Birth Register, by the use of biomarker measurements.

Background

Tobacco smoking

The smoke from a common cigarette typically contains between 4000 and 7000 chemicals, including nicotine, tar, carbon monoxide, arsenic, ammonia, benzene, formaldehyde, hydrogen cyanide, cadmium, DDT and a long list of other toxins and carcinogens.[1] One third of people who have tried smoking become daily users, and the addictive potential of nicotine has been compared to that of heroine and similar drugs.[1,2] The strong addictiveness might contribute to explaining why smoking is still quite common in the world, even though some of its harmful health effects have been known since the early 1960ies when the Surgeon General report (linking cigarette smoking to lung cancer) was first published.[3] However, that the prevalence of smoking is highly dependent on the social and cultural context is evident when comparing percentages of smokers between different countries. The social stigma (or lack thereof) surrounding tobacco smoking, as well as the legislation concerning smoking in public places, smoking taxation and tobacco advertisement, differ between countries, and are likely important factors in determining the degree of smoking.[4]

Worldwide, approximately 22.5 % of adults (32 % of males and 7 % of females) smoke tobacco, which in absolute numbers equals about 1 billion people.[5] It is to note that 12 % of all male deaths, and 6 % of all female deaths are attributable to tobacco use, some of which are due to exposure to second-hand smoke.[6] These numbers are predicted to increase if no measures are implemented to decrease the use of tobacco products.[6]

The prevalence of smoking varies between regions in the world (although variation is lesser than that of for example alcohol), as seen in Table 1 on the next page.

Table 1.
Prevalence of smoking any tobacco product among adults ≥ 15 years of age by WHO region. Data from 2012. (Source: World Health Organization, retrieved online August 2015)

	% Smoking	
WHO Region	Males	Females
Africa	24.2	2.4
America	22.8	13.3
South-East Asia	32.1	2.6
Europe	39.0	19.3
Eastern Mediterranean	36.2	2.9
Western Pacific	48.5	3.4

In this context, Sweden has comparably low numbers of daily smokers (national average about 12 %), but if smokeless tobacco products such as snuff are included, the proportion of daily tobacco users increases substantially (22.7 %) and is on par with the rest of the industrialized countries.[7] Sweden is the only country in the world where smoking is more common among women than men, even if the difference is relatively small (12.4 % of females versus 11.2 % of males).[7] The reason for this is probably multi-faceted, but two plausible contributing explanations could be i) Swedish men have a long-standing tradition of an alternative in snuff, and ii) Swedish women have, in comparison to some other countries, more freedom in deciding their own life-style, allowing them access to habits otherwise typically either reserved for, or traditionally dominated by, men. Indeed, the general trend in most countries is towards a less pronounced gender difference in smoking patterns.[8]

Maternal smoking during pregnancy

Today, on average 6 % of pregnant women in Sweden smoke.[9] In the early 80ies when pregnancy smoking was first registered on a national basis, around 30 % of women smoked during their pregnancy, so a marked decline over the years is evident.[9] However, this decline has plateaued during recent years, and since there is a regional variation in pregnancy smoking, there are areas where the prevalence is markedly higher (amounting to about 10 %).[9] If stratification is made by age, the pregnancy smoking rate in mothers under the age of 19 is around 22 %.[9] Figure 1 on the next page shows the development of smoking during pregnancy over time in Sweden.

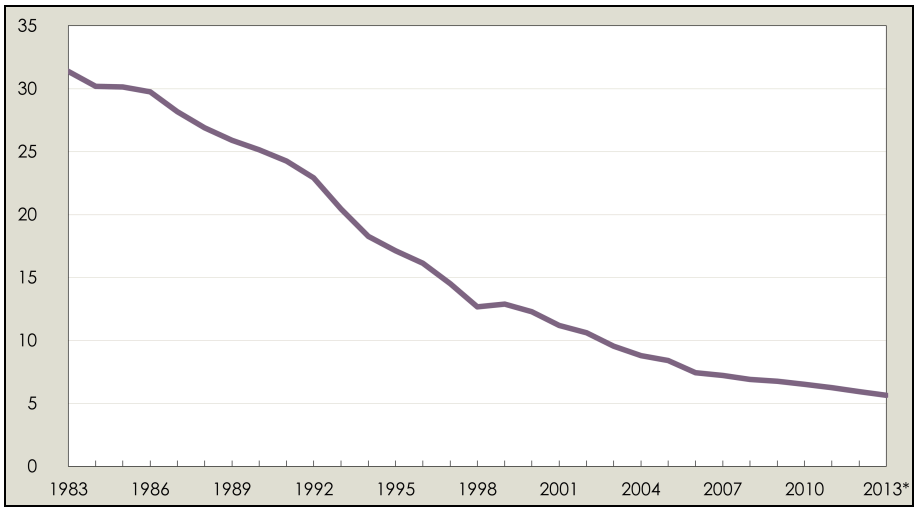

Figure 1.
Percent women smoking during pregnancy in Sweden between 1983 and 2013. (Source: National Board of Health and Welfare, "Graviditeter, förlossningar och nyfödda barn", 2014.[10])

Caution is called for when comparing prevalence rates regarding smoking during pregnancy between countries, as many lack national statistics on these aspects, and the numbers available are collected in non-standardized manners during different parts of pregnancy. But from where data are available, again the prevalence in Sweden is lower compared to many other European countries, where pregnancy smoking amounts to around 12-17 % (in countries like Denmark, Finland, the Netherlands, United Kingdom, Poland, Croatia, Greece and France).[11,12] Many countries in the world, in particular low- and middle-income countries, show substantially lower numbers than Sweden; however, this is likely rather mirroring the generally lower smoking prevalence among women in these countries than a specific pregnancy-related health consciousness.[13]

It should be noted, considering the well-developed infra-structure surrounding maternal health care in Sweden, where almost all pregnant women attend the free maternal health care visits,[14] and where smoking and other life-style behaviors are routinely discussed, the opportunities for reaching and helping women to stop smoking during pregnancy are probably better in Sweden than in many other countries. It is indeed since 2010 an expressively phrased goal of the Swedish government to protect children from the harmful effects of tobacco use of others.[15] In the light of this, and in the light of the comparably strong social stigma surrounding women who smoke when they are pregnant in Sweden, 6 % is not necessarily a low number.

In addition to smoking, some pregnant women use smokeless tobacco, snuff. Although the prevalence is low (around 2 % in Sweden for the years 1999 to 2009),[16] snuff use is becoming increasingly common among young women.[17]

The prevalence of pregnancy smoking is declining in most industrialized countries,[18] but there is an increasing worry that the reported increase in daily smoking among young women could result in this decline coming to a halt, or even be reversed.[19] Another potential cause for concern is that the smoking prevalence among especially women in low- and middle-income countries is expected to increase, following expected societal shifts and aggressive tobacco marketing tailored at these groups.[20]

Who smokes when they are pregnant?

Maternal pregnancy smoking in Sweden and other European countries shows an association with several socio-demographic factors. There are more smokers among younger mothers, and among those with lower educational attainment.[9,12,21] Smoking is more common among women living alone, with many previous children, among women not working and in cases where the pregnancy was unplanned.[12,21] A smoking partner, or own heavy smoking before pregnancy, are also risk factors for continued maternal smoking during pregnancy, as is a presence of substance abuse or alcohol use during pregnancy.[12,21] Figure 2 below shows pregnancy smoking by age, and Figure 3 (next page) by educational attainment, respectively.

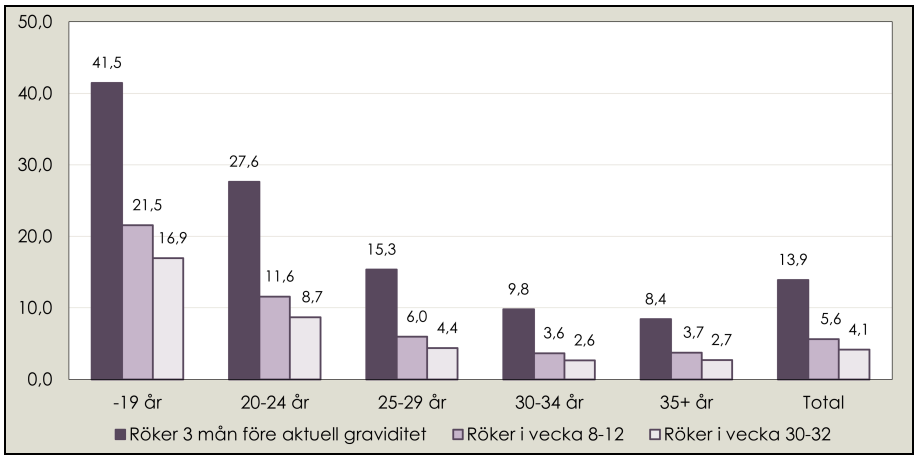

Figure 2.
Percent pregnancy smoking by age in Sweden (2013). Picture labels from left to right: ”Smoking 3 months prior to current pregnancy”, ”Smoking in week 8-12” and ”Smoking in week 30-32”. (Source: National Board of Health and Welfare, ”Graviditeter, förlossningar och nyfödda barn”, 2014.[10])

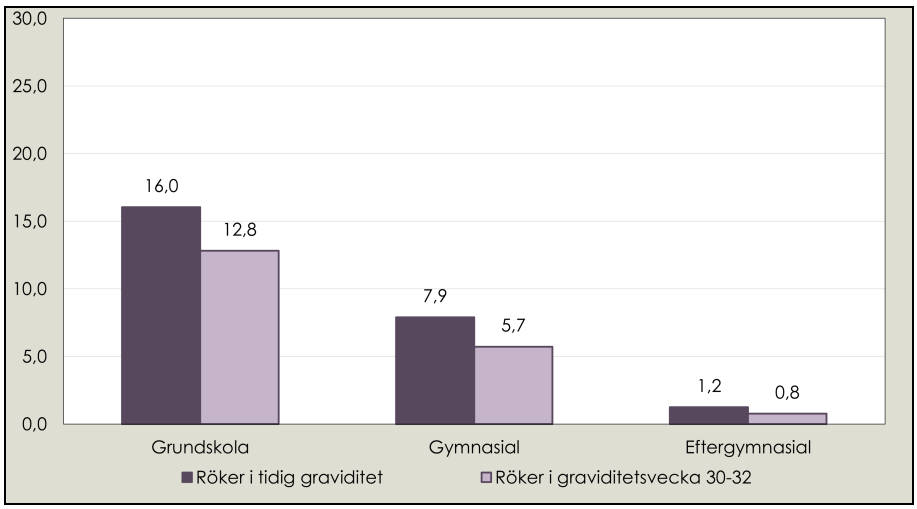

Figure 3.
Percent smoking by educational attainment among Swedish women (2013). Picture labels from left to right: ”Primary school”, ”Upper secondary school” and ”Post-secondary education”, and ”Smoking in early pregnancy” and ”Smoking in week 30-32, respectively. (Source: National Board of Health and Welfare, ”Graviditeter, förlossningar och nyfödda barn”, 2014.[10])

Snuff use among pregnant women is less common than smoking, and does not show the same socio-demographic determinants; it is found almost exclusively among Swedish-born women, and in contrast to cigarette smoking, to a higher degree among older women, women of higher education, and with stable family situations.[16]

Many smoking women do quit smoking at some point before or during their pregnancy, as evident in Figure 2 where there is a notable difference between smoking three months prior to the pregnancy, as compared to smoking at the first antenatal visits. Studies have reported numbers between 20-50 % of women quitting smoking before the first antenatal visit.[16,18] In this context, pregnancy has been identified as a window of opportunity for long-lasting smoking cessation, as many women are motivated to provide a healthy environment for their babies.[22,23]

Adverse health effects of smoking during pregnancy

Placental transfer

Although not all toxic chemicals found in cigarette smoke have been studied in terms of their transfer over the placenta, studies have shown a variety of different xenobiotics and toxic substances in amniotic fluid, umbilical cord blood and

meconium.[24] Many of these are known to be found in cigarettes, such as cadmium, lead, arsenic and other heavy metals, as well as different pesticides such as DDT. It is thus fairly likely that the fetus is exposed to almost the entire content of tobacco smoke. Further, most of the substances in cigarettes have low molecular weights and are water-soluble, facilitating placental transfer.[25]

Indeed, the term ”placental barrier” was never intended as a description of the placenta shielding the fetus from the outside world, but rather, and still somewhat misguidingly, simply denoting the thin layers of epithelial cells keeping the maternal and fetal circulation apart.[26] It is a requirement for fetal survival that this interface is semi-permeable, allowing oxygen and nutrients in, and waste out, through passive or active transport, which has been known for a considerable proportion of medical history.[26]

Smoking during pregnancy may harm the fetus either by affecting the fetus directly, or by affecting the morphology and function of the placenta.[25] The constituents in tobacco smoke most often discussed as substances mediating the physiological effects are nicotine and carbon monoxide, but as noted, there is a wide range of potential toxic culprits.

Nicotine and its metabolites pass readily over the placenta, and are found in fetal circulation, sometimes in an exposure concentration relatively higher to that in maternal serum.[27-29] Nicotine is highly vasoactive and impairs placental development and vasculature, affecting for example uterine blood flow. This may result in decreased oxygen and nutrient supply for the developing fetus, which could possibly explain some of the health effects seen in pregnancies where the mother smokes.[25,30,31]

Offspring short-term health effects

There is robust scientific evidence of adverse perinatal events and short-term infant health effects associated with maternal smoking during pregnancy, including a higher risk of fetal growth restriction, pre-term birth, certain types of congenital malformations (such as oral-facial clefts), stillbirth and spontaneous abortion.[18,32-34] The association between smoking during pregnancy and lower birthweight of the baby (by about 150-200 g) is now generally considered causal.[18]

There are several other pregnancy complications that show an association with maternal smoking, including ectopic pregnancies, placental abruption, placenta previa and premature rupture of the membranes.[35] Additionally, babies of smoking mothers have an increased risk of neonatal mortality and sudden infant death syndrome.[18,36]

In addition to active maternal smoking, similar perinatal outcomes are reported for exposure to environmental tobacco smoke (passive smoking or second-hand smoke) during pregnancy, although effects tend to be of smaller magnitude.[37,38]

Smokeless tobacco, such as snuff, is increasing among women, as well as the use of the product during pregnancy.[17] Snuff use means the exposure to some of the combustion products yielded by cigarettes are avoided, but peak plasma nicotine levels are as high as through cigarette smoking. In fact, the accumulated concentration is arguably higher, as snuff has a longer concentration peak.[39] The harm of snuff use has been debated, but large population-based epidemiological studies have subsequently been published and findings to-date show that babies exposed prenatally are at higher risk to be stillborn, born pre-term and small-for-gestational age.[40-43]

Offspring long-term health effects

The question of whether there exist any long-term adverse health effects, i.e. beyond the perinatal period, has attracted growing interest over the last decades. Scientific literature is rapidly emerging on various health conditions that show an association to having been exposed to tobacco prenatally, ranging from a wide array of metabolic and cardiovascular outcomes, to several aspects of psychiatric functioning and different respiratory and allergic conditions. Many of these studies show an increased risk of disease in childhood, adolescence and adulthood. There are for example reports on a higher risk of obesity,[44,45] increased blood pressure and other markers of cardiovascular health,[46,47] asthma,[48-50] and neurodevelopmental outcomes such as attention-deficit hyperactivity disorder (ADHD).[51,52]

Such findings have lead to the formation of a conceptual framework with proposed theoretical explanations and biological underpinnings for the findings briefly summarized above. This framework is known as the ”Developmental Origins of Health and Disease”.

