

The use of medicinal nicotine in pregnancy for smoking cessation

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Abstract

Background

Smoking during pregnancy is the leading preventable cause of poor birth outcomes for mothers and babies. In 2010, 26% of women smoked during or in the 12 months prior to pregnancy, and 12% smoked throughout pregnancy. 120 trials of nicotine replacement therapy (NRT) have shown that it is effective for smoking cessation in non-pregnant smokers. However, the 9 trials conducted in pregnant smokers, provide no evidence that NRT helps pregnant smokers to stop. In 2012, a large randomised controlled trial (RCT) investigating NRT used in pregnancy for smoking cessation reported that it initially doubled smoking cessation rates, but found no evidence that it enhanced cessation throughout pregnancy. Trial participants reported low adherence to NRT. One possible reason for this is that nicotine metabolism is much faster in pregnancy and so the doses of NRT which have been trialled in pregnancy may not sufficiently treat women's nicotine withdrawal symptoms. This study not only found that children of mothers in the intervention arm had better developmental outcomes at 2 years of age, but also that a dose-response relationship existed between reported higher NRT use and the latter. Using data from this trial, this thesis aims to further explore factors driving/associated with both higher adherence to NRT in pregnancy and improved birth outcomes, and to raise hypotheses about ways in which NRT may be used effectively to help pregnant smokers attempting to quit.

Methods

The studies reported in this thesis used data from 1,050 pregnant women recruited to the Smoking, Nicotine and Pregnancy (SNAP) trial. Linear and logistic regressions, mediation analysis, factor analysis and structural equation modelling techniques were employed to answer the following questions: (1)

which factors are associated with smoking cessation in pregnancy?; (2) which factors, if any, mediate the negative influence of social disadvantage on cessation in pregnancy?; (3) is greater NRT use is associated with poor birth outcomes?; (4) is adherence to NRT associated with greater odds of cessation?; (5) what are the characteristics associated with the rate of nicotine metabolism and does more rapid metabolism of nicotine reduce a woman's chances of achieving cessation?

Results

(1) Within a trial of NRT used for smoking cessation, pregnant women who were more educated (Odds Ratio (OR) at one month post-quit date (one month): 1.82, 95%CI: 1.24-2.67, p=0.002/OR at delivery: 1.89, 95%CI: 1.16-3.07, p=0.010) and less nicotine dependent (OR for baseline cotinine at one month: 0.94, 95%CI: 0.91-0.96, p<0.001/OR for baseline cotinine at delivery: 0.96, 95%CI: 0.92-0.99, p=0.010) / (OR for HSI at one month: 0.50, 95%CI: 0.32-0.76, p=0.001/OR for HSI at delivery: 0.43, 95%CI: 0.23-0.79, P=0.006) were more likely to achieve cessation. (2) There was evidence that the observed relationship between social disadvantage and cessation was mediated by women's nicotine dependence (17.1% of the effect of social disadvantage on cessation), but not by them living with partners who smoked. (3) In an analysis investigating the relationship between use of NRT in pregnancy and birth outcomes, greater reported use of NRT was not associated with either better or worse birth outcomes, either in *a-priori* analyses using birth weight (β: -0.46, 95%CI: -3.58 to 2.66, p=0.773) and being born small for gestational age (SGA) (OR: 1.01, 95%CI: 0.99 to 1.03, p=0.184), or in exploratory analyses using all other SNAP trial birth outcomes. (4) In the first ever detailed analysis of the phenomenon of adherence with NRT in pregnancy, women who adhered more completely had lower pre-treatment cotinine concentrations (β: -0.08, 95%CI: -0.15- -0.01, p=0.020), lower heaviness of smoking index (β: -0.27, 95%CI: -

0.50- -0.05, p<0.001) and were more likely to have been assigned to active rather than placebo NRT (β: 0.51, 95%CI: 0.29-0.72, p<0.001). Greater adherence with NRT was positively and significantly associated with increased odds of smoking cessation (Adjusted OR at one month: 1.11, 95%CI: 1.08-1.13, p<0.001/Adjusted OR at delivery: 1.06, 95%CI: 1.03-1.09, p<0.001), but there was no treatment allocation (i.e. nicotine or placebo)-adherence interaction with respect to cessation (LRT p=0.151). Analyses could not exclude the possibility that the observed adherence-cessation relationship was caused by women who relapsed to smoking consequently also stopping use of NRT. It also remains unclear whether the adherence-cessation relationship may be due the characteristics of individuals who are more likely to adhere (i.e. women who are prone to following instructions to use NRT, might also be more likely to follow instructions to not smoke). (5) In another novel analysis, pregnant women who metabolised nicotine more quickly had reduced odds of cessation (OR at one month: 0.87, 95%CI: 0.76-0.99, p=0.043/OR at delivery: 0.79, 95%CI: 0.66-0.94, p=0.008); however, there was no evidence that NRT was more effective in slower metabolisers.

Conclusions

- Having higher levels of education and lower levels of nicotine dependence were associated with cessation.
- Nicotine dependence mediates the negative effect social disadvantage has on achieving smoking cessation in pregnancy.
- Greater reported use of NRT in pregnancy neither harms the foetus nor protects it from the harms of smoking, with respect to birth weight and SGA, but there is insufficient evidence to draw similar inferences for all birth outcomes.
- Adherence is associated with better smoking cessation outcomes, and women who exhibit greater adherence have differing characteristics

compared to those that adhere less. It was not possible to determine whether or not these better outcomes were a function of women's characteristics or if they were attributable to the treatment they received.

- The rate of nicotine metabolism appears to be important in determining whether or not pregnant smokers will manage to successfully stop smoking, but there was no evidence that NRT effectiveness was influenced by nicotine metabolism.

Quitting smoking during pregnancy is important for both the mother and the child's health outcomes. Socially disadvantaged women are less likely to achieve cessation but this thesis provides the first evidence to suggest that these odds may be improved by addressing nicotine dependence; NRT provides an eminently treatable way of doing this. This thesis's findings suggest that use of NRT is at least as safe as continued smoking with respect to birth outcomes, although probably safer. This thesis also provides the first evidence on the associations between individual pieces of NRT and cessation in pregnancy, as well as the first evidence on nicotine metabolism's association with cessation in pregnancy. Overall, NRT provides a promising way to increase the odds of cessation for disadvantaged pregnant smokers, however, there is a need for further research and the work in this thesis should provide a spur to investigate the effects that adherence to NRT and an individuals' metabolism may have on NRT effectiveness in pregnancy.

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List of abbreviations

3HC	*trans*-3'-hydroxycotinine
95% CI	95% Confidence Interval
AHRQ	Agency for Healthcare Research and Quality
BMI	Body Mass Index
CFA	Confirmatory Factor Analysis
CFI	Comparative Fit Index
CNS	Central Nervous System
CO	Carbon Monoxide
COPD	Chronic Obstructive Pulmonary Disease
Cot	Cotinine
CredI	Credible Intervals
CYP2A6/2B6	Cytochrome P450 2A6/2B6
DAG	Direct Acyclic Graph
DNBC	Danish National Birth Cohort
EFA	Exploratory Factor Analysis
ETS	Environmental Tobacco Smoke
FCTC	Framework Convention on Tobacco Control
FEV	Forced Expiratory Volume
FIML	Full Information Maximum Likelihood
FVC	Forced Vital Capacity
HDL	High Density Lipoprotein
HSI	Heaviness of Smoking Index
IARC	International Agency for Research on Cancer
IFS	Infant Feeding Survey
IMD	Index of Multiple Deprivation
IQR	Interquartile range
LBW	Low Birth Weight
LDL	Low Density Lipoprotein
MI	Myocardial Infarction
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NMR	Nicotine Metabolite Ratio
NRT	Nicotine Replacement Therapy
OR	Odds Ratio
PAH	Polyaromatic Hydrocarbons
PHIAC	Public Health Interventions Advisory Committee
PIS	Patient Information Sheet
ppm	Parts Per Million
PPROM	Preterm Premature Rupture of Membranes
PRAMS	Pregnancy Risk and Monitoring System
RCP	Royal College of Physicians
RCT	Randomised Control Trial
RMSEA	Root Mean Square Error of Approximation
RR	Risk/Rate Ratio
SEM	Structural Equation Model
SES	Socio-Economic Status
SGA	Small for Gestational Age
SIDS	Sudden Infant Death Syndrome
SNAP	The Smoking, Nicotine and Pregnancy trial
SRMR	Standardised Root Mean Square Residual
SSS/P	Stop Smoking Services/for Pregnant women
UK	United Kingdom
USA/US	United States of America
US DHHS	United States Department of Health and Human Services
WHO	World Health Organisation

Chapter 1

Introduction

1.1. Smoking in pregnancy

The World Health Organisation (WHO) global report on tobacco states that nearly six million deaths are caused by tobacco use per year, and that if this pattern continues, this will rise to eight million by 2030.[1] Smoking during pregnancy is a significant public health problem worldwide and it is the leading preventable cause of poor health outcomes for both mothers and babies. Cigarette use exposes pregnant women to over 4,000 chemicals, high levels of carbon monoxide (CO), carcinogens, heavy metals and the addictive substance, nicotine. The detrimental effects of smoking on both the mother and developing foetus make smoking cessation and prolonged abstinence in pregnancy and afterwards of primary importance. In the following sections the prevalence of smoking and harms associated as well as characteristics of women who smoke during pregnancy will be discussed.

1.1.1. Epidemiology of smoking and smoking prevalence in pregnancy

Smoking in pregnancy varies strongly throughout the world, with both country and region. Tobacco use among women was low in the early twentieth century, with women first beginning to adopt regular usage in northern Europe.[2] Since then, point prevalence of smoking in women peaked in northern Europe in the 1970s and 1980s, between 20 and 40%,[2] and declined to between 10 and 20% from the early 2000s onwards.[3] A reduction in prevalence in Nordic countries such as Denmark has been observed since the early 1990s[4] and this has also been seen in the United Kingdom (UK).[5] In the United States (US), the prevalence of smoking in pregnancy is similar to that in northern Europe. Allen *et al.* used data from birth certificates and the Pregnancy Risk Assessment Monitoring System (PRAMS) to estimate that smoking during pregnancy may be 31% higher than the 10.4% prevalence based on reporting on birth certificates, i.e. 15.1% prevalence in 2004.[6] However since questionnaires were completed

two to three months after delivery, these estimates may be subject to recall bias,[7] and could be even higher.

While smoking among young women continued to increase in the southern European states,[2] they seem to now be following the pattern of point prevalence which was previously observed in northern European states. It is important to note that regularly collected prevalence data was not available in northern European states until the 1940s/1950s and the late 1970s in southern Europe, with the exception of Italy.[2] In southern Europe, smoking prevalence amongst mothers is much higher. In Italy in 1990, about 50% of the expecting mothers who smoked, smoked >10 cigarettes per day.[8] A cross sectional study by questionnaire in Valencia, Spain in 1989, reported 60% prevalence of smoking during pregnancy, although this was defined as having smoked at least one cigarette before pregnancy and therefore prevalence may have been overestimated due to differential misclassification.[9]

A recent study found that levels of smoking in pregnancy are higher in eastern European countries (**Figure 1**),[10] but since this study used an anonymous online questionnaire, it may also underestimate the number of pregnant women smoking in these areas. Levels of smoking in Eastern Europe and Latin America have traditionally been low due to historical cultural norms, but are now increasing (15-20%).[3,11] In low income, high-population countries like India and Africa, rates of cigarette smoking amongst pregnant women are currently low (1.7% and 6.1% respectively).[3,11,12] However, rises in the number of younger women smoking indicate that cigarette use has the potential to translate into significantly increased cigarette use in pregnant women in these countries, especially since they may be under-informed on the dangers of tobacco smoking in pregnancy.[13]

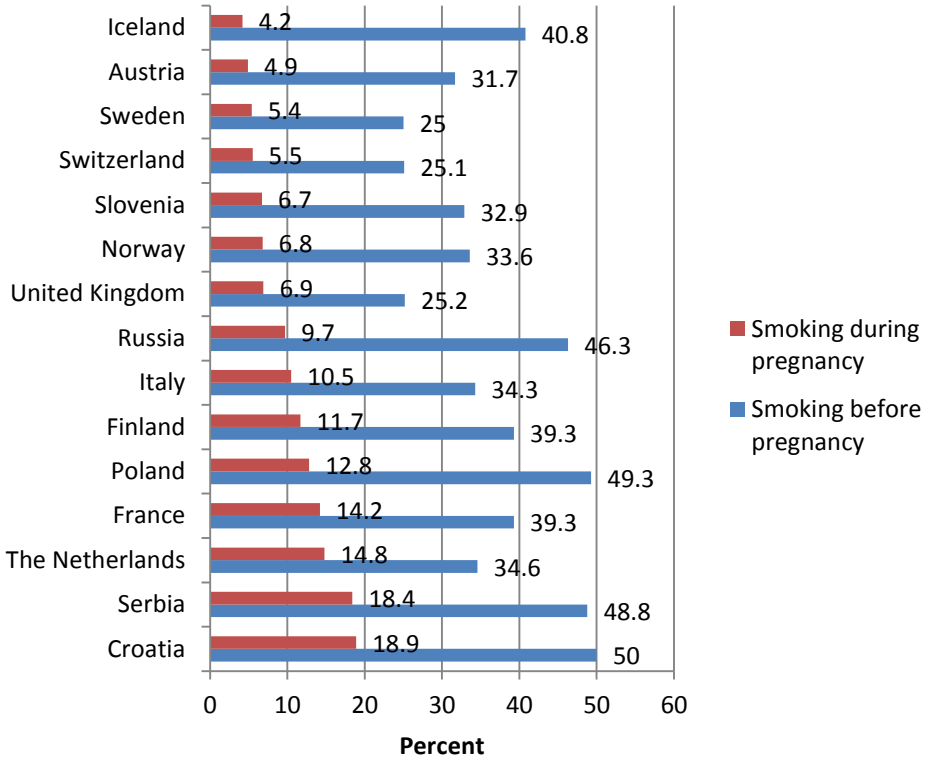


Figure 1 Percentage of women smoking before and during pregnancy in Europe. Adapted from Smedberg *et al.* 2014.[10]

Since the 1980s, smoking prevalence in pregnancy has decreased most in high-income countries.[4,5,14,15] In Sweden, by 2004 smoking in pregnancy had declined to 11% of pregnant women annually.[16] The Netherlands has reported a reduction of more than half between 2001 to 2010, to 6.3% of pregnant women smoking.[17] The 2010 Infant Feeding Survey (IFS) figures reveal that smoking rates in pregnant mothers reduced from 17% in 2005 to 12% in 2010 in the UK.[5] These data, however, are self-reported and not biochemically validated and the true number of pregnant smokers could be higher. Furthermore, in high income countries such as Sweden and the US, the reduction in prevalence among young women has begun to plateau.[4] This could be due to the fact that the decline in smoking prevalence, observed in Sweden and the US over the past three decades has not been seen uniformly in all social demographics. Smoking patterns may be different amongst women with different socio-economic status

or affected by various socio-demographic factors. The following section will detail the characteristics of pregnant smokers.

1.1.2. Characteristics of pregnant smokers

This section describes social determinants that may increase or decrease the likelihood of smoking whilst pregnant. Graham, Hawkins and Law provided evidence on the relationship between socio-economic factors across women's life course and smoking,[18] citing those who are disadvantaged with respect to education, childhood circumstances and current circumstances, being at greater risk of smoking prior to pregnancy. Factors that are known to increase the likelihood of tobacco smoking in pregnancy include being younger and having lower socio-economic classification (**Figure 2** and **Figure 3**),[19] having a lower level of education[20] or having a partner or family member who smokes.[21] Cigarette smoking during pregnancy is a behaviour women from more disadvantaged backgrounds are more likely to indulge in.

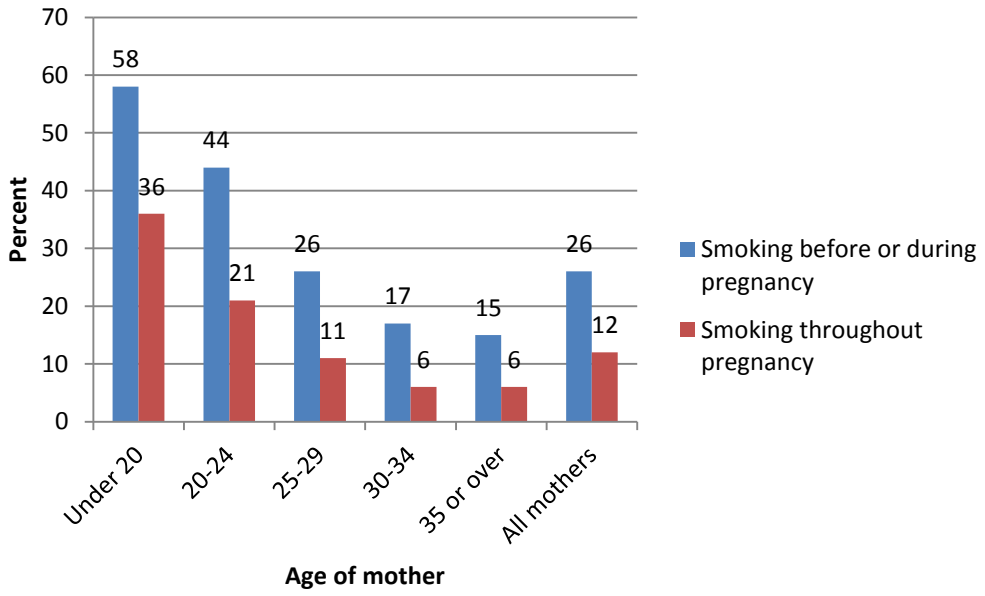


Figure 2 Percentage smoking in pregnancy by age of mother in England and Wales. Adapted from Eastwood 2011.[22]

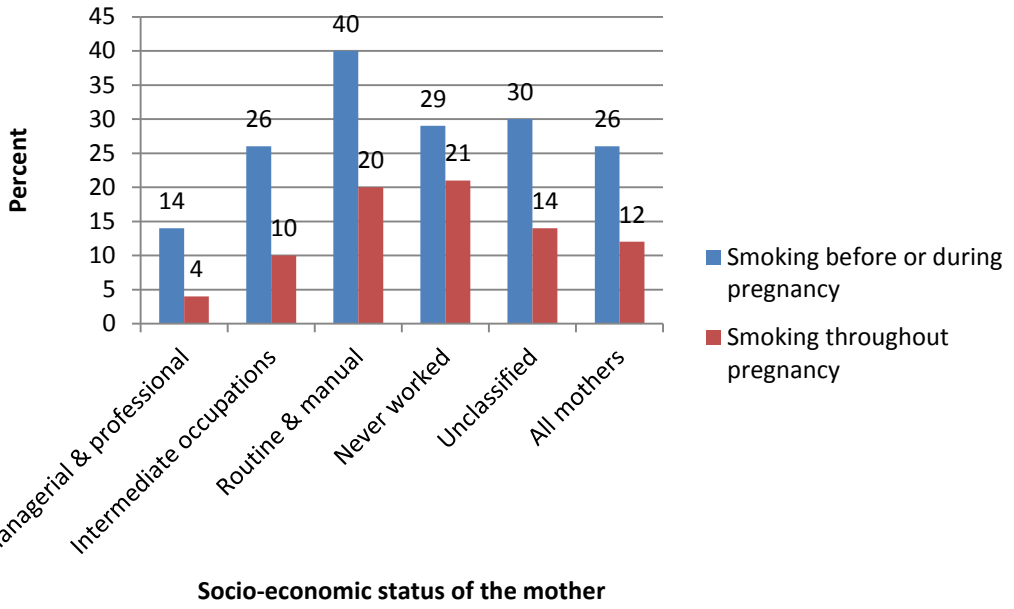


Figure 3 Percentage smoked in pregnancy by socio-economic status in England and Wales. Adapted from Eastwood 2011.[22]

In the UK, the highest number of female smokers is in the 18 and 24 years old age range. **Figure 4** illustrates the distribution of male and female cigarette smokers by age in the UK.