Developmental Origins of Health and Disease

The Developmental Origins of Health and Disease (DOHaD), also sometimes referred to as the Barker hypothesis or fetal programming, is essentially proposing that the foundation for subsequent adult health is laid down already during fetal and early life. Although strictly speaking, they were not the first to have this idea, the spark that ignited this field was when David Barker and Clive Osmond published their report on a geographical association between high neonatal mortality and later cardiovascular mortality, suggesting that poor prenatal nutrition was the explanatory factor for this association.[53]

This set of a surge of observational studies linking birthweight (considered a proxy for prenatal nutrition) to different adverse health outcomes in adulthood, and today there are findings showing associations to so different outcomes as cardiovascular disease,

metabolic diseases including obesity and type 2 diabetes, respiratory disease such as asthma, certain forms of cancer, autoimmune diseases and musculoskeletal problems such as osteoporosis.[54]

The base for the hypothesis is developmental plasticity, which roughly can be interpreted as a stimulus or an insult during a certain critical period having long-lasting effects on function and morphology of tissue and organs.[55] In other words, the organism is, in response to its experiences during this sensitive time, ”programmed” to be better adapted for a stipulated later environment. This adaptive response is an advantage for the individual, provided that these early stimuli correctly predict the future environment.

The risk of disease is increased if there is a mismatch between these fetal or early-life experiences and the later environment. Using the example of fetal nutritional deprivation, some support has been presented by findings from historical ”natural experiments”. Data from two periods of starvation during World War II – the Leningrad siege (1941-1944) and the German occupation of the Netherlands leading to the ”Dutch Hunger Winter” (November 1944 to April 1945, with food rations being so low as 400-800 kcal per day) – indicated that individuals experiencing starvation in utero did not have an increased risk of coronary heart disease, obesity or diabetes when they were born into an environment where nutrition was equally scarce, as was the case during the Leningrad siege.[56] However, when starvation was experienced only during the fetal period, but not postnatally, exposed individuals showed an increased risk of coronary heart disease.[57]

Other than the two quasi-experimental examples of prenatal starvation above, human studies of early-life experiences and adult health are typically purely observational, and experimental testing of the hypothesis and its biological correlates and mechanisms is generally confined to animal models. The latter have been extensively used as a proof-of-principle for the hypothesis, as a wide variety of small and large animals have demonstrated morphological effects on tissue types such as the pancreas, kidneys, muscle and brain following different intrauterine nutritional and toxicological exposures.[58]

Although nutrition was the original determinant of interest, with birthweight as the proxy possible to study, the hypothesis has since then been extended to include other potentially adverse prenatal exposures, such as smoking, maternal stress and different environmental toxins.

Health over the life-course

To understand what determines our health and disease risk over the life-span is immensely difficult, even when focus is confined (correctly or not) to consider

environmental factors only. One epidemiological approach with this goal is life-course epidemiology. It has been defined as the "study of long-term effects on later health or disease risk of physical or social exposures during gestation, childhood, adolescence, young adulthood and later adult life".[59] Reading this definition, some overlap with the concept of Developmental Origins is evident, although the life-course approach expands into a wider consideration of health determining factors in adulthood and the social environment. Crudely, there are two principal models that are often considered in this context – it should be noted that the two models are not mutually exclusive.

Explanatory models

Sensitive/critical period model

The sensitive/critical period model might be seen as two models, but with considerable overlap. The idea is that there are critical or sensitive time periods in an organism's development, during which insults might result in long-term effects on morphology and function of cells, organs and other tissues. If the exposure happens outside the critical period, it has no effect. One example of critical periods are certain phases of fetal development, for example limb or brain development, where exposures might result in irreversible damage (e.g. thalidomide exposure and birth defects). The Developmental Origins of Health and Disease paradigm is one expression of the concept of a sensitive period.

Accumulation of risk model

This model states that it is the accumulation of insults or other adverse environmental conditions or health behaviors over the life-course that determines an individual's health. One aim of this approach is to investigate the extent to which such accumulating conditions damage biological systems, by exploring number, duration and severity of such insults. This is of particular interest when trying to better understand the link between socio-economic context and health.

Thesis health outcomes

There are several potential health outcomes that could be relevant in relation to fetal smoking exposure, as has been shown by previous research findings. We aimed at expanding the existing literature by investigating health endpoints that previously had been linked to smoking exposure, either pre- or postnatally, but where additional research was warranted. Additionally, we wanted to investigate diseases or conditions that are relevant from a public health perspective, and we wanted the outcomes to reflect potential health issues in different parts of the life-course, as noted in the investigation of both childhood and adult health conditions.

Childhood health effects – Type 1 diabetes

Type 1 diabetes (T1D), also known as insulin-dependent diabetes, is an auto-immune disease with a multi-factorial etiology, where a combination of both genetic and environmental factors lead to a permanent destruction of the insulin-producing beta cells in the pancreas. For the patient, this results in a life-long dependence on external insulin, coupled with a high risk of diabetic complications, and at current, there is no permanent remedy for the disease.

The incidence of type 1 diabetes is increasing, and Sweden has, after Finland, the second highest incidence of T1D in the world.[60] The most rapid increase is among the younger age groups, but for the individual the risk of disease onset peaks at 10-14 years.[60] An explanation is lacking as to why the incidence is increasing so markedly, but seeing as genes on a population-level tend to change slowly, environmental factors, or gene-environment interactions, are reasonable hypotheses.

Other than a consensus regarding the interplay between genes and environment, little is still known about the etiology and specific risk factors for the disease. A pathogenic model has been proposed, where a triggering event sets of the auto-immune process (as seen by circulating pancreatic islet cell auto-antibodies) in a genetically predisposed individual.[61] This process is then further driven or modified by additional factors, until the loss of beta cells leads to clinically manifested diabetes. The presence of auto-antibodies (as a sign of auto-immunity) strongly predicts manifest diabetes - almost 90 % of children with diabetes have one or more of these antibodies and the positive predictive value is up to 80 %.[62]

There are generally few environmental risk factors triggering the development of type 1 diabetes that unequivocally are agreed upon, although several have been described and investigated.[63] Since islet auto-immunity can be present long before clinical onset, focus has often been on the pre- and perinatal period and different types of stressors for the mother and baby. Factors that have been suggested to increase the risk include enterovirus infections, being born large-for-gestational age, pregnancy complications such as preeclampsia and Caesarean section, dietary factors such as early introduction to cow's milk, vitamin D insufficiency and short or no breastfeeding.[61,64,65] However, for most of these factors, evidence is circumstantial.

Prior research on the association between maternal smoking during pregnancy and offspring type 1 diabetes risk has repeatedly shown maternal pregnancy smoking to be associated with a reduced risk of offspring diabetes,[66-71] although this has not been consistently demonstrated in all studies.[72-74]

There are few hypotheses providing plausible biological mechanisms for these inverse associations, and notably, none of these earlier epidemiological studies have been able to account for genetic predisposition of the disease in their designs. There is reason to believe that this area could warrant further exploration, because i) there is an important contribution from genetic factors in regards to susceptibility in

development of type 1 diabetes, demonstrated by a probandwise concordance between twins of around 20-50 %,[75,76] and ii) there are increasing epidemiologic evidence of gene-environment interactions for many environmental substances, including those of tobacco smoke, such as for example arsenic.[77]

Genetic predisposition of type 1 diabetes

In regards to genetic predisposition and type 1 diabetes, the human leukocyte antigen system (HLA system) has been identified as the most important area mediating genetic susceptibility (explaining up to around 50-60 %).[78,79]

The HLA region is a highly polymorphic part of our genetic material that plays an important role in immune functioning, since it encodes the cell surface molecules responsible for presenting antigens to the immune cells. This is a central step of normal immune response, as it lets the body distinguish foreign material.

There are certain loci of the HLA region that are specifically important in relation to diabetes susceptibility. The risk associated with a HLA haplotype is determined by the combination of susceptible and protective alleles. Around 90 % of children with type 1 diabetes have at least one of the HLA alleles associated with high-risk haplotypes,[80] and for a sibling sharing the same high-risk HLA with the proband, the risk of auto-immunity has been shown to be 85 %.[81]

Considering the importance genetic plays in diabetes development, some information on this is warranted when studying the relationship between tobacco and development of the disease.

Adult health effects – Obesity

Obesity is recognized by the World Health Organization as one of the most important factors for mortality and morbidity in the world.[82] There has been a steep increase in the prevalence of overweight and obese individuals the past decades, so much so that the term ”obesity epidemic” is sometimes used.[83] This increase in obesity rates is also seen among children, and problems with overweight is now evident in increasingly younger ages.[84]

Genetics plays a role in the risk of becoming overweight or obese, but an obesogenic environmental setting is required for its expression. Although adult life-style factors, such as diet and exercise, explain most of an individual’s risk of becoming overweight, there is an emerging body of literature pointing towards a possible contribution of prenatal determinants of obesity.[85,86]

Reflecting the need for long follow-up times for the study of these associations, the risk of obesity in childhood has been most widely studied, and fewer studies have been published on adult weight status. This has similarly been the case when maternal smoking has been investigated. A large amount of studies have been published, which

subsequently have been reviewed on multiple occasions, showing an increased risk of childhood overweight if the mother smoked during pregnancy.[44,45,87] The results from these studies indicate a relationship quite consistent over different populations.[87]

It is less clear if these associations persist until adulthood, as results from previous research are inconsistent.[88-90] One large Norwegian birth cohort study, reported an association with adult obesity risk in their data.[89] However, the cohort comprises only 38.5% of all women invited to participate, and exposure estimation was based on asking the women if their mothers smoked while pregnant with them.[89] It has additionally been suggested that such associations in reality are due to familial confounding.[88]

One aspect that would strengthen the possibilities to draw casual inferences from these observational studies, would be if a dose-response relationship could be shown. However, very few prior studies have investigated this. One previous study stratified on numbers of cigarettes smoked during pregnancy and showed a dose-response relationship.[88] This study concerned only male offspring, however.

We aimed at replicating the previous finding of an increased adult obesity risk in a different population, with the possibility of minimizing selection bias and investigating if there is a dose-response relationship.

Pregnancy-related health effects – Gestational diabetes

Gestational diabetes is diabetes that occurs or gets diagnosed during pregnancy. It complicates around 1-2% of pregnancies in Sweden,[91,92] but substantially higher numbers have been reported from other countries (such as 14 % in the United States).[93] Diabetes during pregnancy is potentially harmful both for the mother and the baby, as it increases the risk of giving birth to a large baby, which in turn increases the risk of a complicated delivery.[94]

Gestational diabetes is similar to type 2 diabetes, or non-insulin-dependent diabetes, in terms of pathological features, clinical treatment and risk factors. The risk of type 2 diabetes among women who have had gestational diabetes is markedly increased.[95,96] Arguably, they are probably not two different disease entities, but rather, a woman's predisposition for subsequent type 2 diabetes is pushed over the threshold to clinically manifested symptoms during the physiologically demanding event that is a pregnancy.

Risk factors for gestational diabetes include high maternal BMI or excessive weight gain during pregnancy, high maternal age, higher parity, prior birth of a macrosomic baby (i.e. with a birthweight over 4500 g) and family history of gestational diabetes.[95] It also shows an ethnic variation.[95]

There is only one prior study on the risk of developing gestational diabetes following exposure to smoking during the fetal period.[89] The study reported an increased risk for gestational diabetes in exposed women, however, again a potential selection bias and crude exposure estimation warrant replication.

Considering their similarities, one might expect similar findings in regards to the relationship between prenatal smoking exposure and type 2 diabetes risk in the offspring. Studies have yielded inconsistent result, however, with most reporting null or very weak associations.[89,97] In the case where an association was seen, adjusting for offspring own BMI completely attenuated the estimates, suggesting that any increased risk might be mediated through higher BMI.[97]

Pregnancy-related health effects – Preeclampsia

Preeclampsia is a serious pregnancy condition, defined as pregnancy-onset hypertension in combination with proteinuria, and affects around 3-7 % of pregnant women globally.[98] The disease is potentially fatal for both the mother and fetus, as serious complications (such as seizures, known as eclampsia) can develop. The condition, alongside with other hypertensive disorders during pregnancy, is responsible for a major part of the worldwide maternal and perinatal morbidity, but in spite of it being a significant clinical problem, much is still unclear regarding the etiology of the disease.[98]

A two-stage model is generally accepted in regards to preeclampsia development, where the first stage is characterized by defect implantation and formation of the placenta. This leads to an inadequate blood perfusion and oxidative stress, leading to the release of several factors that leak into the maternal blood flow, where they cause inflammation and general endothelial damage resulting in the symptoms seen in the second stage.[99,100] A clinical distinction if often made between early-onset and late-onset preeclampsia, where early-onset develops before week 34 and tends to be of a more serious character. In about 25 % of cases of preeclampsia, it is seen in combination with a small-for-gestational age fetus.

Risk factors for preeclampsia include high BMI, being primiparous and carrying twins.[101] As with gestational diabetes, the prevalence of preeclampsia varies according to different ethnic groups, where women of African-American descent present a higher risk.[101] Additionally, prior hypertension, diabetes, gestational diabetes and kidney disease have been shown to be risk factors for preeclampsia, as has the use of assisted reproductive technologies.[101]

Paradoxically, smoking during pregnancy has consistently in the literature been found, in a dose-response manner, to be associated with a decreased risk of preeclampsia.[102-104] However, if the woman does develop preeclampsia, it is generally in a more severe form, and perinatal outcomes are worse.[102,104]

The relationship with other tobacco products are equally enigmatic. Women using snuff or being exposed to second-hand smoke have in some studies been found to have an increased risk of preeclampsia,[40,105] whereas other studies report no association.[104,106]

Additionally, the timing of the exposure to smoking seems relevant. One Swedish register study reported that when women quit smoking at some point during first and last trimester, the reduced risk of preeclampsia was no longer apparent.[104]

No prior study has investigated the relationship between intrauterine smoking exposure and risk of preeclampsia later in life. This could be relevant as several studies have noted an increased risk among women born low birthweight or small-for-gestational age for both preeclampsia[107-112] and pregnancy-induced hypertension.[113] As being born small-for-gestational age is known to be one consequence of maternal smoking, it would be of interest to examine if there is any independent relationship with being exposed to smoking in utero and later preeclampsia.