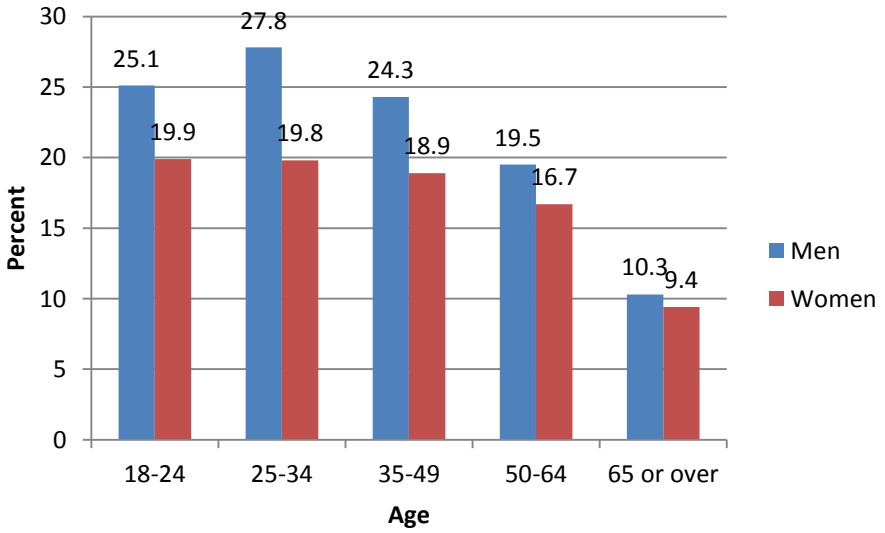


Figure 4 Prevalence of cigarette smokers in the UK by sex. Cancer Research, 2012.[23]

According to the IFS (early 2010), mothers in manual occupations were less likely to quit smoking at some point before or during pregnancy (50%) than those in a managerial or professional roles (72%).[5] Furthermore, women in manual occupations were more likely to smoke than women in managerial or professional occupations in the first instance (40% and 14%).[5] As such, the type of employment women are in, which is a marker of social disadvantage, might also be associated with smoking behaviour.

In **Section 1.1.1** it was shown that smoking prevalence varies in different geographic regions. It is probable that disparities in smoking prevalence within countries are strongly influenced by social disadvantage as well; a WHO report into the social determinants of health suggests that disadvantaged smokers are likely to smoke more, possibly in response to their circumstances.[24] Populations who are more disadvantaged, such as native American, native Alaskan women and Aboriginal and Torres Strait Islander women (46.9%, 55.1% and 53% respectively) have higher rates of smoking prior to pregnancy compared with less disadvantaged women in the same areas.[25-27]

Social pressure to quit and negative attitudes towards smoking during pregnancy may cause underreporting.[28] From previous studies it is known that women who smoke during pregnancy often hide the fact that they currently smoke.[29-31] This may be because of the perceived non-acceptability of smoking during pregnancy. In migrant populations, perceived non-acceptability of smoking has led to different smoking patterns than the rest of the respective populations. For example, migrant populations in Australia, Canada, New Zealand, northern Europe, UK or the US have been found to have lower incidence of smoking compared to women who originate from those countries.[3] Attitudes and motivations for quitting, shaped by circumstances such as migration and factors associated with deprivation, are hypothesised to be important factors for

achieving smoking cessation. As a result of the variation in characteristics and the factors that influence individual's circumstances, there are many trajectories towards cessation. The following section details evidence for different smoking trajectories amongst pregnant smokers.

1.1.3. Trajectories of pregnant smokers

There are several studies that have examined women's smoking trajectories in pregnancy.[32-36] It is estimated that a third of women attempt to stop smoking once they realise they are pregnant.[7] Three studies showed that pregnant smokers change the amount they smoke throughout pregnancy, and although quit attempts are made, relapse whilst women are still pregnant often follows.[32,34,35] A fourth study found that women retrospectively report reduced consumption of cigarettes at booking for maternity care, compared with other points in pregnancy,[36] suggesting pregnancy recognition is a key point. However the reduction in consumption may only be temporary, as reported consumption had increased again by mid-pregnancy.[36] Cigarette consumption in this study may have been affected by recall bias. A fifth study described different trajectories for non/light smokers, non-persistent moderate smokers, persistent moderate smokers and persistent heavy smokers.[33] Smokers of all trajectories had reduced the amount they smoked at 3-4 months into pregnancy. Moderate smokers had a gradual decline in the number of cigarettes smoked per month, while heavier smokers decreased smoking at pregnancy recognition, but increased in the second and third trimesters.[33] Although this study was small, and other trajectories may exist, one conclusion that can be drawn is that it may be necessary to tailor support or the time of intervention by heaviness of smoking since heavier smokers may find it harder to quit than lighter smokers in pregnancy. It is important to reduce or stop any amount of smoking in pregnancy though, because of the associated harms, which are detailed in the following section.

1.2. Harms associated with smoking

There are numerous harms related to active and passive exposure to smoking in adults and children and there is strong causal evidence for the majority of the associations.[37] A recent WHO report estimated that 12% of all deaths among adults aged 30 years and over are attributable to tobacco.[38] The following section outlines the harms that are caused in general by smoking, specific maternal harms, and also those caused in pregnancy that affect the foetus.

1.2.1. Harms caused in general

In 2007 there were 107,071 deaths attributed to smoking in the UK.[39] Smoking in the UK accounts for 84% of all lung cancer deaths and 15% of all circulatory diseases.[19] There is strong evidence for a causal association between smoking and cancer, cardiovascular and respiratory disease.[37]

The US Department of Health and Human Services (US DHHS) Surgeon General's report found that carcinogen exposure from tobacco smoke results in damage to DNA, and increased risk of cancer.[37] Harmful combustion by-products of smoking include polyaromatic hydrocarbons (PAH's), azarenes, N-nitrosamines, aromatic amines, aldehydes, hydrazine, organic benzene compounds, vinyl chloride. Inorganic compounds also found include Nickel, Arsenic, Chromium, Lead, Cadmium and Polonium-210. All of these chemicals have been found to be carcinogenic by the International Agency for Research on Cancer (IARC).[40] The first evidence of an association between smoking and lung cancer was reported in the 1950s,[41] and there is overwhelming evidence that it is causal.[42] Since then, many other forms of cancer have been associated with tobacco smoking, including bladder, pancreas, oesophagus, stomach, kidney, bowel, oral cavity, pharynx, larynx, cervical, colorectal and leukaemia.[43] A 2008 World Cancer Report acknowledges that many associations between smoking

and morbidities are probably causal and estimates that 21% of cancer deaths are attributable to tobacco smoke; in men and women 32% and 8% of all deaths respectively are cancer related.[44]

The risk of cardiovascular disease is increased in individuals exposed to tobacco smoke, in particular to nitrogen, nicotine, CO and oxidising chemicals which contribute to lipid peroxidation; this contributes to endothelial dysfunction and low density lipoprotein (LDL) oxidation.[37] Acute myocardial infarction (MI), stroke, aortic aneurysm and risk of thrombosis are all associated with tobacco smoke exposure.[37] There is a non-linear, dose-response relationship between tobacco exposure and cardiovascular risk that is attributable to smoking, which increases most with low levels of smoking.[37] Cardiovascular disease also has a weaker dose-response relationship with the number of cigarettes smoked per day.[37] Passive exposure to cigarette smoke has also been shown to confer a 30% increase in risk of coronary artery disease, and current smokers have an 80% increased risk.[45,46] While the underlying mechanism for coronary artery disease is currently unknown, there is strong evidence for a dose-response relationship in which greater exposure to cigarette smoke increases the odds of developing heart disease.[47] CO causes cardiovascular disease through oxygen restriction. As CO increases, it replaces oxygen in the blood since it has approximately 200 times greater affinity for haemoglobin than oxygen does.[48] The myocardium extracts more oxygen than other tissues and because of this the effect of hypoxia on it is greater[49]; this increases the risk of angina and MI.

Respiratory diseases are the third main cause of death attributable to smoking. Chronic obstructive pulmonary disease (COPD) is the most common disease associated with smoking, and it in turn is associated with the development of lung cancer, which has the highest mortality rate amongst known associated cancers.[50] COPD is strongly causally linked with smoking,[37] and is driven by

oxidative stress and injury caused by high levels of reactive oxygen species (ROS) in cigarette smoke.[37] COPD has also been linked with genetic mutations involved in the pathogenesis of emphysema.[37] In 2005 alone, in all developed countries, there were 215,000 deaths in men and 122,000 deaths in women attributed to smoking that were thought to be caused by respiratory disease.[39]

1.2.2. Maternal harms

As well as general harms, there are harms that are specifically related to women during pregnancy.

1.2.2.1. Maternal specific complications

1.2.2.1.1. Reproductive complications

A review of the clinical impact of smoking on women found regular smokers are more likely to experience pain, depression, headaches and dysmenorrhea.[51] Smoking has a likely causal association with menstrual changes in women and these occur at a younger age in smokers.[51] In addition there is evidence that smoking results in shorter menstrual cycles which may increase the risk of breast cancer, and may also reduce fecundity as there is a greater chance of egg depletion.[51] Shorter menstrual cycles also mean a greater risk of early menopause. Oral contraceptives have been found to have decreased efficacy when women smoke and are contraindicated for women over 35 years who smoke, as this is associated with increased odds of thromboembolic disease.[51]

1.2.2.1.2. Breastfeeding

Breastfeeding is important for the growth of the child and development of their immune system. It is known that women who smoke are less likely to initiate breastfeeding and also breastfeed for a shorter time.[3] Furthermore, breastfeeding is adversely impacted by smoking, reducing both the amount of milk produced through decreased levels of prolactin, and reducing the fat

content of milk by approximately 19%.[51] Nicotine and its metabolites are also found in breast milk and are therefore transferred to the baby.[51,52]

1.2.2.1.3. Urinary incontinence

Urinary incontinence is the involuntary passing of urine, and is either caused by stress incontinence (urine leaks when there is pressure on the bladder) or urge incontinence (urine leaks after feeling a sudden urge to urinate). Increases in age, pregnancy and childbirth are the most common reasons for reporting urinary incontinence in women. A Norwegian cohort study of 34,755 women observed an increased incidence of urinary incontinence amongst former and current smokers who smoked >20 cigarettes per day.[53] Women who currently smoke have three times the odds of incontinence, whilst former smokers have a two-fold increase.[51]

1.2.2.1.4. Osteoporosis

Osteoporosis, reduced bone density, can lead to fractures later in life. Fractures can lead to disability and loss of independence. There is an increased chance of developing osteoporosis with smoking.[54] A meta-analysis of 29 studies investigating smoking and osteoporosis estimated that one in eight hip fractures were attributable to smoking.[55] Osteoporosis is an especially important issue for women as they often do not achieve the recommended calcium intake, and osteoporosis is more severe in female smokers at the time of menopause, and there is a 7% average decrease in bone density in women who smoke.[51]

1.2.2.2. Pregnancy specific complications

Preterm premature rupture of the membranes (PPROM) is rupture of the amniotic membranes prior to 37 weeks gestation. Heavier smokers are at increased risk of PPROM in early gestation.[56] Smoking during pregnancy is known to reduce the levels of copper and ascorbic acid in the blood plasma

necessary for collagen synthesis, leading to reduced elasticity of foetal membranes and thereby increasing the chance of PPROM.[4]

Placenta praevia, the implantation of the placenta in the lower segment of the uterus, where it is near to or covering the cervix, can lead to severe bleeding and increasing the risk for perinatal death.[4] Smoking during pregnancy is a risk factor for placental complications such as placenta praevia and also for placental abruption, where the placenta separates from the uterus.[57] Placental abruption occurs in 0.4-2% of all pregnancies[4] and women who smoke more cigarettes are shown to be at an increased odds compared with women who smoke less (OR: 1.90, 95%CI: 1.80-2.00).[58] Placental abruption starves the baby of oxygen and nutrients and can lead to restricted growth or increased risk of stillbirth. Women who experience PPROM have a 3.58-fold higher risk of placental abruption when compared with women who do not.[59] While the exact physiological mechanism of these pregnancy complications is not fully understood, it is hypothesised that smoking may increase their incidence.[60]

Preeclampsia is characterised by hypertension and proteinuria. On its own it is a dangerous condition in pregnancy, but it has also been found to increase the risk of being born small for gestation age (SGA), perinatal death and, in a minority of cases, placental abruption.[61] In a meta-analysis of 48 studies, smoking was found to reduce the incidence of preeclampsia by up to 50% and exhibited a consistent dose-response relationship.[62] However, the pathogenesis of this is unclear and requires further research.