Registers in Sweden

Sweden has a long-standing tradition of an extensive population-based data collection and now has a wide array national registers covering different health-related, as well as administrative, aspects. Although there were some national health registers that were initiated earlier (such as the National Cause of Death Register and The Cancer Register), the catalyst event for the establishment of the Swedish Medical Birth register was the thalidomide scandal in the late 1950ies, when children were born with serious limb malformations following exposure to the drug in utero. A need for surveillance mechanisms was identified following this, leading to the initiation of the Register of Congenital Malformations in 1964, which later would be extended into the more comprehensive Medical Birth Register in 1973.[114]

Together with the individual personal identity numbers assigned to all residents at birth or immigration, allowing for linkages between these different registers, there are unique opportunities for certain types of research, particularly those requiring large cohorts.[115,116] The possibilities of linkage is important, as the cost of up-keep and compliance rate in reporting require that the variables included in the registers to be kept at a limited amount.

In order for register-based research to have credibility, validation of the data is necessary. Such studies are performed intermittently for different registers and variables (for example diagnostic accuracy and coverage); for this thesis, the question was raised for the variable on maternal pregnancy smoking.

Validity of smoking data in Swedish registers

This thesis is based on several assumptions. One of the most important from a methodological perspective is that the data on maternal smoking behavior as registered in the Medical Birth Register are a correct and valid estimation of fetal smoking exposure. Indeed, this is true for all epidemiological studies using any type of self-reported data on maternal pregnancy smoking. This assumption, in turn, is based two aspects: i) women report their smoking during pregnancy truthfully and ii) constituents of tobacco smoke pass readily over the placenta, thus reaching fetal circulation.

Although the register data in the Medical Birth Register have one immediate advantage compared to smoking data in some study cohorts, as it is not based on recall of distant events, there is still a pronounced stigma surrounding pregnancy smoking in many cultures, including Sweden, which might lead women to underreport their smoking behavior in fear of a perceived or true risk of being met with judgment.

Obtaining an objective measurement of nicotine exposure is possible through the use of biomarkers. A good biomarker needs to be measurable, and represent the magnitude, frequency and duration of the exposure. Nicotine itself has a half-life of 1-2 hours, a considerable inter-subject variability and is expensive to measure, and due to this, does not lend itself well for these type of measurements.

Cotinine, which is the main metabolite of nicotine, has a longer half-life of around 15-20 hours, and can be measured in hair, saliva, urine and serum.[117] Because of the longer half-life and slower excretion, cotinine levels accumulate over the day, and are hence more stable than nicotine levels. Cotinine measurements are now considered the gold standard of nicotine exposure, and for fetal exposure, the most accurate is to measure levels in umbilical cord blood.[117,118] Another advantage with biomarkers is that it is possible to quantify whether and individual is actively smoking or exposed to second-hand smoke.[117]

Attempts have been made before to investigate how truthfully pregnant women report their smoking behavior. Some of the prior studies have reported satisfying agreement between self-reported smoking data and cotinine measurements.[119-121] Others, on the other hand, have demonstrated a significant underreporting, both among self-reported non-smoker, smokers and quitters.[122-125]

However, it is to note that all prior studies were based on women participating in a study cohort, often in studies requiring several follow-ups. The research setting might lead women to answer differently than they would otherwise have done. Also, it raises the question about who is agreeing to participate. It is known that people participating in research studies, in general, have a higher educational attainment, have an employment, better social support and higher general health consciousness.[126] Individuals with health risk behaviors, such as smoking, alcohol or drug use, are

generally harder to recruit, and are often under-represented in many epidemiological cohorts.[126]

It is therefore of importance to investigate the reporting of life-style habits in a pregnant population that is not participating in such studies, not the least since the proportion of smokers is probably higher in this population. A validation study of the smoking data in the Medical Birth Register based on women from the general population, not participating in an ongoing study, is still lacking.

Summary of research gaps and thesis questions

As has been discussed in the individual sections above, considering the area of Developmental Origins of Health and Disease, there are several research gaps which we have an opportunity to investigate. The main questions which we aimed to address were the following:

i. Will the inverse associations found previously between maternal smoking during pregnancy and offspring type 1 diabetes risk hold if using a design that accounts for genetic predisposition of the disease?

ii. Are the reported increased risks for obesity and gestational diabetes in women exposed to smoking prenatally stable in a different population, without the risk of selection bias, and is there a dose-response relationship present?

iii. Is there an increased risk to develop preeclampsia if having been exposed to tobacco smoking prenatally?

iv. Are the self-reported smoking data in the Swedish Medical Birth Register accurate and valid, i.e. do pregnant women in Sweden disclose their smoking behavior at the antenatal visits?

Materials and methods

Data sources and linkages

This thesis is based primarily on data from national registers, but also on data from a regional quality register, clinical study cohorts and a regional biobank. In the case where linkages between different data sources were made, these were made through the use of the personal identification numbers that each Swedish resident is assigned (at birth or immigration), consisting of the complete date of birth as well as a four digit administrative number. This identification number is essential in all contacts with Swedish authorities and health care providers and constitutes the base for any linkages of otherwise independent data sources.

The Swedish Medical Birth Register

The Swedish Medical Birth Register, established in 1973, is a population-based nationwide register covering 97-99 % of all births in Sweden.[127] It is held and administered by the National Board of Health and Welfare, to which data are sent in the form of copies of standardized antenatal, obstetric and pediatric records, mostly electronically, but paper form is also used in some places.

The register collects a large amount of pregnancy-related information, including demographic and anthropometric data, information on reproductive history and complications during pregnancy, delivery and the perinatal period. This information includes for example maternal height, weight, occupation, family situation (i.e. co-habiting with other parent), health status and life-style habits (smoking, snuff use, alcohol consumption and medication use) from the antenatal period. From delivery and the first neonatal period, data such as maternal age at childbirth, multiple/singleton birth, onset and time of delivery, mode of delivery, still- or live-born, infant sex, birthweight, gestational age, head circumference, Apgar score, and data on complications or medical interventions are collected.

Complications during pregnancy or delivery are classified according to the Swedish version of the International Classifications of Diseases (ICD) system. Over the years, versions ICD-8 (through 1986), ICD-9 (1987-1996) and ICD-10 (from 1997 and onwards) have been used.

Beginning with the woman's first antenatal visit (usually at 8-12 weeks of gestation), all information onwards is collected prospectively. Information is collected by staff responsible for patient care. For the antenatal period and births without complications, data are collected by trained midwives; information on diagnoses is summarized by obstetricians and pediatricians, respectively, when the woman is discharged from hospital. The data should be reported to the National Board of Health and Welfare one month after the birth of the child at the latest.

The main structure of the register has remained since 1973, and the form of reporting has been constant since 1982, when also information on smoking during pregnancy was added. The register categorizes maternal smoking into non-smoker, smoking 1-9, or 10 or more cigarettes per day. Smaller modifications in register content have been made in 1990, 1994 and 1998, mainly variable additions.[127]

Over the years, several quality analyses have been performed (published in 1977, 1988, 1990 and 2002).[127-130] Data quality of the variables differ, as does the quality of some of the specific variables over time. Generally, "hard" data, such as birthweight, perinatal survival, fetal presentation and mode of delivery and similar variables tend to be of good quality.[128] Some of the variables are more difficult to use in research, for reasons such as i) high proportion of missing data, ii) information not coded, but registered in free text with inconsistent spelling and/or classifications, and iii) regional inconsistencies in diagnoses, mainly due to inexact diagnostic criteria in clinical practice.

Perinatal Revision South Register

The Perinatal Revision South Register is a regional, population-based clinical quality register, established in 1994. The register aims to provide a comprehensive database for surveillance and quality improvement of clinical care within obstetrics and neonatal medicine. This goal can not be reached through the use of the Medical Birth Register, as the national register only provides data for research, or aggregated data for descriptive statistics, and not for improvement of specific aspects of clinical care.

The uptake area for the register comprises the southern region of Sweden, with a population of around 1.8 million (2015). The delivery clinics are university departments with approximately 3000-5000 deliveries/year, central hospitals (1500-2000 deliveries/year) and county hospitals (<1000 deliveries/year). Pregnancy-related and neonatal data from all delivery clinics in the uptake area are collected prospectively, and the information is sent to the register, which is held and administered from the university hospital in Lund.

In regards to content, it is very similar to the national Medical Birth Register, however, it contains more detailed clinical data, especially from the neonatal period. Such additional clinical data from the peri- and neonatal period include for example

information on amniotic fluid, placental weight, laboratory tests from the umbilical cord, length of stay at the hospital, complications such as brain hematomas and need of respiratory care, and any medical interventions provided.

Around 20,000 children are born annually within the uptake area, and the register now contains information on more than 350,000 births. For the whole duration of this register, data on maternal smoking have been collected, categorized in the same manner as in the Medical Birth Register.

Diabetes Prediction in Skåne Cohort

The Diabetes Prediction in Skåne Cohort (DiPiS) is a population-based prospective study cohort aiming at determining genetic and early-life environmental risk factors for the development of type 1 diabetes in children. In brief, children were tested at birth for their genetic risk profile of developing diabetes (i.e. HLA-typed) and those considered having an increased risk were followed until the age of 15 years.

Between 2000 and 2004, parents expecting children in the region of Skåne were informed about the study through their maternal health care visits. At delivery, oral informed consent was obtained before sampling of blood from the umbilical cord of the newborn, as well as a venous sample from the mother. At two months, parents received a written invitation to participate in DiPiS, and after written informed consent, they filled out questionnaires regarding family history of type 1 diabetes as well as information of various other health-related and psychosocial factors.

Of the 48,058 children born in Skåne during 2000 and 2004, cord blood were drawn and HLA-typed for 35,683 (74 %) children. Apart from determining HLA genotypes, cord blood was also analyzed for a presence of islet auto-antibodies (as a sign of an initiated auto-immune process).

For the children with an increased risk of diabetes, parents were contacted again for a follow-up when the children reached 2 years. Increased risk constituted mainly certain HLA-types, but inclusion was also based on the following factors: heredity for type 1 diabetes (mother or father with the disease), being born large-for-gestational age, and having experienced infections during pregnancy. Based on these inclusion criteria, around 6,000 children were invited to participate and of these, 3,680 children continued to participate in the yearly follow-ups. The reason for inviting children considered to be a an increased risk was to have a better chance at reaching a sufficient number of children with diabetes in the cohort, since type 1 diabetes is a rare disease.

The participating families contributed with yearly blood samples that were analyzed for islet cell anti-bodies, as well as by filling out yearly questionnaires, covering aspects such as nutrition, diseases, medications and different stressors. If the child developed anti-bodies, the parents were contacted had they expressed such a wish. However, due

to a decision by the Ethics Committee, this was only possible after 2004. In case of development of multiple auto-antibodies, the children were followed in closer intervals.

For this study, the DiPiS cohort was expanded by including all children born in Skåne between 1999 and 2005, who had received a diagnosis of type 1 diabetes. These children were registered in the Skåne Study (1999-2005) and The Better Diabetes Diagnosis Study (from 2005 and onwards). In total, out of the 84,039 children born in Skåne during these years, 344 of them had developed type 1 diabetes by May 1st, 2013. This expansion of the study cohort was done for reasons concerning statistical power.

Malmö Maternity Unit Serum Biobank

In the city of Malmö, the largest city in the region of Skåne, nearly all deliveries occur at the Malmö University Hospital delivery unit. Based on these deliveries, the Malmö Maternity Unit Serum Biobank has between 1969 and 2000 been collecting serum samples from the umbilical cord of the newborn, sampled at delivery, as well as venous samples from the mother during early labor. It now contains samples from around 70,000 deliveries. As written informed consent was not implemented during the time when this biobank was established, samples were collected after informed oral consent by the mothers.

Study designs and statistical approach

Terminology and main concepts

This thesis concerns long-term health effects of prenatal smoking exposure. The principal design in the majority of the studies spanned over two pregnancies and three generations (G1-G3), although the third generation was not studied. Women in the first generation (generation G1) were pregnant and smoked or not during these pregnancies (G2 generation intrauterine exposure, i.e. exposure of interest). G2 women subsequently became pregnant themselves and smoked or not during these pregnancies (considered potential confounder). We thus studied the intrauterine exposure and adult outcomes of the G2 generation, adjusting for characteristics in the same generation.

All covariates are denoted by a G1 or G2 prefix, indicating to which generation/woman (not pregnancy) they refer. For example, ”G2 mode of delivery” means the way G2 women were born themselves.

In all studies, smoking habits were based on the categorizations made in the Medical Birth Register. Smoking 1-9 cigarettes per day is hence referred to as moderate smoking, and smoking 10 or more cigarettes as heavy smoking. The figure below illustrates the main concept and the basic structure of the analyses used in this thesis.

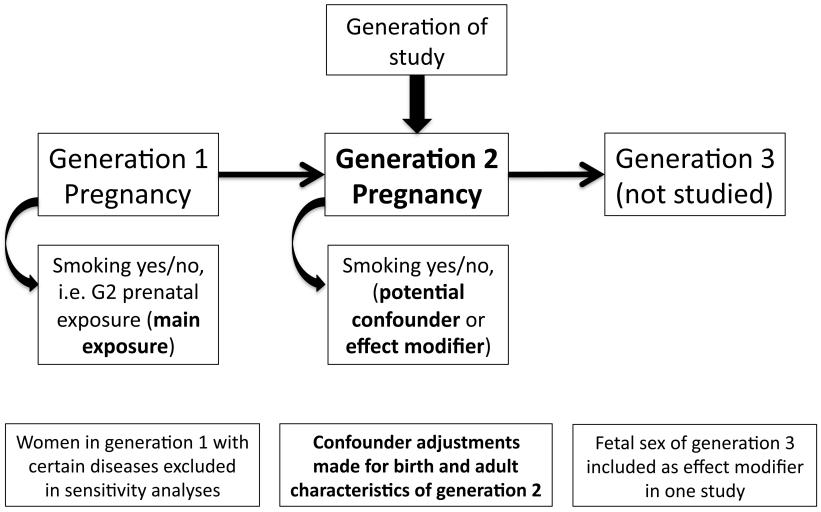

Figure 4.
Conceptual model and main research design. The figure shows from which generation different variables are used.

Study populations

Type 1 diabetes study

The data for this paper, designed as a case-control study, come from the DiPiS cohort complemented by two registers capturing all children with type 1 diabetes in Skåne, and the Perinatal Revision South Register. During the years 1999-2005, 84,038 children were born in Skåne, and by May 1st 2013, 344 of them had developed diabetes. All children in the uptake area were diagnosed according to the American Diabetes Association,[131] and case ascertainment constituted a clinically registered diagnosis of type 1 diabetes.

From the DiPiS study cohort, three control children were chosen for each child with diabetes, matched for year of birth and HLA-type. The HLA matching was done

exact if possible, where an exact match constituted the same DQA alleles and the same DQB alleles. In case no exact match was possible, a child with a random HLA-type considered low-risk was chosen. For the majority (81 %) of the children there were at least two exact matches available, for 4 % no exact HLA-match was found. Controls were born within one to three years of their matched case; 94 % of the controls were born within two years.