An ectopic pregnancy occurs when the ovum implants outside the uterus. The rate of ectopic pregnancy in developed countries was 100-175 per 1 million women during the 1980s and 1990s.[63] Implantation usually happens in the fallopian tubes. A plausible mechanism for linking ectopic pregnancy with

smoking is through fallopian tube motility and oviduct function.[37,64] A meta-analysis of nine studies by Castles *et al.*[65] showed a strong association between smoking and ectopic pregnancy (OR: 1.74, 95%CI: 1.21-2.27). Furthermore, a large French case control study found a dose-response relationship with number of cigarettes smoked.[66]

1.2.3. Foetal harms

In addition to the aforementioned harms caused in general and specific maternal harms, there are several that are specifically related to the foetus. The following section is a summary of the possible harms caused, using review level evidence.

1.2.3.1. Low birth weight

Low birth weight is most often defined as being below the 10th percentile of birth weight, or being born <2,500g at ≥37 weeks gestation. It is estimated that maternal smoking is responsible for 30% of babies born with low birth weight (LBW) in the United States (US).[67] The association between maternal smoking and LBW was discovered by Simpson, in 1957.[68] A recent systematic review of smoking in pregnancy and children indicated that babies of smoking mothers weigh an average of 250g less than babies of non-smoking mothers.[69] A dose response relationship between risk of LBW and smoking more cigarettes, was suggested in a report to the US Surgeon General,[37] and a cohort study by Habek *et al.*[70] supports this; birth weight decreased by around 250g for women smoking 5-20 cigarettes per day and 350g for women smoking >20 cigarettes per day.

1.2.3.2. Small for Gestational Age (SGA)

Being born small for gestational age (SGA) is defined as a baby being born with a weight less than the standardised average for a given gestation. Smoking is considered to have a causal relationship with intra-uterine growth restriction

(IUGR) and this affects the birth weight regardless of gestation.[4] Although the exact mechanism is not fully understood, animal studies have shown cigarette smoke reduces uterine blood flow causing foetal hypoxia-ischemia and malnutrition which may lead to IUGR.[71]

1.2.3.3. Preterm delivery

Preterm delivery, i.e. giving birth at <37 weeks gestation, may explain some of the decrease in birth weight associated with smoking.[70] It is an outcome which is caused by similar factors to those related to low birth weight and SGA birth.[72] The reduction in gestation by continued smoking during pregnancy has been consistently observed, and a recent Cochrane review investigating interventions for smoking cessation in pregnancy found that women who were randomised to cessation interventions had an 18% reduction in preterm births, providing evidence for causality.[3] A large retrospective study found that women who were former smokers, up to and including those who smoked ≥10 cigarettes per day, also had increased odds of having a baby born preterm.[73]

1.2.3.4. Congenital abnormalities

Congenital abnormalities are a category of birth defects that are structural abnormalities. These include cardiovascular, musculoskeletal, facial, gastrointestinal, genitourinary, central nervous system (CNS), respiratory system and skin defects. A meta-analysis of 38 studies found a borderline association in the pooled odds between maternal smoking and all birth defects (OR: 1.01, 95%CI: 0.96-1.07).[74] However, smoking is not associated with some chromosomal birth defects e.g. Down syndrome, and including these has previously biased the effect towards the null. Consequently, when restricting the 38 studies to the three largest prospective studies with >2000 participants, i.e. the most reliable, the pooled OR was 1.04 (95%CI: 1.01-1.07),[74] indicating a positive but borderline association between smoking and the odds of the foetus

developing any congenital abnormality, including minor and major ones. A 2010 Royal College of Physicians (RCP) report found that smoking was not related to major congenital malformations like heart defects, musculoskeletal defects, genitourinary defects or central nervous system defects.[69] The report did find an increased risk of orofacial malformations (OR: 1.90, 95%CI: 2.27-3.07).[69]

1.2.3.5. Foetal and infant demise

Smoking during pregnancy has been shown to increase the number of miscarriages (spontaneous abortions) and stillbirths. Miscarriages are defined as foetal demise which happens ≤23 weeks into pregnancy. There is a positive association between the number of cigarettes smoked and the odds of having one or more miscarriage, and the increase in odds of miscarriage plateaus with women who smoke more than ten cigarettes per day.[75] A systematic review and meta-analysis found that any active smoking increased risk of miscarriage (summary Relative Risk Ratio (RRR): 1.23, 95%CI: 1.16-1.30), with a stronger association when restricted to those studies where smoking occurred specifically during that pregnancy (RRR: 1.32, 95%CI: 1.21-1.44).[76]

Stillbirth is when a baby is born dead at ≥24 complete weeks of gestation. The population-attributable fraction of stillbirths that would not occur if all women in the US stopped smoking is estimated to be 11%.[57] An approximate two-fold increase in the rate of stillbirth has been seen in women who smoke.[57,77] The risk of stillbirth is increased in general within people in lower socioeconomic groups but the mechanism remains unclear.

1.2.3.6. Sudden infant death syndrome (SIDS)

Sudden infant death syndrome (SIDS) is defined as the sudden death of a child within the first year of life, which after autopsy, review of death circumstances and clinical history, remains unexplained.[78] There are approximately 4,000 cases

of SIDS in the US each year.[79] It has been estimated that 61.3% of SIDS cases in women who smoked during pregnancy were caused by smoking, and 21% of all SIDS cases are attributable to smoking.[80] However, it is unclear whether this is due to smoking in pregnancy or postnatally. A literature review of factors associated with SIDS concluded that the aetiology of SIDS is unclear and is likely to be affected by multiple factors including maternal age and education, marital status, previous drug use, sleep environment, genetic polymorphisms in serotonin transport genes, infections and cardiovascular factors.[81] However, it seems likely that smoking would be at least as, or more, influential than social factors such as education or marital status.

1.2.4. Harms caused by Environmental Tobacco Smoke (ETS)

Most research relating to the effects of tobacco smoke on the foetus has been in actively smoking pregnant women, yet, when non-smokers are in proximity to smokers, they are exposed to environmental tobacco smoke (ETS). It has been estimated that 22% of non-smoking pregnant women in the UK live with a partner that smokes, and are potentially exposed to ETS.[69,82] A RCP report on passive smoking and children investigated the effects of ETS on a range on foetal outcomes.[69] It found evidence of increased time to conception of between six and 12 months in ETS exposed women.[69] A meta-analysis in three studies from the RCP report, found that paternal smoking was associated with a 72% increased risk for any congenital malformation (i.e. minor or major).[69] A meta-analysis by Leonardi-Bee *et al.* revealed associations between ETS exposure and 60% increased risk of perinatal mortality, and a 23% increase in the risk of stillbirth.[69] A pooled analysis of nine prospective studies found maternal exposure to ETS was associated with an increase in the risk of low birth weight (OR: 1.32, 95%CI: 1.07-1.63).[83] However in an analysis using eight prospective studies, ETS was not associated with being born SGA.[83] This review also noted that there was no significant increase in the risk of preterm delivery (OR: 1.14,

95%CI: 0.92-1.41).[83] The existing literature provides demonstrable evidence of an association between ETS, passive or paternal smoking and poor foetal outcomes. As a result, indirect exposure to tobacco smoke must be considered a possible source of foetal harms.

1.2.5. Summary: harms associated with smoking

There are numerous harms caused in general, to mothers and to the foetus associated with smoking tobacco. Since smoking causes many harms, one might hypothesise that stopping smoking might reduce them. Smoking cessation provides a way to reduce the incidence of these harms in general, to mothers and to the foetus. The following section reviews the evidence that stopping smoking can improve outcomes.

1.3. Benefits of smoking cessation

In this section I will briefly outline the benefits of smoking cessation in both the general and pregnant populations.

1.3.1. Non-pregnant smokers

The advantage of cessation in the non-pregnant population is the reduction in the risks of cardiovascular disease, cancers and respiratory diseases. Evidence from 50 years' observations in male British doctors suggests that smoking cessation at 60, 50, 40 or 30 years of age gained 3, 6, 9 and 10 years of life expected respectively,[42] highlighting that earlier cessation can reduce mortality from smoking. Tobacco use is also the largest known attributable cause of cancer worldwide, and since 12% of all adult deaths over the age of 30 years are also attributable to tobacco smoke,[38] cessation should be encouraged as this can, over a number of years, reduce the risk of cancer, cardiovascular disease and respiratory disease.[37]

1.3.2. Pregnant smokers

There are potential benefits of smoking cessation in pregnancy that affect people at the individual level and the population level. At the individual level, stopping smoking would reduce the odds of the harms to the mother and developing foetus. A Cochrane systematic review and meta-analysis showed smoking cessation reduced the incidence of preterm births (RR: 0.82, 95%CI: 0.70-0.96) and infants born with low birth weight (RR: 0.82, 95%CI: 0.71-0.94).[3] Smoking cessation has also been found to influence the incidence of infertility and placental abruption.[4] Furthermore, a large retrospective study of 30,524 women found that recent quitting (within the last 12 months) resulted in significantly lower odds of babies being born SGA (OR: 0.43, 95%CI: 0.33-0.57).[84] A systematic review of the benefits of reduced tobacco consumption, which included 25 studies, found that a reduction to less than 50% of baseline tobacco consumption was associated with reduced cardiovascular risk factors (increase in high density lipid (HDL)/low density lipid (LDL) ratio, lower cholesterol, lower pulse rate) and respiratory disease symptoms (forced vital capacity (FVC), forced expiratory volume (FEV)).[85] However, this evidence comes from many small studies and studies with longer term follow-up data are needed to investigate the effect of cessation in pregnancy on these outcomes further.

Smoking cessation is likely to benefit mothers who have other children that may be exposed, since multiparous women are more likely to continue smoking in pregnancy than nulliparous women.[4] Children who have parents that smoke are also more likely to smoke themselves, and addressing maternal smoking in pregnancy may help prevent uptake of smoking and the high levels of morbidities and premature mortality predicted by 2025.[86] Smoking cessation in pregnancy is hypothesised to have benefits irrespective of the length of time smoking. In **Section 1.1.3**, the trajectories of light and heavier smokers suggest that self-initiated smoking cessation occurs at recognition of pregnancy or in

preparation before becoming pregnant. There was also evidence of a reduction in cigarette use 3-4 months into pregnancy, i.e. the second trimester. As the harms associated with smoking occur with increased exposure to cigarette smoke, it is logical to hypothesise that stopping smoking earlier in pregnancy would confer better maternal and foetal outcomes. In the next section, the factors that influence stopping smoking are investigated.

1.4. Influences on smoking cessation

In addition to self-initiated quitting, there are other influences on whether women decide to quit and these are detailed in the following section. In this section I will examine which factors are thought to be associated with smoking cessation in pregnancy. These are important because they could provide potential therapeutic targets for manipulation by smoking cessation interventions.

1.4.1. Pregnancy recognition

Pregnancy recognition can be a motivating event in a woman's life. Smoking cessation rates have been found to be three times higher during the year of pregnancy,[87] although few women make a quit attempt after the first trimester.[77] Pregnant women, women in general, younger smokers and those who are more deprived have lower rates of short-term quitting than other groups.[88] There are potential reasons for this, including that women may be less confident in quitting and the association between gender and level of deprivation.[89] Another possible explanation for the reduction in cessation rates amongst women is that they may be more addicted to nicotine and thus less likely to achieve cessation. However, pregnancy provides a unique opportunity to quit. Pregnant women are more likely to recognise risks to themselves and to the foetus, and this is more likely to elicit strong emotional response,[90] prompting women to quit. In addition, women's smoking is associated more with

the social stimuli of smoking than men, suggesting that women may be more susceptible to behavioural reinforcement of smoking.[91] Many interventions have been trialled to support cessation if a quit attempt has been made and investigating factors that influence cessation in pregnancy may provide evidence to inform when and how pregnant smokers are treated. In the UK, one intervention can be to refer smokers to specialist Stop Smoking Services (SSS), and the following section details their influence on smoking cessation.

1.4.2. Stop Smoking Services (SSS) and Stop Smoking Services in Pregnancy (SSSP)

National Health Service (NHS) SSS and stop smoking services for pregnant women (SSSP) provide an avenue to receive support for quitting. SSSP are offered for free to all pregnant women, and are unique to the UK despite it being in accord with Article 14 of the Framework Convention on Tobacco Control (FCTC).[92] A systematic review of NHS SSS studies found that 37.2% of pregnant women making a quit attempt remained abstinent at four weeks when using CO validation,[88] but longer term abstinence is unknown. There is evidence of relapse over time, with 20.3% remaining quit at 12 weeks, and 12.7% remaining quit at 52 weeks after quit date.[88] However, factors such as 'being afraid of disappointing myself if I failed' and 'not tending to seek help for this kind of thing', which are common reasons pregnant women give for not attending stop smoking courses during pregnancy in the first instance.[93] As a result, most pregnant smokers who attempt to quit do so unaided. From surveys in antenatal classes there is a trend for heavier smokers to make more cessation attempts though,[94] providing some evidence that even heavier smoking individuals may still want to quit. Yet the reach of the SSS for pregnant women remains unclear and more evidence is required to assess what proportion of pregnant women these services reach and therefore influence.

1.4.3. Which factors influence pregnant women making a quit attempts and their success?

In the general population,[95] as well as during pregnancy,[96] there is an inverse relationship between factors that measure social disadvantage and the number of quit attempts made. Previous studies that have investigated factors associated with cessation have found that partners' smoking, greater nicotine dependence and multiparity have been associated with lower likelihood of achieving cessation in pregnancy.[97] Furthermore, it has been found that pregnant women with lower socio-economic status and lower levels of education have lower chances of cessation.[98] Nicotine dependence has been found to be inversely associated with odds of cessation in many studies in the non-pregnant and pregnant populations.[95,97] In addition higher educational levels of partners of smoking women and increased income have been shown to be positively associated with cessation in pregnancy.[28,97,99] Women who have partners that do not smoke, that smoked fewer cigarettes initially, that started smoking at an older age and were primiparous had greater odds of achieving cessation.[98] Currently, no studies have investigated factors associated with cessation in women using NRT for smoking cessation.

1.4.4. Summary: benefits and influences on smoking cessation in pregnancy

Quitting smoking reduces harm to pregnant mothers, their foetuses, and household members. Self-efficacy, pregnancy recognition, provision of services and factors associated with the social demographics of women that smoke, are all likely to influence smoking cessation in pregnancy. However, it is unlikely that these factors act in isolation or directly upon cessation. Further investigation is required to determine any causal associations between these factors, their associations with cessation, and whether any factors mediate the associations with cessation. One hypothesis is that these factors' influences might in fact only

proximally influence cessation. One factor that could mediate these influences on cessation is nicotine dependence. Therefore pharmacological treatments, as well as psychosocial treatments that are used to aid smoking cessation and treat nicotine dependence in pregnancy, are described in the following section.

1.5. Treatments for smoking cessation

1.5.1. Effective treatments in the general population

1.5.1.1. Behavioural therapy/support

Behavioural support may take the form of advice from a professional, pharmacist or other trained individual. A Cochrane review of individual behavioural interventions found that they improved the relative risk of smoking cessation when used alone (RR: 1.39, 95%CI: 1.24-1.57), and to a lesser extent when used in combination with NRT (RR: 1.27, 95%CI: 1.02-1.59).[100] Group behavioural therapy has also been found to increase the odds of cessation compared with self-help (RR: 1.98, 95%CI: 1.60-2.46), but group interventions were not found to be more effective than similar levels of individual counselling.[101] Yet, although behavioural support alone can achieve smoking cessation, it does not address nicotine addiction and metabolism of nicotine directly,[14] and more dependent smokers may require these factors to be addressed to quit. Pharmacological treatments that attempt to address nicotine addiction by substituting the addictive substance derived from cigarette smoking may be required.

1.5.1.2. Nicotine replacement therapy (NRT)

The aim of NRT use is to provide adequate substitution of nicotine, the addictive substance derived from smoking, thereby reducing the craving and withdrawal symptoms associated with quitting smoking. This is hypothesised to reduce the chance that the quitter will relapse to smoking. There are many forms of nicotine replacement therapy (NRT) that have been used for smoking cessation. These

can broadly be categorised into longer acting forms (transdermal patches) that deliver a steady supply of nicotine over the course of a day and intermittent forms (gum, lozenges, inhaler, sublingual tablet, nasal spray) that deliver a more rapid dose of nicotine and are designed to be used *ad libitum*, in place of individual cigarettes.

A systematic review and meta-analysis including 111 trials with over 40,000 participants by Stead *et al.* concluded that, in the non-pregnant population, the rate of cessation was increased between 50-70% depending upon the type of NRT used.[102] NRT has been found to approximately double the odds of cessation (OR: 1.84, 95% credible intervals (CredI): 1.71-1.99).[103] However, a meta-analysis including 31 randomised clinical trials of NRT found that despite the lower rates of smoking cessation amongst women who use NRT for smoking cessation, there was no significant difference in the efficaciousness of NRT between men and women.[104] There was a non-significant decline in odds of cessation in women compared with men at 12 months follow-up though, and this decline in odds was greater in women who received lower intensity behavioural support.[105] A more recent meta-analysis of 14 placebo controlled NRT trials found that while absolute abstinence rates were higher for those assigned to NRT than placebo, at 6 months follow-up, 20.1% of men remained abstinent while only 14.7% of women did.[106] An interaction test between gender and NRT found men to have significantly higher odds of quitting (OR: 1.40, 95%CI: 1.02-1.93, p=0.04).[106] This indicates that women might be less likely to benefit from NRT although the reasons for this are not yet clearly understood. Overall though, NRT for smoking cessation in the general population is effective.[102]

1.5.1.3. Recommended adherence with NRT

As mentioned in the previous section, the main formulations of NRT available are either short acting (e.g. nicotine gum, oral inhaler) or longer acting (e.g.

transdermal patch). The British National Formulary (BNF) provides guidelines for the recommended use of NRT in pregnancy.[107] Nicotine gum is recommended to be used when the urge to smoke arises. A maximum of 10-12 pieces per day is suggested with participants using it for between one and three months. Outside of pregnancy, reduced gum use is started after three months with completion of cessation by six months. The nicotine inhaler, which is available in 10 or 15mg, provides small amounts of nicotine per puff, and it is recommended that patients do not exceed a maximum of 12 doses per day. Nicotine inhaler use is reduced after four months, with treatment permitted for up to 24 months. Transdermal nicotine patches are given in a variety of strengths (e.g. 7, 14, 21mg). Participants are instructed to apply one patch daily and to use 16hr patches, which are removed at night, for 14-20 weeks, and to use 24hr patches for 8-12 weeks.[107] These formulations of NRT are used with the same frequency in pregnancy but there is no consensus about how long courses of NRT should be. In pregnancy, NRT use is preferred when compared with continued smoking and intermittent forms may be used to minimise nicotine exposure to the foetus.[107]

Longer acting NRT formulations such as the patch may be preferable as adherence might be easier to maintain and could avoid the spikes in blood nicotine levels seen with the use of intermittent therapies.[108] Yet, guidance from the National Institute for Health and Care Excellence (NICE) suggest intermittent formulations are preferable,[109] because of reduced overall exposure to nicotine. *Ad libitum* forms of NRT that simulate the high initial levels of nicotine derived from smoking and intermittent forms of NRT may be useful in conjunction, as a combination therapy. However, for either longer or shorter acting NRT, levels of nicotine liberated vary with how they are used, e.g. puffing frequency and depth of inhalation from a nicotine inhaler. It is possible that a lack of adherence to NRT might undermine its efficacy.