The reason for matching on HLA-type (i.e. having the two same alleles) rather than including such information as a covariate, was due to the complexity of risk mediation through HLA alleles. There are different allele combinations considered to generate a low and neutral risk, as well as different degrees of increased risk. There are also combinations of alleles considered to be protective.[132]

For the children born 2000-2004 and participating in the DiPiS study, HLA-genotyping was made at birth; for children born 1999-2005 and not participating in DiPiS, HLA-genotyping was made at time of diagnosis.

A presence of islet cell auto-antibodies precedes clinical diabetes, often by many years, and a child with multiple auto-antibodies is extremely likely to develop diabetes later on.[62] Therefore, in order to avoid that children with an initiated diabetes development were misclassified as diabetes-free controls, we only included children as controls if they had presented a negative test for multiple auto-antibodies.

Lastly, we only included children in our analyses if they had complete data on maternal smoking during pregnancy, yielding a final cohort of 319 children with type 1 diabetes, and 956 healthy controls.

Studies on adult and pregnancy-related health effects (obesity, gestational diabetes and preeclampsia)

The study populations for these papers come exclusively from the Medical Birth Register. Inclusion was based on the following criteria: all women born in 1982 (i.e. smoking habits during pregnancy first registered) or later, who had given birth themselves were included.

For the paper on gestational diabetes, data until 2010 were retrieved, for the paper on preeclampsia until 2013, yielding a total of 80,189 and 195,922 eligible pregnancies, respectively. Some of the G2 women contributed with more than one pregnancy (27.3 % and 32.3 %). Women were included if they had valid smoking data from both generations (G1 and G2), as well as on all the other covariates of interest, leaving 50,012 and 153,885 pregnancies for the final analyses. Most of the excluded women were due to missing data on smoking habits, and most of these were in turn in the first (G1) generation.

Register validation study

The study population for this study comprises the control group from an earlier project on neuropsychiatric outcomes of prenatal exposure to environmental toxins (The Fetal Environment and Neurodevelopmental Disorder in Epidemiological Research [FENDER] Project). No woman was asked to participate in a specific research study for the acquisition of the data used in this paper, as all data come exclusively from the Medical Birth Register and the Malmö Maternity Unit Serum Biobank. Verbal informed consent was retrieved before sampling of maternal venous samples and umbilical cord blood from the newborn, but not additional commitment was required by the women.

The children were chosen randomly from the Medial Birth Register to match children with ADHD born between 1982 and 2000 according to birth year and maternal country of origin. The children were selected in pools of ten eligible controls, and the first child with a sample in the biobank was chosen. In the case no biobank sample was available for the eligible controls, the principle of next-baby-born in the biobank was used. The selection procedure has been described in more detail previously.[133] For this study, we had 204 mother-child-dyads.

Laboratory analyses

Analysis of HLA and islet cell auto-antibodies

HLA genotypes were analyzed by polymerase chain reaction (PCR) on dried blood spots, and auto-antibodies were analyzed by means of radio ligand-binding micro assay. Laboratory methods are described in more detail elsewhere.[132,134,135] All samples were analyzed in terms of HLA-type and islet cell antibodies within DiPiS at the same laboratory, using the same methods.

Analysis of cotinine

Cotinine levels were analyzed in the stored samples from the Malmö Maternity Unit Serum Biobank. In brief, aliquots of 100 µl sera were added with isotopically labeled internal standards and analysis was then performed using a hybrid triple quadruple linear ion-trap mass spectrometer.[136]

The limit of detection was 0.2 ng/ml. To increase the accuracy, the value used for the analyses is the average of two measurements from the same sample worked up and analyzed on different days. All analyses were made at the laboratory at the Division of Occupational and Environmental Medicine, Lund University, Lund.

Cotinine cut-off levels denoting active smoking

The choice of an appropriate cut-off for cotinine levels denoting active smoking is dependent on the distribution of cotinine among active smokers and among those

exposed to environmental tobacco smoke. Prevalence of smokers in the study population as well as the proportion of non-smokers exposed to passive smoking is therefore relevant. If smoking, and exposure to second-hand smoke, is more common, a higher cut-off level would be expected. Thus, different cut-offs could be relevant for different cultural contexts and different time periods.

14-15 ng/ml has been suggested in many earlier studies as an appropriate level.[137,138] This has been revised later, and 3 ng/ml is the most commonly used cut-off in recent literature.[139] We argued that, for our study, the higher cut-off is probably more accurate, seeing as there were few regulations in place in order to minimize the public's exposure to environmental tobacco smoke (particularly indoors) during the years studied (1982-2000). However, in order to avoid misclassification due to an arbitrarily chosen cut-off, we also investigated if the results were stable at the 3 ng/ml cut-off. Those under the limit of detection were considered non-smokers.

Statistical methods

Main analyses

The following statistical approaches were used in the present thesis: basic associations between prenatal smoking exposure and the outcomes obesity, gestational diabetes and preeclampsia were investigated through logistic regressions, generating odd ratios (OR) and 95 % confidence intervals (CI). For the study of type 1 diabetes, the matching between the cases and controls based on HLA-type was essential, and we therefore used conditional logistic regressions, which compares the cases to their specific control/-s, rather than comparing the whole groups to each other.

To investigate the agreement between different smoking variables in the validation study we used Kappa coefficients (κ) as measures of agreement (between register-data on maternal smoking and maternal cotinine levels; and between fetal and maternal cotinine levels as categorical variables, respectively).

To explore the correlation between maternal and umbilical cord cotinine levels treated as continuous variables, we used Spearman's rank correlation coefficient (r_s). Additionally, a ratio was calculated between umbilical cord cotinine levels and maternal serum levels for those mother-child dyads where the mother smoked actively as well as was exposed to environmental tobacco smoke, according to the cotinine levels.

Confounders and intermediate variables

For all the papers in this thesis, confounders and intermediate variables were considered on an a priori basis. This was generally derived from prior literature on risk factors for the outcomes and their relation to smoking behavior.

Figure 5 below shows the concept of thought behind the associations we were testing in this thesis, in the form of an directed acyclic graph.

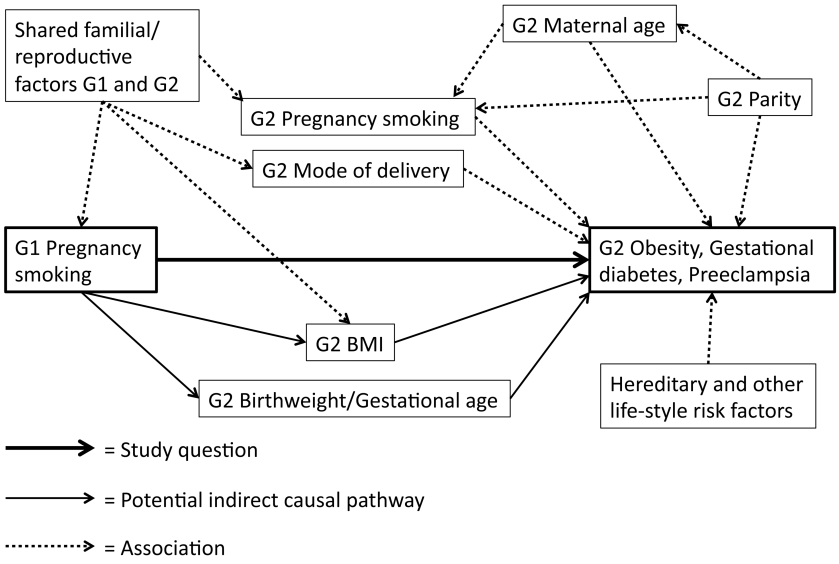

Figure 5.
Conceptual model depicting the hypothesized relationship between relevant covariates.

In the figure above, there is no arrow from G1 pregnancy smoking to G2 pregnancy smoking, as it was hypothesized that this association rather was mediated through shared familial factors and view on health behaviors than a direct causal effect from G2 intrauterine exposure to G2 own smoking.[140] Some of the variables in the models could be argued to potentially act as either confounders or intermediates (e.g. birthweight, gestational age, BMI), and therefore, these variables were included in separate steps, to better illustrate their effects.

In Table 2 (next page), the confounders that were included in the analyses for the different outcomes are shown. All confounders refer to the G2 generation, i.e. both adult and birth characteristics from that same generation were adjusted for.

Table 2.
Overview of included confounders in the different outcome models. All confounders refer to the G2 or offspring generation.

Outcome	Type 1 diabetes	Obesity	Gestational diabetes	Preeclampsia
Confounder				
Maternal age	x	x	x	x
Parity	x	x	x	x
Mode of delivery	x	x	x	-
Own smoking	-	x	x	x
BMI	-	-	x[1]	x[1]
Birthweight	x[1]	x[1]	x[1]	x[1]
Gestational age	x[1]	x[1]	x[1]	x[1]

[1] Included as an additional separate step.

Sensitivity analyses

To investigate the robustness of the results in our studies, a series of sensitivity analyses were performed. Mainly, they were directed at attempting to account for i) a possible impact of heredity (excluding, when possible, women from G1 generation with same outcome as studied in the specific model), ii) assuring that all pregnancies were independent observations (including only non-related primipara in the G2 generation), iii) taking into account possible ethnic differences (including only Swedish/Nordic born G1 women). Table 3 on the next page shows the sensitivity analyses performed in the different studies.

As screening practices for gestational diabetes differ regionally in Sweden, where some areas provide an oral glucose tolerance test to all pregnant women, and others only offer it to women with risk factors for diabetes (which factors to be considered are not standardized), we also conducted a subset of analyses including only women born in a region (Skåne) where all women are offered the test.

Table 3.
Overview over sensitivity analyses performed for the different outcomes.

Outcome	Type 1 diabetes	Obesity	Gestational diabetes	Preeclampsia
Sensitivity analysis				
Including only G2 primipara	x	x	x	x
Including only G1 Swedish/Nordic-born mothers	x	x	x	x
Excluding G1 type 1 diabetes	x	-	x[1]	x[1]
Excluding G1 gestational diabetes	x	-	-[2]	-
Excluding G2 gestational diabetes	-	-	-	x
Excluding G1 preeclampsia	x	-	-	x
Excluding G1 hypertension	-	-	-	x
Excluding G1 obesity	-	x	x[3]	-
Stratifying on G2 smoking behavior	-	x	-	x
Mandatory glucose tolerance test during pregnancy only	-	-	x	-

[1] No distinction of diabetes type in the Medical Birth Register, "prior diabetes" used for these analyses.
[2] Not possible, gestational diabetes not registered in the Medical Birth Register until 1987.
[3] By treatment as a confounder in separate analyses.

The data in these cohorts are clustered in terms of G2 women being related (sisters) as well as some of them giving birth to more than one baby. Dependent data might lead to an underestimation of standard errors and false statistical precision. To address this, we also performed analyses where we only included independent G2 women giving birth for the first time, as illustrated in Figure 6 on the next page.

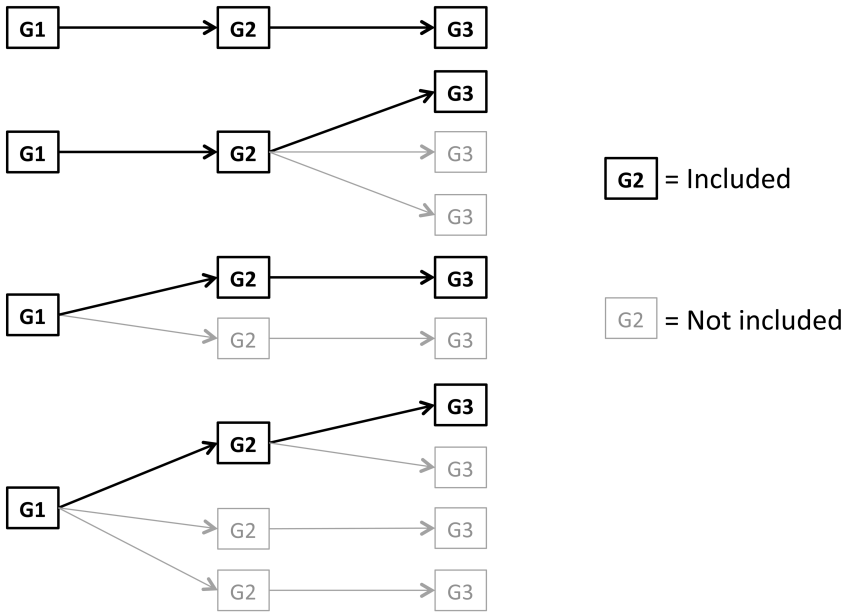

Figure 6.
Schematic picture showing how G2 pregnancies were selected in order to be independent observations in regards to both generation G1 and G3. This was done in a sensitivity analysis.

Testing the exposure accuracy

Two of the papers were written before the validation study had been undertaken, so in order to evaluate the exposure estimation (maternal self-report), we checked the smoking data towards mean offspring birthweight for each smoking category among singleton births, where a dose-response association of lower birthweight by increasing smoking category was considered a proxy for validation.

In the preeclampsia paper, the results from the cotinine validation study were published, and hence, these were used as an indication of the accuracy of the exposure variable, and retrospectively also for the study on gestational diabetes and obesity. The exposure data for the study on type 1 diabetes come formally from a different register, but seeing as the same data from the maternal health care visits are forwarded to both registers, it can likely be assumed that the validation results are relevant for that register also.

Results

Childhood health effects

Type 1 diabetes

Maternal smoking during pregnancy was associated with a higher risk of her child to develop type 1 diabetes (OR 2.83; 95 % CI: 1.67, 4.80 for moderate smoking exposure and OR 3.91; 95 % CI: 1.22, 12.51 for heavy smoking exposure). These results were robust and point estimates similar over the different confounder adjustments, as well as through all the sensitivity analyses. When including only those controls who were an exact HLA-match to their respective case, the results remained, but with wider confidence intervals, see Table 4 below.

Table 4.
Odds ratios (ORs) with 95 % confidence intervals (CI) for offspring type-1 diabetes by maternal smoking during pregnancy.

	Crude OR (95% CI)	Adjusted OR[1] (95% CI)
Complete sample		
Non-smoker (ref)	1 (-)	1 (-)
1-9 cigarettes/day	2.83 (1.67, 4.80)	2.71 (1.58, 4.65)
>9 cigarettes/day	3.9 (1.2, 12.5)	4.1 (1.2, 13.8)
Exact HLA-matched controls only		
Non-smoker (ref)	1 (-)	1 (-)
1-9 cigarettes/day	2.72 (1.52, 4.87)	2.66 (1.46, 4.84)
>9 cigarettes/day	4.8 (1.2, 19.6)	5.8 (1.3, 25.7)

[1] Models adjusted for maternal age at childbirth, parity and mode of delivery.