1.5.1.4. Alternatives to treatment with nicotine

Despite a possible reduced benefit for women (**Section 1.5.1.2**), NRT is likely safer for use than the alternatives to treatment with nicotine.[110] There are two main alternatives to NRT which have been used outside of pregnancy: bupropion and varenicline. Bupropion is a non-nicotinic anti-depressant that causes increased levels of dopamine through blocking of reuptake by working on neurons in the locus coeruleus. It also causes a release of adrenaline from the adrenal medulla. It is a nicotinic acetylcholine receptor antagonist.[111] Though Bupropion is significantly better than placebo for smoking cessation in the general population (OR: 1.82, 95% CredI: 1.60-2.06),[103] its exact mechanism is not currently known.

Varenicline is a partial agonist of the nicotinic acetylcholine receptor. Varenicline works by binding to nicotine receptors and has a greater affinity for these receptors than nicotine. When it binds to $\alpha_4\beta_2$ receptors, dopamine is released and the pleasurable effect of smoking is achieved.[112] If the individual chooses to smoke whilst using varenicline, nicotine from cigarettes is blocked, thus making smoking less pleasing due to decreased dopamine release. Cravings are also decreased as more nicotine receptors are occupied by varenicline. Varenicline has been successfully used for smoking cessation in the general population (OR 2.88, 95% CredI: 2.40-3.47).[103] Varenicline was found to be more efficacious than Bupropion and nicotine patches, gum or inhalers, sprays, tablets and lozenges, but not more efficacious than combination NRT.[103]

However, to date, there have been no trials of bupropion or varenicline for smoking cessation in pregnancy. Evidence from bupropion exposed mothers suggests that there is no increased risk of congenital malformations, but a greater risk of spontaneous abortion has been observed (14.7% versus 4.5%, p=0.009);[113] though this risk is not greater than that of using other anti-

depressants.[113] Bupropion may potentially be safe in pregnancy, and evidence of transplacental transfer and metabolism of bupropion and its metabolites found placental tissue to be unaffected,[114] meaning foetal tissue could also be protected. More evidence is needed to confirm safety. Varenicline has a similar structure to nicotine and is therefore expected to be associated with similar risks as nicotine. While there is no evidence to suggest that the metabolism of bupropion or varenicline is altered in pregnancy, it is necessary to consider the effect of exposure to the foetus. There is insufficient evidence to assess the safety of either of these medications to mothers or babies and until there is more data regarding birth outcomes associated with either varenicline's or bupropion's use, neither can be recommended for use in pregnancy.

1.5.2. Effective treatments in pregnancy

1.5.2.1. Behavioural therapy/support

A systematic review of 86 trials of psychosocial interventions for smoking cessation in pregnancy found that counselling was effective when compared with usual care (RR: 1.44, 95%CI: 1.19-1.75),[3] although these effects varied when provided alongside other types of interventions i.e. those aiming to improve maternal health.[3] The effectiveness of different counselling strategies did not appear to be different from each other (RR: 1.15, 95%CI: 0.86-1.53).[3] Importantly, for pregnancy, there were no adverse effects from psychosocial interventions.[3] There is mixed evidence of the effectiveness of social support, health education and feedback interventions, but there is evidence to suggest that behavioural support is more effective for pregnant women when the focus is not only on individual change, but also on social and motivational factors as well.[3] The influence of receiving support from a recognised professional may amplify the effect of behavioural support, meaning women may be more likely to be receptive.[115]

1.5.2.2. Nicotine replacement therapy (NRT)

NRT has been the most thoroughly investigated pharmacological agent for smoking cessation in pregnancy. This is probably due to the uncertainty behind actions of other drugs, caution towards use of medications in pregnancy, and the fact that smokers are already exposed to nicotine in pregnancy. The following section details previously identified good quality placebo controlled trials of NRT for smoking cessation in pregnancy.

A Cochrane review of pharmacological interventions for smoking cessation identified six trials of NRT and none for bupropion or varenicline.[112] This review included 1,745 pregnant women. Four trials were placebo RCT's[116-119] and two were non-placebo controlled trials.[120,121] A pooled analysis of all six studies showed NRT was not associated with cessation (RR: 1.33, 95%CI: 0.93-1.91).[112]

Previous RCT studies in pregnancy have had several limitations including not being able to validate cessation using a low enough cut point, being underpowered or prematurely stopping recruitment due to adverse events found in the treatment arm.[112] One factor that could influence the significance of these findings from these trials was whether blinding was used. When split into placebo and non-placebo controlled trials, non-placebo controlled trials showed a greater effect for NRT (OR: 7.81, 95%CI: 1.51-40.35) than placebo controlled trials (OR: 1.20, 95%CI: 0.93-1.56).[112] Another source of difference in the outcomes of these trials could lie in the heterogeneity of the treatment type given. One study provided 2mg nicotine gum[119] and in another trial one third of participants was offered the option of NRT gum, lozenges or 21mg/16hr patches[121]; the remainder received 15mg/16hr NRT patches. The heterogeneity in types of treatment used in these trials makes it difficult to assess if some participants received higher doses of nicotine or if smoking cessation was associated with treatment type. However, in a recent NRT RCT not included in

the Cochrane review, Berlin *et al.* investigated pregnant women randomised to receive high doses of NRT in patches which were adjusted to nicotine levels generated from smoking by using cotinine measurements.[122] This study was carried out in 476 pregnant women between 12 and 20 weeks' gestation, with 203 randomised to nicotine patches and 199 to placebo patches. It was found, that higher doses adjusted to levels from nicotine patches that were adjusted to those received from smoking did not substantially increase the rate of cessation in pregnant women (OR: 1.08, 95%CI: 0.45-2.60).[122] This provides evidence to suggest that women who received greater doses of nicotine might not have had greater likelihood of achieving cessation.

1.5.3. Summary: findings from trials of NRT in pregnancy

Current evidence shows that use of any type of NRT is effective for smoking cessation in the general population (RR: 1.58, 95%CI: 1.50-1.66),[102] but there is no evidence for this in pregnancy (RR for smoking cessation in later pregnancy with NRT: 1.33, 95%CI: 0.93-1.91).[112,122] Since NRT works by replacing the nicotine derived from cigarette smoking, to stem nicotine craving and withdrawal, there are two potential reasons why NRT may not be as efficacious during pregnancy. Firstly, adherence to treatment was low in many trials included in this review (7.2-29% of women used recommended course of NRT)[112] and this may be a potential explanation for NRT not being efficacious in pregnancy;[115,123] NRT cannot work if it is not used. Secondly, there is some evidence that the rate of nicotine metabolism increases with gestation,[124] and this could mean that the dose of nicotine provided by NRT would be insufficient. **Section 1.7** and **1.8** highlight the evidence for adherence and metabolism as factors hypothesised to be associated with the efficacy of NRT in pregnancy. However, before assessing whether these factors influence smoking cessation with NRT, the safety of NRT use in pregnancy must be investigated.

1.6. Safety of NRT in pregnancy

Few studies have investigated whether NRT use is associated with increased risk of adverse events in pregnancy or not, i.e. whether NRT is safe for use in pregnancy and its association with adverse birth outcomes. This section will present the current evidence for how safety can be proven and whether NRT is safe for use in pregnancy.

1.6.1. How can safety be demonstrated?

One way of demonstrating the safety of using NRT for smoking cessation in pregnancy is to compare the birth outcomes from NRT users versus those who continue to smoke in trials of NRT, or use placebo, and determine whether they differ. Use of NRT is theorised to reduce the number of adverse birth outcomes by reducing exposure to approximately 4,000 chemicals, including tar and CO. There are concerns about the safety of nicotine itself though and potentially adverse associations with development.[125,126] Whilst it is theorised that NRT is safer in pregnancy due to lower levels of nicotine provided by NRT compared with tobacco smoking,[127,128] caution is warranted as exposure to any level of nicotine in the developing child also has to be considered. NRT is an effective medication in non-pregnancy and increases the rate of cessation two-fold,[102] therefore worth investigating for cessation in pregnancy. Investigating this relationship would provide evidence to allay concerns about NRT use in pregnancy and the associated concerns regarding higher doses of NRT, which may be necessary in pregnancy.[129] Evidence is required demonstrating similar or reduced numbers of poor birth outcomes in those using NRT, compared with women continuing to smoke. The following section will detail the current evidence for safety of NRT use in pregnancy.

1.6.2. Evidence for safety of NRT in pregnancy

1.6.2.1. Evidence from systematic reviews and trials

The effects of nicotine in pregnancy have been studied and there is evidence to suggest that it is a foetal toxin.[60,126] Tobacco smoke though, contains numerous other toxins in addition to nicotine, and since NRT contains only nicotine, NRT use during pregnancy may represent a harm reduction compared to continued smoking. A Cochrane review and meta-analysis of all NRT RCTs in pregnancy that investigated birth outcomes for women recruited to these trials, found that amongst NRT and placebo patch users, there were no statistically significant differences in the rates of miscarriage, stillbirth, premature birth, birth weight, admissions to neonatal intensive care unit or neonatal death between these groups.[112] Of the studies included in this review, only one found higher rates of adverse birth outcomes in the nicotine treatment arm.[121] Three trials included in this systematic review represent the largest previous placebo controlled RCT's to assess NRT's safety by investigating birth outcomes; however the results of the systematic review are limited in their interpretation of the safety of NRT since the proportion that completed treatment in these trials was low (11%)[117] and use of NRT was not accounted for in any of the studies.[117,119,121] Additionally, a recent RCT by Berlin *et al.* found that use of NRT patches did not significantly increase the birth weight of babies born to pregnant smokers compared with pregnant smokers given placebo.[122]

There is no evidence from previous trials that NRT is associated with reduced numbers of poor birth outcomes.[112] The use of NRT has been associated with some non-serious side effects such as developing headaches, dizziness, fatigue, heartburn, nausea or vomiting,[119] or minor skin irritation where a patch is placed.[112] However, it is not known if some of these minor side effects are causally associated with use of NRT or not. Serious adverse events from NRT trials, summarised in a systematic review and meta-analysis, showed no strong evidence that of NRT had higher rates of miscarriage/spontaneous abortion (RR: 1.24, 95%CI: 0.37-4.17, in three studies), stillbirth (RR: 1.98, 95%CI: 0.55-

7.07, in four studies) or preterm births (RR: 0.85, 95%CI: 0.57-1.26, in three studies).[112] It was noted, however, that in data from the Smoking, Nicotine and Pregnancy (SNAP) trial, there was a higher risk of Caesarean section amongst NRT users (20.7% versus 15.3%).[116]

1.6.2.2. Evidence from observational studies

Whilst trials provide more reliable evidence with regards to causal associations, they are necessarily limited by their size and their ability to detect associations between adherence to NRT and rare birth outcomes. This section briefly summarises the evidence from observational studies that investigated these rarer outcomes.

Research using the Danish National Birth Cohort (DNBC) has compared the prevalence of malformations amongst Danish women that used NRT (n=250), smoked (n=16,812) and were non-smokers (n=55,915) in pregnancy.[130] Women who were exposed to smoking did not have higher odds of congenital malformations than those who did not smoke.[130] However, non-smoking women who used NRT had higher odds of malformations than non-smoking women who did not use NRT (RR: 1.61, 95%CI: 1.01-2.58), and when this was restricted to major or musculoskeletal malformations the risk ratio was higher (RR: 2.63, 95%CI: 1.53-4.52).[130] This finding must be interpreted carefully though as the greater number of malformations in the NRT group was marginal[131] and only a small number of women used NRT. Furthermore, heavier smokers may be more likely to have been prescribed NRT in the first instance, so any association between NRT and congenital malformations may have been confounded by previous smoking in this observational cohort study. In the same Danish cohort, prevalence of stillbirth, a serious adverse outcome, was investigated in NRT users, smokers who used NRT concurrently and smokers who used no NRT.

Smoking increased the risk of stillbirth, but women who used NRT either while smoking or abstinent showed no evidence of an increased risk.[132]

1.6.3. Low adherence: an explanation for the lack of difference in birth outcomes demonstrated by NRT in pregnancy

Adherence to NRT in studies conducted during pregnancy has generally been found to be poor.[112] Two previous studies investigating adherence and birth outcomes categorised NRT as either used or not used,[117,119] so these may have been biased by exposure misclassification. For example, when analysing the use of NRT in the SNAP trial, having used NRT could take any value between 1-56 days' worth of patches, which makes it difficult to assess the impact of NRT on birth outcomes.

Scant evidence is available on NRT use in pregnancy, and non-use or differing use may be the reason for no strong evidence found thus far for a protective effect on birth outcomes when using NRT. Several randomised control trials that have investigated the use of NRT for smoking cessation in pregnancy have also recorded participants' adherence to NRT. In RCTs of NRT in pregnancy, women's NRT use ranged between one and six weeks,[117-121] with an average of approximately three weeks. The largest three trials, all of which were placebo controlled, placed median adherence at 14[117] and 37.8 days,[119] with participants in the largest trial reporting a median of 10 days' worth of patches used.[116] Further analyses of the safety of NRT in pregnancy should account for individuals NRT use; in the next section I will summarise the evidence on adherence to NRT.

1.7. Adherence to NRT

Adherence may be a key factor to account for when determining the efficacy of NRT in pregnancy. As such, in this section, I will examine what is currently

known about adherence to NRT in and outside of pregnancy, and any relationship between adherence and cessation.

1.7.1. Adherence to treatment

Adherence is a key component in the efficaciousness of drugs for any condition. Adherence has been defined by the World Health Organisation (WHO) as "the extent to which the patient follows instructions";[133] logically, drugs cannot work in patients that do not take them. Pregnant women may have particular concerns when following a treatment regime, as they must consider the foetuses wellbeing as well as their own. Consequently, when using medication such as NRT, many women wish to reduce nicotine exposure to the child, and this may result in them minimising their use of NRT, lowering their adherence. **Section 1.7.2** explains the characteristics of smokers which may be related to their treatment adherence in more detail and postulates how this may affect treatment with NRT.

1.7.2. Epidemiology of adherence

The definition of adherence varies with treatment type, but adherence to NRT in the general population is poor.[115] As mentioned in **Section 1.6.3**, adherence with NRT in pregnancy is generally low but there is relatively little data on adherence with NRT outside of pregnancy as well. A WHO report on adherence with long term therapies found that there is a sharp decline in adherence rates to NRT in the first six weeks of use.[133] A study amongst Chinese smokers found that 16% of participants were adherent (at least four weeks use of gum or inhaler in first three months) and that adherence was a significant predictor of achieving cessation, with 40% of adherent participants achieving cessation, compared to only 25% of those who were non-adherent.[134] Several population based studies in Australia and the US have investigated the association between adherence to NRT and cessation in non-pregnancy,[135-137] but relatively few

indicate how long participants were adherent for, or detail factors associated with adherence. There is also evidence to suggest that adherence is important for NRT to have an effect on smoking cessation.[138] Early abstinence experience appears to be an important factor for adherence to treatment; this has been observed in a study investigating bupropion or varenicline, which found high adherence with these drugs and behavioural support in those who had early tobacco abstinence experience.[138] Furthermore, a study investigating NRT for cessation found adherence within the first three weeks improved the odds of cessation at a six week follow-up.[139] The next sections investigate what is known about the relationship between adherence with NRT both within and outside of pregnancy.

1.7.3. Studies investigating impact of adherence to NRT in the general population

In the general population, greater adherence with NRT has been found to be associated with increased odds of achieving cessation;[102] Raupach *et al.* conducted a systematic review of studies that assessed the association between adherence to NRT, or other smoking cessation medications, and cessation in the non-pregnant population.[140] This review aimed to determine the extent to which the included studies attempted to determine whether or not an adherence-cessation association might be explained by 'reverse causation'. Reverse causation occurs when an observed adherence-cessation relationship is not causal but is a simple consequence of the fact that smokers who fail in quit attempts generally stop using cessation medications when they re-start smoking.[141] Of 150 studies that reported an association between adherence to NRT and abstinence, only five with data on adherence and cessation attempted to investigate whether or not relapse to smoking was the cause of reduced adherence to NRT and hence explained any adherence-cessation association.[141-145] Furthermore, the association with adherence and cessation was not a primary

outcome in these studies, and two had small sample size (n<100) with a high proportion of participants lost to follow-up. In two studies that controlled for relapsing to smoking as a cause of non-adherence, a significant weak association was found between adherence to NRT and cessation.[141,142] However, it was determined that further evidence is needed due to the heterogeneity in definition of adherence and the use of self-reported measures of adherence in these studies.

1.7.4. Studies investigating the impact of adherence to NRT in pregnant smokers

Current evidence on the effect of adherence to NRT on cessation during pregnancy comes from only one study. This conducted secondary analyses of data from a clinical trial in 104 pregnant women. It investigated adherence to NRT and the effects of this among pregnant smokers, finding that using NRT as directed in the first 48 hours of a quit attempt was a strong predictor of longer term NRT use.[146] The same study also reported that longer term NRT use was associated with quitting at 38 weeks gestation.[146] However, findings are difficult to interpret as only a univariable analysis of retrospective cohort data was conducted and other explanations, such as relapse to smoking causing a reduction in adherence, may also explain this association. These results could suggest that adherence to NRT may be important for longer term cessation in pregnancy but, given the nature of the analysis used, there may be other explanations for observed associations. Further research investigating the relationship between adherence with NRT in pregnancy and subsequent cessation, including multivariable analyses from larger studies, is needed to determine whether or not this association is causal. The potential reasons for lack of adherence are detailed in the following section.

1.7.5. Potential reasons for low adherence with NRT

Adherence may affect the assessment of the efficacy and safety of NRT. Therefore, it is important to understand the causes of non-adherence and account for these in subsequent analyses. Several possible reasons exist why pregnant women may not adhere to NRT, which are summarised below.

1.7.5.1. Relapse to smoking

Women who achieve abstinence might stop using NRT because of concerns about foetal nicotine exposure or a belief that they have permanently stopped smoking, and may believe that NRT is no longer needed. Some women may give up for the duration of their pregnancy, many women return to smoking after giving birth. Short term cessation after a period of adherence might be seen as symptomatic of women wanting to protect the unborn child from exposure to nicotine and women believing themselves to have quit. But stopping use of NRT too early may be hypothesised to increase the rate of relapse. These women may reduce their use of NRT causing lower blood nicotine levels and resulting in a relapse to smoking. Alternatively, women may relapse to smoking while using NRT, and therefore reduce and discontinue NRT use because they are smoking, i.e. the 'reverse causal' hypothesis. If evidence was found to support this hypothesis, it would suggest that an association between adherence and cessation resulted from the women who have lapsed, using less patches, and adherence to standard dose NRT not increasing the odds of cessation.

1.7.5.2. Side effects/medical reasons

Another reason for participants being less likely to adhere to treatment is to reduce the possibility of side effects. One proposal has been to recommend transdermal patches in pregnancy due to continuous release of nicotine, eliminating the high peaks of nicotine derived from intermittent forms, thereby reducing the likelihood of side effects.[131,147] It is, however, more difficult to assess the safety of NRT when participants are not adherent. Evidence from

good quality placebo and non-placebo randomised control trials show that adherence to treatment is low.[112] Within these, non-serious side effects or adverse events have been reported in three trials,[116,117,119] with the largest of these trials reporting a non-significantly higher rate of non-serious adverse events in the NRT group (535 events in 521 NRT versus 450 events in 529 Placebo).[116] To further validate the safety of NRT in pregnancy, investigations must account for adherence to NRT and side effects must be considered a reason for non-adherence.

1.7.5.3. Low motivation to continue

Low motivation to continue with treatment can arise from a number of sources. Cost of NRT and running out of treatment may be seen as two of the main barriers to adherence. However, the cost of NRT use is not dissimilar to that of smoking and studies of NRT provide an adequate supply of NRT for free, as does the NHS which provides all prescriptions for free during pregnancy. Another cause of low motivation may be having a partner or household member that smokes, which has been shown to be negatively correlated with smoking cessation in pregnancy in many studies.[97] Additionally, having a partner or household member who smokes is associated with socio-economic gradient amongst those trying to achieve cessation in the non-pregnant population[148,149] and so pregnant participants with lower SES might also have lower motivation to quit.

1.7.5.4. Nicotine metabolism

Variation in nicotine metabolism is a potential reason for non-adherence or reduced adherence to NRT. Nicotine metabolism may increase in pregnancy[124] and thus the fixed amount of nicotine derived from adhering to NRT might not be enough to suppress craving and withdrawal symptoms; these symptoms are supressed by maintaining a certain level of free nicotine in the blood. If this is

not maintained, women could stop or reduce NRT and relapse to smoking. The pathways of nicotine metabolism and factors that might influence it are explained in greater detail in **Section 1.8**.