Obesity

Prenatally exposed women were more likely to be obese than non-exposed. A dose-response relationship was present, where heavy smoking exposure was associated with a higher risk (adjusted OR 1.58; 95 % CI: 1.48, 1.68) compared to moderate exposure (adjusted OR 1.36; 95 % CI: 1.28, 1.44). Models that additionally adjusted for birthweight and gestational age resulted in somewhat higher point estimates (Table 5).

Table 5.
Odds ratios with 95 % confidence intervals (CI) for the associations between maternal smoking during pregnancy and daughters' risk of obesity (BMI>30).

	Crude OR (95% CI)	Adjusted[1] OR (95% CI)	Adjusted[2] OR (95% CI)
Obesity			
Non-smokers (ref)	1 (-)	1 (-)	1 (-)
1-9 cigarettes/day	1.40 (1.32, 1.48)	1.36 (1.28, 1.44)	1.45 (1.36, 1.54)
>9 cigarettes/day	1.65 (1.54, 1.75)	1.58 (1.48, 1.68)	1.71 (1.60, 1.83)

[1] Models adjusted for G2 maternal age at childbirth, parity, mode of delivery and own smoking.
[2] Models additionally adjusted for G2 birthweight and gestational age.

There was an interaction between prenatal smoking exposure and own adult smoking in the G2 generation ($p<0.001$). Thus, separate analyses were performed, analyzing the risk for obesity for smoking G2 women and non-smoking G2 women separately. When G2 women smoked themselves, the odds ratios for obesity where attenuated, but still significant (Table 6 on the next page).

Table 6.
Odds ratios (OR) with 95 % confidence intervals (CI) for obesity by exposure to maternal pregnancy smoking, stratified according to own smoking in generation 2 (G2).

	G2 Non-smokers	
	Crude OR (95% CI)	Adjusted OR[2] (95% CI)
Obesity		
Non-smokers (ref)	1 (-)	1 (-)
1-9 cigarettes/day	1.39 (1.30, 1.49)	1.38 (1.29, 1.47)
>9 cigarettes/day	1.72 (1.59, 1.85)	1.70 (1.58, 1.83)
	G2 Smokers[1]	
Non-smokers (ref)	1 (-)	1 (-)
1-9 cigarettes/day	1.20 (1.04, 1.37)	1.19 (1.04, 1.37)
>9 cigarettes/day	1.23 (1.07, 1.41)	1.23 (1.07, 1.41)

[1] Smoking ≥1 cigarette/day.
[2] Models adjusted for G2 maternal age, parity and mode of delivery.

Gestational diabetes

Maternal smoking during pregnancy was associated with an increased risk of gestational diabetes in her daughters (Table 7 below). The results were robust over all models, including those where G2 BMI, own birthweight and gestational age were adjusted for.

When only including women from a region where all pregnant women receive an oral glucose tolerance test, point estimates were somewhat higher (adjusted OR 2.01; 95 % CI: 1.21, 3.34 for moderate smoking exposure and adjusted OR 2.05; 95 % CI: 1.20, 3.50 for heavy exposure) compared to the complete sample.

Table 7.
Odds ratios (OR) with 95 % confidence intervals (CI) for gestational diabetes by prenatal smoking exposure.

	Crude OR (95% CI)	**Adjusted[1] OR (95% CI)**	**Adjusted[2] OR (95% CI)**
Gestational diabetes			
Non-smokers (ref)	1 (-)	1 (-)	1 (-)
1-9 cigarettes/day	1.73 (1.33, 2.26)	1.82 (1.39, 2.38)	1.62 (1.24, 2.13)
>9 cigarettes/day	1.68 (1.25, 2.27)	1.81 (1.34, 2.46)	1.52 (1.12, 2.06)

[1] Models adjusted for G2 maternal age, parity, mode of delivery and own smoking during pregnancy.
[2] Models additionally adjusted for G2 BMI.

Preeclampsia

The results for preeclampsia were not consistent over all manifestations of preeclampsia. For any type of preeclampsia, as well as early-onset preeclampsia, result were generally insignificant. For late-onset preeclampsia there was a small risk increase for women that were heavily exposed to smoking during their fetal life (Table 8 below). No association remained significant when G2 birthweight-for-gestational age and gestational age were included in the models.

Table 8.
Odds ratios (OR) with 95 % confidence intervals (CI) for any type of preeclampsia and late onset preeclampsia by prenatal smoking exposure.

	Crude OR (95% CI)	Adjusted[1] OR (95% CI)	Adjusted[2] OR (95% CI)
Preeclampsia (any)			
Non-smokers (ref)	1 (-)	1 (-)	1 (-)
1-9 cigarettes/day	0.99 (0.93, 1.06)	1.06 (0.99, 1.13)	0.98 (0.92, 1.05)
>9 cigarettes/day	1.07 (0.99, 1.15)	1.18 (1.10, 1.27)	1.07 (0.99, 1.15)
Preeclampsia (late onset)			
Non-smokers (ref)	1 (-)	1 (-)	1 (-)
1-9 cigarettes/day	1.02 (0.94, 1.10)	1.09 (1.01, 1.17)	1.01 (0.94, 1.09)
>9 cigarettes/day	1.08 (0.99, 1.18)	1.20 (1.10, 1.31)	1.08 (1.00, 1.18)

[1] Models adjusted for G2 maternal age, parity and own smoking during pregnancy.
[2] Models additionally adjusted for G2 BMI.

There was an interaction between fetal smoking exposure and own smoking during pregnancy ($p < 0.05$). Table 9 on the next page shows the risk for any type of preeclampsia, when G2 smokers and non-smokers were considered separately. The risk estimates for non-smoking women that were heavily exposed during fetal life were somewhat higher and were robust over adjustment for G2 BMI (adjusted OR 1.10; 95 % CI: 1.02, 1.20) for heavy smoking exposure.

Table 9.
Odds ratios (OR) with 95 % confidence intervals (CI) for any type of preeclampsia by exposure to maternal pregnancy smoking, stratified according to own smoking in generation 2 (G2).

	G2 Non-smokers	
	Crude OR (95% CI)	Adjusted OR[2] (95% CI)
Preeclampsia (any)		
Non-smokers (ref)	1 (-)	1 (-)
1-9 cigarettes/day	1.05 (0.98, 1.12)	1.07 (1.00, 1.15)
>9 cigarettes/day	1.20 (1.11, 1.30)	1.24 (1.14, 1.34)
	G2 Smokers[1]	
Non-smokers (ref)	1 (-)	1 (-)
1-9 cigarettes/day	0.86 (0.70, 1.05)	0.87 (0.71, 1.06)
>9 cigarettes/day	0.82 (0.67, 1.01)	0.84 (0.68, 1.03)

[1] Smoking ≥1 cigarette/day.
[2] Models adjusted for G2 maternal age and parity.

Validity of exposure estimations

Demographic data and representativeness of the cohort

The study population for this report is a sub-group of women from the general population giving birth in the region of Skåne. As it was considered of interest whether, and to what degree, these women represented the population of Skåne (and thus, according to prior data on selected socio-demographic factors,[141] likely the whole of Sweden), demographic and perinatal information have been presented in more detail for these women, compared to earlier results in this thesis.

Investigating the representativeness of the study cohort revealed only small differences between the cohort and the general population in Skåne during the same years (1982-2000), mainly differences in birth characteristics such as birthweight and gestational age. There was no indication that there was a more health-conscious group of women in the cohort compared to the general population, judging by pregnancy smoking rates. Selected maternal and child characteristics are presented in Table 10 on the next page.

Table 10.
Selected maternal and child characteristics in the study cohort and in the general population in the region of Skåne between 1982 and 2000.

Perinatal characteristic	**Cohort prevalence n** (%)	**Regional prevalence** (%)[1]
Maternal age at childbirth (years)		
<20	2.9	2.6
20-24	20.1	19.9
25-29	36.3	37.2
30-34	27.0	27.9
35-39	9.3	10.6
≥40	4.4	1.8
Maternal country of origin		
Sweden	82.8	84.6
Parity		
1	51.0	41.8
2	33.8	36.1
≥3	15.2	22.1
Birthweight (g)		
<2500	2.5	4.3
2500-4000	73.0	78.6
>4000	24.5	17.1
Gestational age (weeks)		
<37	3.4	5.9
≥37	96.6	94.1
Mode of delivery		
Vaginal birth	85.8	86.3
Elective Ceasarean section	2.0	2.6
Emergency Ceasarean section	4.9	7.5
Forceps/vacuum extraction	7.4	3.6

[1] Numbers from The Medical Birth Register.

Table 11 on the next page shows the smoking prevalence by different 10-year time periods in the study cohort and in the region of Skåne. The proportion of smokers was somewhat higher in the cohort compared to the regional prevalence for the same years. There was a decrease in smoking prevalence over time in both groups, most evident in the strata with heavy smokers.

Table 11.
Smoking prevalence and background characteristics among pregnant women in the study cohort and in the general population in the region of Skåne between 1982 and 2000.

Smoking during pregnancy	Cohort prevalence (%)	Regional prevalence[1] (%)
Whole period (1982-2000)		
Non-smoker	66.7	75.5
1-9 cigarettes/day	17.6	15.1
>9 cigarettes/day	12.7	9.4
1982-1991		
Non-smoker	65.8	70.0
1-9 cigarettes/day	16.7	18.4
>9 cigarettes/day	17.5	12.0
1992-2000		
Non-smoker	73.1	81.5
1-9 cigarettes/day	20.5	11.8
>9 cigarettes/day	6.4	6.7

[1] Numbers from The Medical Birth Register.

Transfer of cotinine from mother to fetus

The correlation between maternal and umbilical cord cotinine levels at delivery was 0.90 ($p<0.001$). The correlation is visualized in Figure 7 below.

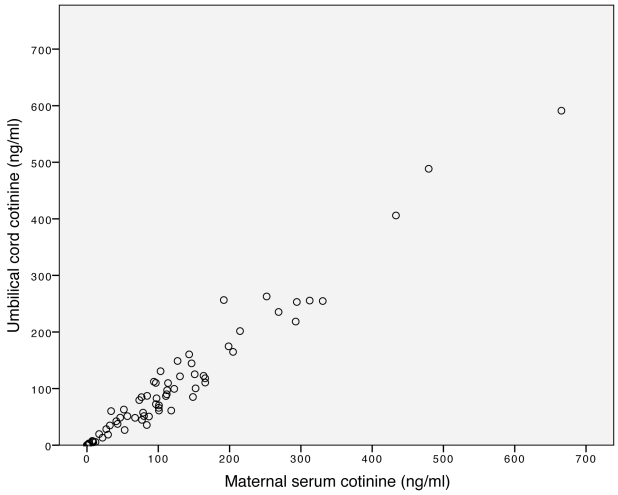

Figure 7.
Correlation of maternal serum cotinine and umbilical cord cotinine.

The analysis of agreement between cotinine levels treated as categorized variables generated a kappa coefficient (κ) of 0.75 (Table 12). The cotinine levels in umbilical cord blood were 88 % (95 % CI: 82 %, 94 %) of maternal levels when the mother was actively smoking, and 92 % (95 % CI: 79 %, 105 %) when the mother was exposed to environmental tobacco smoke.

Table 12.
Smoking at delivery based on maternal and umbilical cord blood cotinine levels. Numbers are individuals.

	Umbilical cord serum cotinine levels			
Maternal serum cotinine levels	Non-smoker (<0.2 ng/ml)	Passive smoking exposure (0.2-14.9 ng/ml)	Active smoker (≥15 ng/ml)	Total
Non-smoker (<0.2 ng/ml)	68	19	0	87
Passive smoking exposure (0.2-14.9 ng/ml)	12	34	0	46
Active smoker (≥15 ng/ml)	0	1	57	58
Total	80	54	57	191[1]

[1] Of a total of 204 mother-child dyads, 1 child and 12 mothers had missing data on cotinine levels and were not included in the analyses above.

Agreement between register data and biomarker measurements

The agreement between self-reported smoking in the register and maternal cotinine levels was high (κ = 0.82). Of the self-reported non-smokers, 95 % (95 % CI: 89 %, 97 %) were also classified as non-smokers after cotinine measurements at delivery. Inversely, out of those who reported that they were smoking in early pregnancy, 87 % (95 % CI: 75 %, 94 %) were also smoking at the time of delivery.

Table 13 on the next page shows the distribution of the different smoking variables. To investigate the robustness of our results, we additionally examined the agreement between register data and biomarker data with a different cotinine cut-off denoting active smoking. When 3 ng/ml was used as the cut-off, the agreement was strengthened (κ = 0.85). Using this cut-off, 94 % (95 % CI: 88 %, 97 %) of the self-reported non-smokers were non-smokers after cotinine measurements, and 92 % (95 % CI: 83 %, 97 %) of the women smoking in early pregnancy were also smoking at delivery.

Table 13.
Smoking status in early pregnancy as registered in The Medical Birth Register, and at delivery based on serum cotinine measurements.

Maternal serum cotinine levels	Medical Birth Register		
	Non-smoker	Smoker (≥1 cigarettes/day)	Total
Cotinine cut-off: ≥15 ng/ml			
Non-smoker (<15 ng/ml)	124	7	131
Smoker (≥15 ng/ml)	7	48	55
Total	131	55	186[1]
Cotinine cut-off: ≥3 ng/ml			
Non-smoker (<3 ng/ml)	123	4	127
Smoker (≥3 ng/ml)	8	51	59
Total	131	55	186[1]

[1] Of a total of 204 mothers, 12 had missing data on cotinine levels and 6 on smoking from the Medical Birth Register and were not included in the analyses above.

Lastly, we also ran the analyses stratifying by maternal country of origin to test if there were any differences. The results are shown in Table 14 below, and do not reveal any differences in the overall trend, although the statistical uncertainty was higher in the foreign-born group due to few included individuals.

Table 14.
Smoking status in early pregnancy as registered in The Medical Birth Register, and at delivery based on serum cotinine measurements, stratified according to maternal country of origin.

Maternal serum cotinine levels	Medical Birth Register		
	Non-smoker	Smoker (≥1 cigarettes/day)	Total
Swedish-born mothers[1]			
Non-smoker (<15 ng/ml)	98	6	104
Smoker (≥15 ng/ml)	6	44	50
Total	104	50	154
Foreign-born mothers[2]			
Non-smoker (<15 ng/ml)	26	1	27
Smoker (≥15 ng/ml)	1	4	5
Total	27	5	32

[1] $\kappa = 0.82$.

[2] $\kappa = 0.76$.

Discussion

General discussion

Novelty and consistency

This thesis reports on several associations between exposure to tobacco smoking in utero and health outcomes in childhood and adulthood, some of which have not been reported earlier, or to little extent.

Type 1 diabetes

The finding that fetal smoking exposure is associated with an increased risk for type 1 diabetes is new and contrary to prior studies on the topic, where a majority have found a reduced risk,[66-71] and some no risk.[72,73] There are some methodological aspects with the previous reports that warrant consideration. In some of the studies, estimates became insignificant, or only borderline significant, when confounders were adjusted for.[66,67] Most studies did not have data allowing for quantification of number of cigarettes smoked,[66-68,71] and several based the exposure estimation on recall of distant events.[68,71]

Although there are more studies reporting a reduced risk in the literature, our study has one advantage, since it is the first study that could account partly for genetic predisposition of the disease. One previous study, investigating the risk of islet auto-immunity in relation to perinatal determinants (among which smoking was included), stratified the analyses on higher genetic risk of type 1 diabetes.[74] However, the outcome was not clinical diabetes (only 14 of the 52 included children subsequently developed diabetes), and risk grouping was based on both risk-associated HLA-types and parental diagnosis of type 1 diabetes.

The reason that our results differ from earlier reports is not clear at this stage. We hypothesized that earlier studies lacked the opportunity to account for unmeasured genetic confounding, but it is not known whether smoking habits show a relation to different HLA-alleles. It would be useful to further use the data in the DiPiS-cohort to explore this aspect.

Another area that might hold explanatory potential is gene-environment interactions. As noted, cigarettes contain a large spectrum of harmful substances, and for some of

these, notable interactions that have implications for disease risk have been reported in the literature. One such example is arsenic.[77] This highlights the need to consider other tobacco toxicants apart from nicotine and carbon monoxide.

Animal studies unfortunately give little guidance in determining which association might be more plausible. There are studies showing that nicotine exposed mice had a lower incidence of type 1 diabetes, where the hypothesized explanation is modulation of the immune system,[142] but there are also others showing a decreased beta cell mass in the pancreas, which arguably would speak in favor of an increased risk.[143]

Since this is the first study reporting a positive association, replication in further studies using other data is needed before making strong interpretations. This is particularly important as there are some methodological considerations in this study that might warrant caution, which will be discussed further below.

Obesity

The risk of obesity following prenatal smoking exposure is fairly widely studied, although most studies concern the risk of childhood weight status. The studies on childhood weight consistently show an increased risk of obesity.[44,45,87,144] Whether this risk is transmitted into adulthood is less clear, some reports find it to be consistent into adulthood,[89,90,145,146] whereas others suggest that the associations could be due to confounding. One study found that when discordantly exposed brothers were compared to each other, the risk was no longer significant,[88] and other studies found similar risks associated with paternal smoking as with maternal, suggesting that the increased risk is mediated through other familial factors rather than intrauterine effects.[147-149] However, it can not be ruled out that the passive smoking the mother is exposed to through her partner's smoking lacks a biological effect on the fetus, seen as passive smoking shows an effect on, for example, short-term birth outcomes.[37,38]

Although due to design, our study could not definitively answer the question whether the association with offspring obesity is causal, it adds to the many earlier studies reporting a relationship, by being able to show that additionally, there seems to be a dose-response relationship.

Gestational diabetes

This outcome in relation to maternal smoking has been very sparsely studied in previous literature, likely due to the fact that large cohorts with long follow-up times are required for comparatively rare outcomes like gestational diabetes.

Our results are in line with those from one prior Norwegian study on the subject.[89] Our study expands on the previous report by being able to include a cohort with less risk of selection bias, as well as examining if there was a dose-response relationship. For gestational diabetes, we did not see such a relationship. This might be because by using the fairly crude categorizations in the Medical Birth Register regarding numbers of cigarettes smoked, we were not able to distinguish such a relationship, or women

might have been misclassified, but it is also possible that there is no dose-response relationship biologically.

Preeclampsia

At the time of writing, this is the first study examining maternal smoking during pregnancy and daughters' risk of different manifestations of preeclampsia during their own subsequent pregnancies. The rationale for performing this study was that earlier studies have reported an increased risk of preeclampsia and gestational hypertension if the mother was born small-for-gestational-age or low birthweight herself.[107-113] We therefore hypothesized that there could be a relationship also with intrauterine smoking exposure.

Preeclampsia is dependent on both placental and maternal factors.[99] We did not expect a woman's smoking exposure in utero to have a direct effect on the formation and implantation on the placenta in her own subsequent pregnancies, but rather that it could potentially modify maternal constitutional factors, making her more susceptible to, or less able to compensate for, the factors released by the preeclamptic placenta that reach maternal circulation and cause inflammation.

Our analyses indicated that there could be a weakly increased risk of late-onset preeclampsia if the woman was exposed to tobacco in utero, however, the results were not robust over more severe types of preeclampsia, such as early onset and preeclampsia in combination with a small-for-gestational-age fetus. This is in accordance with our hypothesis, as maternal factors have been shown to be more important for late-onset preeclampsia, whereas early-onset preeclampsia is associated with more placental pathology.[150]

The associations were most apparent when the woman herself was a non-smoker and heavily exposed during her own fetal life. If measures for birthweight and gestational age were included, results were insignificant regardless of type of preeclampsia manifestation, indicating that these factors might be mediating the associations. Similar interpretations could also be made for the women's BMI.

In terms of explaining the etiology behind preeclampsia, intrauterine smoking exposure does not seem to contribute to a large degree in relation to other clinical risk factors. However, taken together with the previous findings of an increased preeclampsia risk if being born small-for-gestational age, it can not be ruled out that some of the maternal susceptibility can be modified by fetal or early-life factors.

Register-data on smoking

Prior studies investigating this question have yielded mixed results. Some find a good agreement between self-report and biomarkers,[119-121] whereas others do not.[122-125] Such differences might have several explanations. Studies are performed in different cultural contexts, which could influence the results due to varying stigma surrounding pregnancy smoking or different maternal health care routines. However, among the

studies from Sweden or other Nordic countries, results also vary.[119,121,123] The main contribution from this study is that the women were not part of a research project unlike in the earlier studies, and as such, arguably more representative of the general population.

Another possible explanation is the use of different cotinine cut-off levels. As discussed previously, optimal cut-offs differ and different levels have been suggested in the literature (ranging from 3 ng/ml to 15 ng/ml denoting active smokers). Of the studies reviewed in this thesis, one used 3 ng/ml,[121] and four used 10-17.5 ng/ml as the cut-off.[119,120,123,125] One study used urinary cotinine, precluding a comparison of cut-off levels.[122] To test that our findings were not only valid for a certain cut-off, we used both the lower and the higher level suggested. Only four individuals had cotinine levels between these values and needed to be re-classified when using other levels, why this had little effect in our data.

About 5 % of the women had to be re-classified from self-reported non-smokers into smokers. A larger proportion were smokers in early pregnancy according to self-report, but non-smokers at delivery, than vice versa. This seems reasonable, as some of the women were expected to quit smoking at some point during the pregnancy. If these numbers reflect a true smoking cessation, our estimates are in line with the previously reported between 11 % and 29 % quitters.[18,151,152]

Lastly, the data in our study showed a ready transfer of cotinine from the maternal side to the fetus, as fetal concentrations on average were 90 % of the maternal levels. This is coherent with previous reports.[153,154]

Biological plausibility and possible mechanisms

Although the causal mechanisms behind the associations discussed above are not fully clear, there are several plausible biological pathways that have been suggested. One likely explanation is that they could be due to epigenetic changes as a response to certain environmental cues. Epigenetic changes are heritable changes in gene expression, that are not due to changes in the actual DNA sequence (such as mutations). The genes are in essence turned on or off in response to environmental stimuli. Such epigenetic changes have indeed been found in offspring to smoking mothers.[155] However, much is still unknown as to which effects such changes have in relation to disease development.

Parts of the hypothesis of Developmental Origins have been tested in a wide variety of different animal model systems, including mice, rats, guinea pigs, sheep and pigs, and the majority of the studies show permanent effects on various organs and tissues following different adverse exposures, such as maternal nutritional deprivation.[58] Potentially relevant findings from animal studies are that prenatal nicotine exposure has been shown in rats to be linked to increased adiposity, blood pressure and

impaired glucose metabolism,[156] which proposes one possible link to the risk of obesity and gestational diabetes. A higher rate of beta cell apoptosis has also been seen in rats exposed to nicotine in utero.[157] Morphological alterations in systems involved in regulation of appetite, and the hyperphagia and weight gain seen upon smoking cessation, could be other pathways involved in obesity development.[158,159] A recent human study has also reported higher levels of the appetite stimulating hormone ghrelin in young adults exposed to maternal cigarette smoke in utero.[160]

However, as most of these mechanistic studies for practical and ethical reasons have to be undertaken in animal models, and since there are caveats with a simple translation from animal model systems into human biology, the field of underlying mechanisms is still an area where much remains unclear.

Criticism of the concept of Developmental Origins

The hypothesis of developmental programming and subsequent adult health, and its relevance, has been questioned. The criticisms that have been put forward concern mainly that epidemiological results are conflicting, effect sizes small and inflated due to publication bias, and the study designs are unable to account for unmeasured confounding from genetics or familial factors.[54]

Studies in humans testing these hypotheses are of observational nature, and evidence as of today is often conflicting, including the results from ”natural experiments” (historical famines, twins).[58,161] This sometimes makes interpretation difficult. However, speaking in favor of the hypothesis is that several of the reported associations are remarkably stable over different populations, cultural contexts and over time period of study.

An aspect that needs to be considered when judging the current state of the literature on this topic, is the influence of publication bias. Small studies showing an association will be easier to publish than those reporting null effects. Even when efforts are made to account for this when conducting meta-analyses, this will still likely affect the conclusions drawn from the literature. A possible explanation for the reduced estimates found in larger cohorts compared to smaller studies in regards to certain topics within the field, is that the smaller studies are more sensitive to random error.[58]

Additionally, many of the studies use proxies for other exposures of interest, e.g. birthweight or anthropometric measures at birth as a proxy for fetal growth or maternal nutrition. It has been questioned how valid these proxies are, as for example, there is no consistent evidence that maternal diet influences offspring birthweight.[58] This particular area would be improved by studies being able to use several measures of fetal growth, such as through ultrasound measurements.

That the associations might be due to confounding by genetics or socio-economic factors is an important objection. Such objections must inevitably arise when

considering the complex hypotheses tested within this field. In response to this it has been argued that, for example, the association between birthweight and adult cardiovascular risk factors is present in all socio-economic groups.[54] To better disentangle the intrauterine contribution from that of genes and adult environment, different approaches are needed. A potential approach in regards to prenatal smoking exposure is to compare effects of maternal and partner's smoking, respectively. If these effects are of similar magnitude, this could indicate that any effect is mediated through familial factors, whereas if maternal effects are larger, there might be a true physiological effect from the uterine environment. Some studies have used this approach and generally, no or a very small extra effect from maternal smoking compared to partner's smoking, have been reported.[46,162]

Other methods for addressing this thematic include sibling or family designs (e.g. siblings from the same family are compared to each other), or studies using data from assisted reproductive technologies (where donated gametes allow for different genetic kinship between family members). These studies often find that the associations are partly or completely attenuated when discordantly exposed siblings are compared to each other, or when genetic inheritance is ruled out by donated gametes or embryos. Outcomes that have been investigated with these types of designs include for example obesity,[88] hypertension,[163] long-term cognitive functioning, externalizing behavior,[164,165] and ADHD.[166]

Although these methods present many strengths, there are also potential biases with these designs.[167] These biases mainly concern confounding by factors not shared between siblings, and measurement error of the exposure. This becomes relevant as sib-pairs likely are more similar, in terms of for example exposure risk, than two unrelated individuals. When using a discordant-sibling design, we are "forcing" them to be more different than expected, implying some kind of selection due to unmeasured non-shared factors.

Related to this, attenuation of associations due to random measurement error in the exposure will be increased: random error is one non-shared factor between siblings by which discordant pairs could be selected, and as a result, there will be proportionally more misclassification among the discordant sib-pairs than in the general population.[167] This could yield results of weaker or absent exposure-outcome associations in the within-pair comparisons, even when familial confounding is not present.

Another aspect in sibling studies regards generalizability (twins, or families that by design must have more than one child, might not be representative of the population). All in all, the question cannot be said to have been finally resolved with the advent of these studies, although they arguably provide much insight. Lastly, there are studies in the field of Developmental Origins, linking birthweight and asthma, that have found positive associations to be robust also when sibling designs were used.[168]

It has also been put into question how important these associations indicating fetal programming are, even if they should be causal.[161] Many of the cohorts that are used for the study of early-life determinants were established several decades to half a century ago, and whether the circumstances driving health and disease during that time are relevant for contemporary populations is not certain. However, some of the circumstances could still be valid for some low- or middle-income countries.

Another major criticism regards effect sizes. In a review of studies on birthweight and subsequent blood pressure (including some 400,000 subjects, aged up to 84 years), Huxley et al. concluded that 1 kg increase in birthweight resulted in a lowered blood pressure by only about 2 mmHg.[169] This has subsequently been met with the claim that, from a clinical perspective, hypertension might be a more relevant outcome and when this was considered in another large American study, about a 40 % decrease in the prevalence of treated hypertension was found over the birthweight spectrum.[54,170]

The scientific dialogue regarding the validity and importance of Developmental Origins is on-going; unfortunately, the methods used in this thesis cannot further with certainty determine whether the relationships between prenatal experiences and adult health are causal.

Methodological considerations

When the quality of observational studies are considered, the risk of three main pitfalls are often judged: selection bias, information bias (misclassification) and confounding. The following discussion will delineate relevant aspects of these biases, as well as provide some other additional considerations, in regards to the studies in this thesis.

Exposure assessment

Use of self-reported data

As has been stressed on multiple occasions, the importance of having an accurate measure of exposure cannot be over-estimated when performing epidemiological studies. Since it is often not feasible or possible to use laboratory measurements, self-reported data are most commonly used. Concerns have been raised that self-reported data on smoking during pregnancy might under-estimate the true fetal smoking exposure, as there is a stigma surrounding these types of health behaviors during pregnancy leading to under-reporting.[122-125]

If all smoking pregnant women under-report such behavior, thus shifting true smokers into the non-smoking group, the result would be an under-estimation of

effects. The problem is that a misclassification of self-reported pregnancy smoking would likely be differential. One recent study that constructed a statistical model to predict proportions of under-reporting, found it to be disproportionately common among women who were college-educated, married, and over the age of 30.[171] It must be noted that the model they used was not derived from pregnant women exclusively, although it was validated against a dataset of pregnant women that had cotinine-calibrated smoking data available. If this is true, estimating effects of differential misclassification provides a tougher challenge, particularly when little is known regarding who under-reports.

The first two studies in this thesis, conducted before the validation report was undertaken, used the association between maternal smoking and offspring birthweight as a validation proxy. The birthweight association is widely studied, with such compelling consistency over time, populations and methodologies, that it is more or less considered causal. Not only that, we know also that there is a dose-response relationship and we know by which magnitude smoking results in a decrease in birthweight (just little below 200 g). In the light of this, the smoking-birthweight association might carry some information as a validation proxy, although some might consider it crude.

The preeclampsia study was performed after the validation was conducted and we were thus able to use this as contributing evidence of exposure accuracy, also lending support to the earlier studies. Our study found the validity of the self-reported data to be high, with the vast majority (95%) of reported non-smokers classified as non-smokers after biomarker measurement. Another relevant finding was that the majority of smokers in early pregnancy (87%) also were smokers at the end of pregnancy.

Another important objection concerning how to estimate fetal exposure to smoking is that, often, only one measurement is used that is supposed to represent the whole pregnancy. This could of course be problematic. There are data in the Medical Birth Register on last trimester smoking collected at around 32 weeks of pregnancy. This variable has been added later to the register (in 1990) and suffers from a high proportion of missing data (75.2 % for the years 1990-1999), precluding the use of it to improve the exposure estimation in the current studies.

It could be used, however, to get an approximation of quitting rates in the G2 generation. Of women with valid smoking data in the first trimester, the proportion of smokers decreased from 13.4 % to 10.4 % of smokers in general, and from 2.6 % to 2.0 % if only heavy smokers were considered. This seems to be coherent with findings from earlier studies, where an estimated 11-29 % of Swedish women stop smoking at some point during pregnancy, out of which the majority quit before the first antenatal visit (and hence would not affect the exposure variable used in this thesis).[151]

Considering the support from the validation study, this indicates that birth register data on maternal self-reported smoking likely are a fairly accurate estimation of fetal

exposure throughout pregnancy. Some methodological aspects in this study should be mentioned, however.

Methodological considerations in the validation study

The validation study included in this thesis has important strengths, one of which is the fact that the women were not asked to participate in a research study. However, some other aspects might need addressing. Although the sample is population-based and randomly selected from the Medical Birth Register, the population is confined to the region of Skåne (the southernmost region in Sweden). The women were pregnant between the years 1982 and 2000, and thus, generalization might not be possible to today's childbearing women and to the whole of Sweden. This also limits global generalizations on the quality of self-reported smoking data. However, as these older register data are often used in today's research, it is of importance to investigate the validity also of historical data.

Another potential issue is timing between measurements. The self-reported data are from early pregnancy, whereas the cotinine measurements come from the time of delivery, meaning that we have no information on a large part of the pregnancies. Occasional smokers, or those with changing smoking habits, might have been misclassified in either direction. The variable on smoking in last trimester in the Medical Birth Register did not have a good enough quality during the study period covered in this paper, and was not helpful.

For the correlation between maternal cotinine levels and levels in offspring umbilical cord blood, the same objection can be voiced: maternal blood was drawn during early labor, whereas the sample from the umbilical cord was collected after delivery. This might be more relevant in the case of pregnant women, where cotinine metabolism has been seen to be faster (around 8-9 hours).[172] However, those studies having used simultaneous measurements, find correlations similar to ours.[173]

The usefulness of the findings are tightly linked to cotinine being the proper objective measure, and that the cut-offs we have chosen have true validity in separating active smokers from non-smokers and non-smokers exposed to second-hand smoke. There are potential alternatives to measuring cotinine, for example breath carbon monoxide levels or serum thiocyanate, however cotinine is generally preferred due to specificity and the longer half-life.[174] The issue of cut-offs was circumvented by the use of two different cut-off levels in this study.

Another important aspect is the possibility of misclassification due to differences in cotinine metabolism, that is, the rate by which nicotine/cotinine is metabolized might differ biologically between women. Such inter-individual and inter-ethnic variation have already been described previously.[175] The transformation of nicotine into cotinine is catalyzed by the CYP2A6 gene, which is a region that has been shown to be highly polymorphic in humans, which could contribute to explaining this variation.[175] Due to our small sample size, and lack of genetic data, the possibilities

were limited to explore in depth whether the agreement between self-reported and biological measures of smoking status differed between different sub-groups of women. But stratification by Swedish born versus non-Swedish born women did not indicate a substantial difference in our data.

We did not have the means to explore the fact that nicotine levels, and hence cotinine, are not only dependent on the amount of cigarettes smoked, but also on the nicotine content per cigarette as well as the depth and force of inhalation.[175] It is conceivable to imagine that someone who is trying to cut back on smoking inhales deeper in order to "get the most" out of the cigarette. This could affect the correspondence between number of cigarettes and cotinine levels.

Cotinine reflects nicotine levels, and there are other sources of nicotine than smoked tobacco. Snuff, as noted earlier, contain high levels of nicotine, and snuff is becoming more prevalent among women. However, during the time frame studied here (1982-2000) the prevalence of snuff use among non-pregnant women was only 1-2 %.[17]

Timing of exposures in relation to outcomes

To have any possibility of making even weak causal assumptions, it is a requirement that it can be determined that the exposure took place before the outcome. This cannot be made in ecological or cross-sectional studies. If the exposure is determined in retrospect after the outcome is known, such as for many case-control studies, there is a risk of recall bias. In this thesis, all exposure data were retrieved from registers before any outcomes were known, so recall bias is unlikely. Further, no women were aware of the study objectives at the time of data collection minimizing reporting bias due to such circumstances.

Separation of pre- and postnatal exposures and exposure to second-hand smoke

This thesis aims to address prenatal smoking exposure. However, it is likely that women who did not quit smoking during pregnancy, also smoked after the baby was born. In addition, there is a correlation between smoking during pregnancy and having a partner that smokes.[21] National statistics from 2012 corroborate the relevance of postnatal smoking: 12 % of the babies had at least one parent smoking the first month of the child's life, or put differently, 5 % of the mothers smoked and 10 % of the other parent/-s.[176] This means that babies exposed to maternal smoking prenatally, are likely to be exposed to second-hand smoke in early-life and childhood. There are also circumstances where the mother ceases to smoke during her pregnancy, but resumes after the baby is born. We had no means of separating these effects in the current setting, which is a considerable limitation.

Assessment of outcomes

All outcomes in this thesis, except for obesity, were based on clinical diagnoses using the International Classification of Diseases system (Swedish versions, 8th-10th revisions). Patients are diagnosed according to international guidelines from corresponding clinical associations.

That all individuals with a certain disease, and only those, get the correct diagnose, is based on several steps: i) afflicted individuals seek health-care at a health-care facility, ii) the patients at these facilities get the correct diagnose, iii) this information is correctly reported and registered in databases/registers.

Regarding type 1 diabetes it is likely that all children with the disease are captured, as it is a serious disease that usually requires in- or out-patient hospital care at some point. In Sweden, all children with type 1 diabetes are referred to a specialist and diagnostic definitions are clear.

Similar arguments can likely be made for preeclampsia. Blood pressure is closely monitored during the maternal health care visits, and any cases of suspected preeclampsia are referred to hospital for further evaluation. Seeing that almost all pregnant women in Sweden avail themselves of maternal health care, we likely capture almost all cases of preeclampsia.

Gestational diabetes in the Medical Birth Register is probably under-estimated, due to a lack of uniform screening practices throughout the country. The cohort in this study was young at childbirth (13-30 years), which probably partly explains the low prevalence noted in our cohort (0.5 %). In some regions in Sweden, all pregnant women receive a diagnostic test for gestational diabetes, whereas in most, only women with risk factors receive the test. What risk factors that should be considered also vary regionally. Indeed, when we performed sensitivity analyses restricted to a region where all women receive the test, the prevalence of gestational diabetes was higher (1.2 %). That some women are falsely classified as non-diabetic would dilute our findings, but since the chance of a woman getting the diagnose is not related to our exposure, this would not constitute a major cause for concern.

Selection bias

The risk of selection bias is a serious and potentially very problematic issue with some study designs. It arises when selection of participants, groups or data for analysis in a study are related to exposure/outcomes status, and thus not representative of the defined source population intended to study. Selection bias can distort results from a study in any perceivable way. Thus, it affects both internal and external validity. It can be the result of for example inappropriate control group selection, differential entry into a cohort (e.g. volunteer or ascertainment bias) and loss-to-follow-up (e.g.

drop-out or non-response). Once selection bias is a fact, it is usually hard to remedy the problem.

Considering the above, having a study design that precludes or minimizes the potential risk of selection bias is a notable strength. The two studies based exclusively on data from the Medical Birth Register arguably avoid selection bias to a high degree, as they include practically all births in Sweden between the defined study period.

However, for the study on type 1 diabetes, there is a risk of selection bias during several steps during the construction of the cohort. The first potential issue was when delivering women were asked for verbal consent for the sampling of blood: women not speaking Swedish, having a complicated birth or when the baby was in distress were not asked as frequently. There was no information on the women declining at this step.

Another potential problem regards continued participation in the study cohort (which involved yearly follow-ups). This concern was further raised when it became evident that the smoking prevalence among mothers of children with diabetes was substantially higher than that of mothers to controls (11.6 % versus 4.8 %). Seeing this, we were worried that the control group consisted of more health-conscious women agreeing to participate.

As the children with diabetes come from two other regional registrations of type 1 diabetes in addition to the DiPiS-cohort, whereas the controls come only from DiPiS, we checked whether this difference remained when restricting the sample to only including those participating through all of DiPiS. The difference was a little attenuated, but the pattern remained (10.6 % versus 4.8 %). At present, we lack an explanation for this difference, but there is little risk that it is related to the risk status of the child, as parents were only allowed to be informed on this after 2004, due to a decision by the ethics committee. The information was also given first *after* the parents had agreed to participate in DiPiS, upon request at the follow-up at two years.

Future steps for this cohort in relation to this question could be to use the information on parental education in the questionnaire data. This was not used in the present setting as it was not expected a priori to have an importance (see below on socio-economic status), and since there was no information on this in the other registrations of diabetes that were used in this study. To summarize, as the risk of some selection in this cohort cannot be excluded, interpretation of the findings must be done very carefully.

Confounders, intermediates and residual confounding

It is of importance in epidemiology to consider alternative explanations for any reported associations. The problem of confounding is one reason for this. In this

context, with very long time periods unaccounted for, the problem is further highlighted. The increased disease risks found in these data could be due to other health behaviors in the G2 women, possibly transferred from the parental generation. These include life-style habits like diet, exercise (however, parts of this might be captured by including maternal BMI), or other potential risk- or health-promoting behavior. Other explanations include genetic susceptibility or different types of gene-environment interactions.

The case of socio-economic status

It is well-known that there is a social gradient in general health, as well as in many specific health conditions, and it is also known that smoking during pregnancy is more common among women with lower educational attainment. This raises the concern that the associations reported in this thesis in reality are due to confounding by socio-economic status. Such reports have already been published in regards to prenatal smoking exposure and overweight risk in sons.[88]

Optimal confounder control in epidemiology requires both that the variable is indeed a confounder and that it is possible to measure it satisfactorily (otherwise there will still be residual confounding). The formal definition of a confounder states that the variable should be i) correlated to the exposure, ii) an independent risk factor of the outcome and iii) that is should not lie as an intermediate on the causal pathway, see Figure 8 below. The likely scenario is probably that socio-economic status is both a confounder and an intermediate.

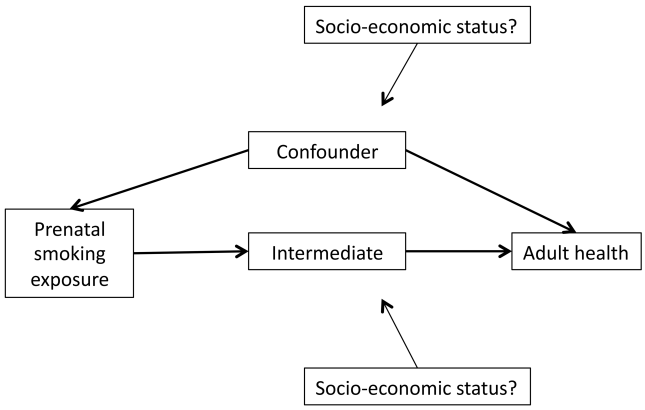

Figure 8.
Model depicting the possible interrelations between the exposure, outcome and socio-economic position, highlighting the difference between a confounder and an intermediate.

Socio-economic status as a confounder poses potentially a problem both in the formal definition as well as in the measurement requirement. First, it is difficult to establish the order of events and that it does not lie on the causal pathway (do you get sick because of your adverse socio-economic position, or do you have a low socio-economic status because you have had ill-health throughout your life and could not work?). Further, its relation to several of the outcomes is still not unequivocally established, which is discussed below.

Socio-economic status and type 1 diabetes.

There are some prior studies on the relationship between different measures of socio-economic context and the risk of type 1 diabetes, yielding mixed results. Some find the risk to be increased with poor socio-economic status,[177] and that children with diabetes have a smaller proportion of mothers with high educational attainment.[178,179] Other studies report that parents of children with type 1 diabetes had more educational qualifications.[68,180] Area-level deprivation has been inversely associated with type 1 diabetes risk in one study, i.e. a higher incidence of diabetes was shown in affluent areas,[181] but was found to be unrelated in another.[180]

Socio-economic status and obesity

That socio-economic context has implications for body size is undisputed. The relationship between socio-economic position and body weight is country dependent in the sense that the pattern differs between high-income and middle-/low-income countries: there is an association between low socio-economic status and overweight/obesity risk in the former, whereas the opposite is the case in the latter.[182] There is a relationship between low socio-economic position and obesity also among pregnant women, and there are indications that this social gradient is increasing in Sweden.[183]

Socio-economic status and gestational diabetes

Although being overweight or obese is a very strong risk factor for gestational diabetes,[184] and obesity in turn relates to socio-economic position, evidence is less clear regarding an independent social gradient in gestational diabetes. Much of this is due to few studies having investigated the relationship, but also, the results that have been reported are conflicting. One recent Australian study found a relationship between low socio-economic status and gestational diabetes risk,[185] but other studies using residential area deprivation as a determinant did not report an association.[186] Recent preliminary data from the region of Skåne, however, using socio-economic measures such as educational level and household income, find an increased risk of gestational diabetes in relation to lower income levels and lower educational attainment (E. Malmqvist, 2015, personal communication).

Socio-economic status and preeclampsia

Studies have in general failed to demonstrate a significant relationship between socio-economic status and risk of preeclampsia. Although one study reports such a relationship,[187] most do not find an association.[188-192] In studies on fetal growth and subsequent preeclampsia, no effect modification was seen when including maternal education,[107,108,110] and another study found that the relationship between educational level and gestational hypertension was largely mediated through BMI.[193]

Measuring socio-economic status

Maybe the greatest problem when trying to incorporate socio-economic status in research relates to how it should be captured both quantatively and qualitatively. An individual's social context is complex, and the effect of it is likely mediated through some other factors. By that is meant that it is not a high education *per se* that makes you healthy, but rather other health-related choices, possibilities and circumstances that a person with higher education, or higher financial means, is more likely to make or have. Figure 9 suggests a conceptual model on how socio-economic status could influence an individual's health.

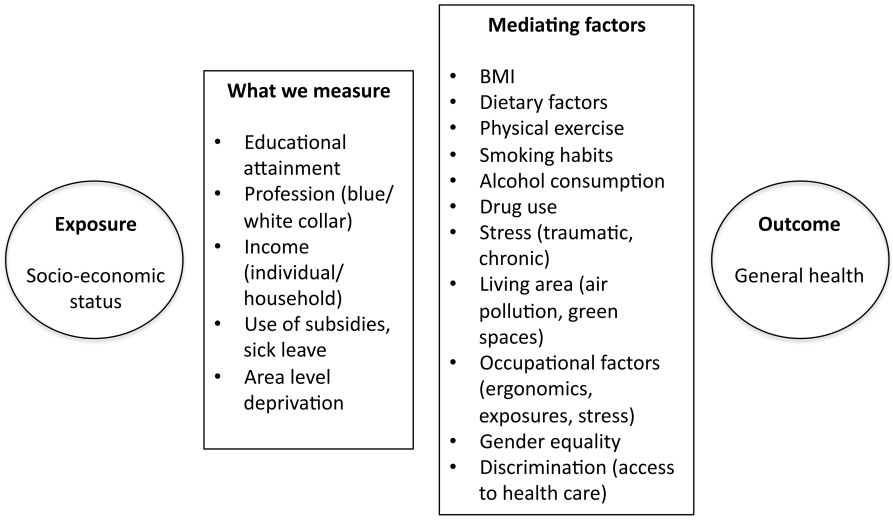

Figure 9.
Overview over the challenges with measuring the effect of socio-economic status on health outcomes.

Common proxies for socio-economic status that are used in research include educational attainment, income levels (individual or family-level), occupation, area-level deprivation and sometimes utilization of government subsidies or sick-leave. For

several of these variables there are registers in Sweden that can be used. It would have increased the validity of the risk estimates in this thesis, had a measure of socio-economic status been included. This is a limitation in the present designs. The question remains, if inclusion of these variables had changed the estimates to a large degree. As seen above, the relationship or the direction of the social gradient for many of the diseases in question are far from clear. For example, in the Norwegian cohort used to study prenatal smoking exposure and risk of gestational diabetes, including education as a confounder resulted only in a 5 % attenuation of the results (L. Cupul-Uicab, 2012, personal communication).

Additionally, for the pregnancy-related outcomes (gestational diabetes, preeclampsia), BMI is the completely overriding clinical risk factor. This speaks against any treatment-altering clinical implication of the main findings of this thesis, but it partly ameliorates the concern that including socio-economic status would make large differences in the results, as the effect not mediated through the women's BMI would probably be smaller than the effect captured by BMI.

Converging the arguments above, it is possible that the findings in this thesis would not hold if measures of socio-economic status were included in the analyses, although it cannot be assumed that this must be the case. Regardless, it is of importance that further efforts are undertaken to test these complex interplays between health and socio-economic context, and this would be the next step in regards to these findings. One interesting aspect to explore would be to examine if there are certain sub-groups of women that might be more vulnerable to prenatal tobacco exposure. Potential methods for doing this include register linkages to educational and income data registers held by Statistics Sweden, or by other designs such as sibling designs. Less useful, but possible with effort, would be to use the data on maternal occupation in the Medical Birth Register, which is registered in free-text.

Missing records and missing data

Although the register aims for completeness, there will always be missing records and missing data. There can be several reasons for this. The data are collected using standard forms in order to make sure all relevant health aspects are covered, however, it is possible that certain questions can be forgotten, omitted or that the woman is unwilling to report. There is often a latency between the introduction of a new variable to the register and a sufficient coverage of that specific variable (such as smoking in last trimester), which could be due to inertia in changing clinical routines. Records can be missing for administrative reasons: both paper or electronical forms could be forgotten or delayed and not sent to the National Board of Health and Welfare, particularly in clinical situations where unexpected or critical situations arise. Such complete missing is not due to maternal characteristics, however, and therefore of less concern in this thesis.

Lastly, records can be missing for unknown reasons or mishaps. For example, all information on maternal weight for the years 1990/91 are missing in the Medical Birth Register. However, these data should be missing completely at random, and thus have little impact on any exposure-disease risk estimates.

Consequences of missing data and possible ways to handle it

Some of the women were excluded from the analyses due to missing data on smoking behavior (the majority) or any of the other included variables (mainly BMI); thus a complete case analysis approach were used for the statistical analyses. There are alternate ways to handle missing data, for example with methods such as multiple imputation, which could have resulted in the inclusion of more data. However, as these registers contain such a vast amount of data, meaning that any power issues generally are absent, there is little reason to use constructed data when we have measured data at hand, specifically if we were to construct data on the exposure variable of interest.

The essential aspect for judging whether these missing data pose a problem is whether the reasons for missing are related to the exposure/outcome status for these women. In the registers, all data are collected prospectively. Whether women in the G1 generation have missing smoking data might not be random (i.e. it could be the women smoking that do not want to disclose their habits, or it could be related to some other characteristic), but this will not be related to the outcomes in her daughter's future pregnancy. Likewise, what the women in the G2 generation report will not be dependent on what their mothers reported decades earlier.

Prior analyses from the Medical Birth Register have indicated that women with missing smoking data were younger or older than average, primiparous or of very high parity, had low or unknown educational level and were more often immigrants.[194] Although women with less education are known to smoke more, the opposite is true for primiparas, immigrants and older women, indicating that the rate of missing is likely similar among smokers and non-smokers.[194] Table 15 on the next page shows selected characteristics of women in our cohort with valid and missing smoking data, respectively, between the years 1982 and 2013.

Table 15.
Characteristics of women with valid and missing smoking data in both generations. Data from 1982-2013.

%	First generation		Second generation	
	Valid smoking n=167,833	Missing smoking n=28,089	Valid smoking n=188,312	Missing smoking n=7,607
Age at childbirth				
<20	6.3	6.8	7.9	11.3
20-29	68.5	66.3	90.6	87.1
30-39	24.1	25.6	1.5	1.4
≥40	1.2	1.3	-	-
Body Mass Index				
<18.5	8.4	8.9	3.4	3.4
18.5-24	74.0	74.8	59.0	58.7
25-29	14.3	13.3	24.0	22.6
30-34	2.9	2.8	9.4	10.6
35-39	0.3	0.2	3.1	3.8
≥40	-	-	1.1	0.7
Missing	30.5	61.2	3.9	85.9
Maternal country of birth				
Sweden	90.0	87.1	100	99.6
Parity				
1	39.5	40.0	67.2	65.5
2	33.7	34.8	27.4	27.1
≥3	26.8	25.2	5.4	6.4
Mode of delivery				
Vaginal	89.2	89.1	79.5	78.2
Emergency Cesarean section	10.0	10.5	8.6	10.3
Elective Cesarean section	0.4	0.2	4.2	4.8
Forceps/Vacuum extraction	0.3	0.2	7.7	6.6
Gestational diabetes[1]	0.2	0.1	0.6	0.6
Preeclampsia	1.8	1.2	3.3	3.9

[1] Prevalence from 1987 and onwards and thus under-estimated in first generation.

The availability of smoking data in G1 was not related to the prevalence of the outcomes in G2. In most aspects, the groups stratified on valid smoking were similar,

although the G2 generation with missing smoking data had a higher proportion of missing also on BMI.

It is possible to imagine that missing smoking data in an antenatal record rather reflects the midwife's style of working and perception of what is important information, than a pregnant woman refusing to answer a question, especially since the stigma around pregnancy smoking was less pronounced for a long duration of the Medical Birth Register. Indeed, most missing on smoking is from the first generation, and in G2 there was only missing for 3.9 % of the women, in spite of the stigma having increased during this time. This, together with the mishap regarding maternal weight data mentioned above, might contribute to explaining the higher proportion of missing BMI among those with missing smoking data.

Future research and challenges

From a scientific perspective, in order to advance the field of Developmental Origins and having an opportunity to unveil any potential causality behind these associations, other types of studies are needed than the pure testing of one risk factor and one outcome, regardless of how much confounder adjustment that is attempted. Outlined below are some areas which still require exploration, as well as some methodological opportunities.

Separation of pre- and postnatal exposures, exposure to second-hand smoke

One issue that was not possible to account for in this thesis was the separation of pre- and postnatal exposure. As children tend to be more vulnerable to harmful exposures than adults (due to other exposure routes and accumulation of substances, as wells as immaturity of the immune system and other organs), post-natal exposure to second-hand smoke might confound the associations in the present thesis. To better disentangle these aspects, linkage to data from other study cohorts, or the sole use of such cohorts, will be needed, as the Medical Birth Register does not collect information regarding this.

Contribution from a potential partner

In the Developmental Origins area, much of the focus lies on the parent giving birth to the child. Very little is known about possible contributions from a potential partner's behavior. Again, other data sources than the Medical Birth Register would be required to explore this further.

Familial confounding, socio-economics and family-based designs

Although the difficulties have been discussed above, it is important to make efforts to further incorporate and address the effects of socio-economics in this context. Such

information can of course be included as confounders in the classical sense, but one of the best methods we have so far are the family-based methods, or sibling designs. Investigating whether an exposure-outcome association remains not only in the between-family analyses, but also in the within-family analyses (comparing for example two discordantly exposed siblings), would give some support to causal claims. However, the risk of introducing bias should not be overlooked.[167] This approach was unfortunately not possible for many of the outcomes in this thesis, such as gestational diabetes and preeclampsia, due to power issues. It could arguably have been possible for obesity.

Multiple exposures/synergistic effects

Epidemiology often concerns one exposure and one outcome, in some cases mediators and effect modifiers are included in the models. However, this bears very little resemblance to the manner in which we are exposed to our environment in reality. We are not exposed to one thing at a time, but rather to a virtual cocktail of different substances. These might act separately to affect disease risk, but may also counteract each other or have synergistic effects. Trying to account for this, or getting closer to the way we are exposed in reality, pose an important but methodologically difficult challenge in epidemiology.

Genetic contribution, gene-environment interactions and assisted reproductive technologies

Many diseases have a heritable component, including all the outcomes in this thesis. Behavior (such as smoking habits) also tends to be inherited, although it is not fully clear to what extent the heritability is genetic or social. Gene-environment interactions have been seen for several exposures – indeed it could maybe explain why some individuals get sick from certain life-style habits and exposures, whereas some do not. Research possibilities for better understanding genetic versus environmental contribution are for example the use data from twins, or families that have used assisted reproductive technologies. The latter could provide situations where the fetus is not genetically related to one or both of the parents, which would help to determine the contribution from for example the intrauterine environment.

Biological pathways

This is a fairly intensively researched area today, and to little surprise. Better understanding how a fetus is influenced or ”programmed” in the uterine environment, would greatly aid in determining whether the reported epidemiological associations are more than just associations. Promising in this area is the field of epigenetics. Epigenetic changes have already been reported following environmental exposures, also from prenatal smoking,[155] but more knowledge is needed as to their biological and clinical relevance, and connection to certain diseases.

Intervention studies/"natural experiments"

The chance of performing intervention studies, or using information from natural experiments, occurs intermittently within epidemiology. The historical famine studies have been mentioned earlier in this thesis. One example of a more recent opportunity is studying the effects of enforcement of smoking bans in public places. One recent study from England concluded clinically significant reductions in stillbirths, low birthweight and neonatal mortality following the introduction of smoke-free legislations.[195] Although, as was pointed out by the authors, one limitation in this report was the absence of individual data on maternal smoking during pregnancy – and hence no possibility to distinguish whether the effects were due to lower active maternal pregnancy smoking or lower exposure to second-hand smoke – such studies at least argue a strong case for the perinatal health benefits of reducing smoking in society.

Future needs from a clinical perspective and safe alternatives

From a clinical perspective, total smoking cessation during pregnancy should be a goal. There is little reason to be content with 6 % of all pregnant women in Sweden smoking, or 19 % of pregnant mothers in the younger age spectrum, considering the extent of maternal health care participation in Sweden, and the general willingness to provide unborn children with a healthy environment. There is a need to better understand the context, motivation and needs of women who continue to smoke during their pregnancy, in order to be better equipped to reach and help these women stop smoking. It is crucial to be perceptive to individual patients and adopt a strategy that does not judge or alienate those seeking maternal health care, as this comes with the risk of an opposite effect than the desired. It has been noted that having a partner who is smoking is an important risk factor for continued maternal smoking, why any interventions might have to be directed to the family constellation as a whole.

In regards to safe alternatives that can be offered, existing literature on the use of nicotine replacement therapies such as chewing gum and patches during pregnancy generally do not show an increased risk of adverse perinatal outcomes.[196,197] Although efficacy is yet to be determined,[198] further delineating potential risks and health benefits of replacing smoking with such therapies would be highly valuable, since these could perhaps provide a treatment option for smoking patients where cessation is otherwise impossible. Additionally, there is insufficient data on whether smoking cessation therapeutics, such as varenicline or bupropion, could be a safe treatment option.[199] Another nicotine-delivering device that is becoming increasingly popular is the electronic cigarette, the e-cigarette. Since this exposure is new, very little is known about its health effects.[200] This should be further addressed.

Lastly, it must not be forgotten that nicotine is a highly addictive substance, and thus, drawing expertise from other areas, such as addiction medicine, would probably be beneficial. What measures of action that would be feasible in the clinical setting of maternal health care needs to be further evaluated.

Public health implications

Under the assumption that the associations found in these studies are causal, the public health implications associated with smoking during pregnancy deserves mentioning. For the outcomes that are fairly rare (such as gestational or type 1 diabetes), the effects would probably be small with a lower prevalence of maternal smoking. For obesity however, there might be considerable gain with less smoking during pregnancy, in particular since we have little knowledge yet regarding possible synergistic and interacting effects.

The findings in this thesis probably have limited implications for the practical routines in maternal health care, as there are arguably other more immediately relatable consequences of pregnancy smoking that might serve as a better motivation for smoking cessation. It rather highlights the gain that could be made by primary prevention, that is, limiting smoking in society as a whole. In countries where very few women in general smoke, it follows that very few smoke during pregnancy. Such prevention would of course also have effects beyond the pregnancy-related health benefits. This could also possibly be a more successful way to reach those last 6 % who are still smoking.

Thoughtfulness is key when epidemiological findings are communicated. The main reasons for this are mainly that the inherent uncertainty with observational designs that precludes strong causal assumptions, is sometimes lost along the course of communication, or is erroneously too much inflated in the interpretation. Additionally, a population-level risk might be hard to translate into risk on an individual-level in a meaningful way. As indicated above, the main audience for the these findings may not necessarily be pregnant women, as there is a risk of further criticizing a group that is already a common target of this, but instead health policy-makers or others who have influence over which public health measures that should be implemented in our health care systems, or on a societal level.

Conclusions

The first conclusion of this thesis is that exposure to tobacco smoking during fetal life might have long-term consequences, as our data show:

i. An increased risk for type 1 diabetes in childhood.

ii. An increased risk for obesity and gestational diabetes in adult women.

iii. Weak, and less consistent, indications of an increased risk of some manifestations of preeclampsia if heavily exposed to tobacco in utero.

The second conclusion is that women seem to report their smoking behavior during pregnancy truthfully, which has two possible interpretations:

iv. Data on self-reported smoking in Swedish registers are valid and can be used for research.

v. Most women seem to experience the maternal health care visits as safe, allowing them to disclose health behaviors surrounded by stigma.

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Original publications

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