1.7.6. Summary: adherence to NRT in pregnancy

There is evidence to suggest that, outside of pregnancy increased adherence with NRT is associated with longer term smoking cessation but there is no such evidence from studies conducted in pregnancy. The possible causes of non-adherence with NRT in pregnancy should be investigated further and a first step towards doing this would be to describe the characteristics of pregnant women who are particularly adherent to NRT. Future analyses should also investigate whether or not adherence with NRT in pregnancy is associated with smoking cessation and, if so attempts should be made to determine whether or not any relationship is explained by reverse causality.

The potential for increased nicotine metabolism in pregnancy causing women to have lower adherence has been previously mentioned. The following section relates what is known about nicotine metabolism and the extent to which changes in the metabolism of nicotine might affect smoking cessation both within and outside of pregnancy.

1.8. Metabolism of nicotine

The majority of studies that have investigated the influence of nicotine metabolism on cessation have been conducted outside of pregnancy. The rate of nicotine metabolism and the potential implications of this on pregnant women's smoking and their likely success in quitting is discussed in the following section.

1.8.1. Nicotine and cotinine metabolism pathways

Small amounts of nicotine enter the body by buccal absorption and most of the

nicotine derived from smoking is absorbed through the lung mucosa. The alkaline nature of tobacco smoke facilitates nicotine absorption in the alveoli and bronchioles.[52] In addition the amount of nicotine derived from cigarettes is determined by intensity of puffing, smoke dilution with the air and depth of inhalation, which allows the smoker to titrate the amount of nicotine.[52] Nicotine metabolism occurs in the liver and it is converted into several downstream metabolites. The main nicotine metabolic pathway converts nicotine into cotinine,[150] which is then converted to *trans*-3'-hydroxycotinine (3HC) by the same enzyme,[151] cytochrome P450 (CYP) 2A6 (CYP2A6). This is quantitatively the most important route for metabolism as 70-80% of nicotine is converted to cotinine via this pathway.[52,152] Cotinine is the primary metabolite of nicotine, and since it has a longer half-life than nicotine, is used as a marker of exposure to tobacco smoke and nicotine dependence.[52] **Figure 5** illustrates the main metabolism pathway.

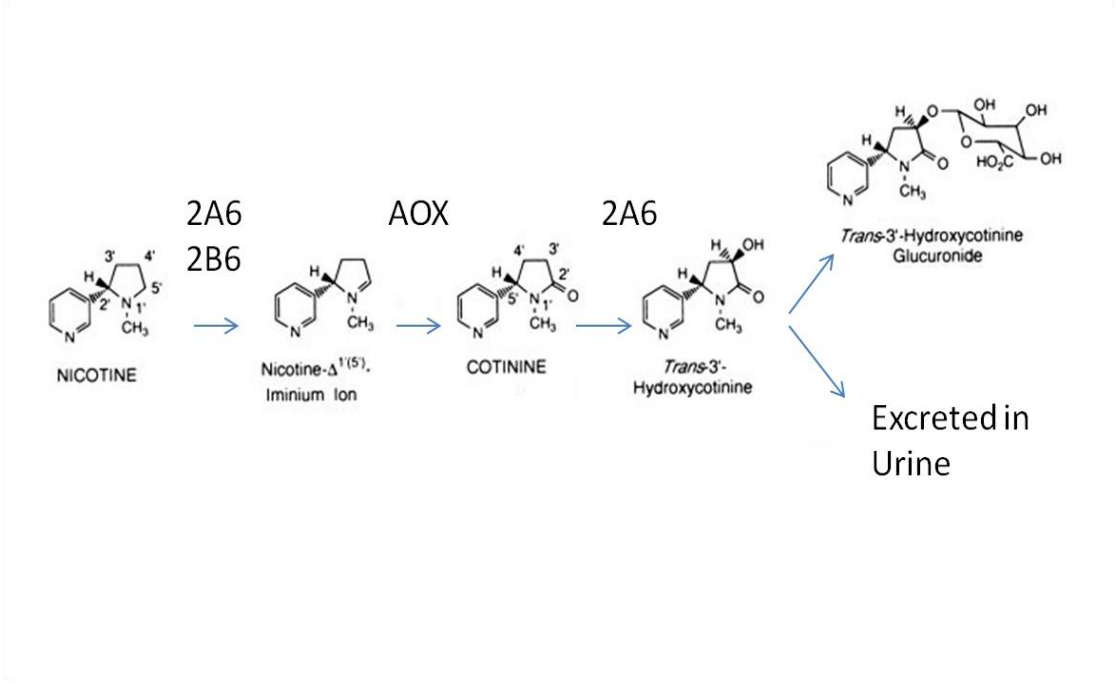


Figure 5 Main nicotine metabolism pathway. Adapted from Hukkanen *et al.*[153] 2005. 2A6 = P450 CYP 2A6, 2B6 = P450 CYP 2B6, AOX = Aldehyde Oxidase.

CYP2A6 and P450 (CYP) 2B6 (CYP2B6), are part of the cytochrome P450 super family of xenobiotic metabolising enzymes, meaning they metabolise foreign

compounds introduced into the body. As a result of CYP2A6 mediating the two steps in the pathway for metabolising nicotine, the metabolism of nicotine to the nicotine iminium ion and also of cotinine to 3HC (**Figure 5**), the speed of CYP2A6 metabolism defines the rate of nicotine metabolism. A twin study showed that CYP2B6 is associated with variation in nicotine metabolism in individuals with reduced CYP2A6 activity outside of pregnancy.[154] While bioinformatic data has shown that the two areas of the chromosome that code for CYP2A6 and CYP2B6 are related, linkage disequilibrium analyses showed no association.[155] This indicates that it is unclear whether the phenotypes for CYP2A6 and CYP2B6 are related and modify the overall rate of metabolism. In addition, research in the non-pregnant population that shows the rate of trans-3'-glucuronidation does not alter the rate of CYP2A6 mediated nicotine metabolism.[156] The rate of nicotine metabolism can be influenced by genotype though,[157] and this is expanded upon further in the next section.

1.8.2. Genotype influence on nicotine metabolism and the NMR

Smoking is a complex behaviour and is influenced by both environmental and genetic factors. Studies in twins have found that liability to initiate smoking results from approximately 60% genetic influences (~20% environmental, ~20% unknown) and genetic factors may be responsible for ~70% of the influence in transition to nicotine dependence.[158] Likelihood of smoking initiation has also been found to be a heritable trait,[159] indicating that persistence and dependence may be influenced by genetic factors. There are many different functional alleles which have been found to confer differing rates of nicotine metabolism. This varying of the rate of nicotine metabolism may influence adherence to treatment; slow nicotine metabolisers may exhibit greater adherence to NRT compared with faster metabolisers. In men and women, Benowitz *et al.* found three distinct groups of enzymatic activity; assuming the

wild type allele to have 100% activity, there was a second group that had 80% activity and a third with 50% of normal activity.[160]

1.8.3. Phenotypic changes in NMR

The cytochrome P450 liver enzyme system is responsible for the metabolism of nicotine to cotinine. Nicotine clearance via the CYP2A6 pathway varies in speed with different alleles, but it appears that phenotypic variation using serum cotinine measurements explains more of the variation in rate of nicotine metabolism than genotype differences might.[159] There is great variation in the rate of nicotine metabolism in the wild type allele and with combinations of other alleles (**Table 1**);[161] classification of rate of nicotine metabolism by phenotype is more accurate.[52]

The nicotine metabolite ratio (NMR) is a phenotypic measure of the speed of nicotine metabolism;[155] it is the ratio of 3HC: cotinine. This ratio represents one step in the metabolism of nicotine (**Figure 5**) and is a proxy for the rate of nicotine metabolism.[162] The NMR is preferable to use of a cotinine: nicotine ratio, as the half-life of the NMR is more stable and thus provides a more accurate measure of the rate.[163] Studies investigating properties of the NMR and of nicotine metabolism itself are summarised below. The NMR is stable measure of nicotine metabolism in plasma and saliva samples but ratio measures from urine samples were less accurate with repeated measures.[164] A brief list of alleles and the NMR's associated with them is summarised in **Table 1**.

Table 1 Alleles of CYP2A6 found in non-pregnant individuals, NMR and their functional % compared with wild type allele. Adapted from Lerman *et al.* (2010).[161]

Allele	Genotype	Observed frequency	Baseline mean NMR ratio	SD	% Mean
Normal metaboliser genotype	*1/*1	362	0.42	0.19	100
CYP2A6*2	*1/*2	21	0.24	0.10	57
	*2/*2	3	0.11	0.08	29
CYP2A6*4	*1/*4	9	0.31	0.17	74
CYP2A6*9	*1/*9	58	0.29	0.11	69
	*9/*9	3	0.15	0.08	36
CYP2A6*12	*1/*12	11	0.21	0.09	50
Two or more different alleles	*2/*9	1	0.65	-	155
	*4/*9	1	0.04	-	10
	*4/*12	1	0.02	-	5
	*2/*2/*9	1	0.32	-	76
Reduced metaboliser genotype	All variants	109	0.26	0.13	62

Different genotypes of CYP2A6 have been found in different ethnic populations, with white and black populations generally expressing faster metabolising phenotypes than Korean or Japanese individuals.[165] One reason for a weak overall association between NMR and genotype that has been observed might be that there are novel alleles yet to be discovered that would improve the association.[163] As such, the following section will discuss the evidence of phenotypic variation of nicotine metabolism in the general population and in pregnancy, as well as characteristics associated with the NMR.

1.8.4. Nicotine metabolism in the general population and measurement using the Nicotine Metabolite Ratio (NMR)

A systematic review of 27 studies on NMR in the non-pregnant population found that the ratio was weakly related to both measures of nicotine dependence and cigarette consumption.[166] This review investigated the validity of the NMR and factors associated with the NMR. Both plasma and saliva NMR correlated well with orally administered nicotine (r=0.79 and r=0.78)[167] and the plasma NMR

from *ad libitum* smoking correlated strongly with orally administered nicotine (r=0.96).[168] However, NMR was found to be weakly related to an increase in the number of cigarettes smoked but a higher NMR was indicative of reduced response to nicotine patches;[161,166] smokers who were identified as 'fast metabolisers' (i.e. having a high NMR) showed lower levels of cessation when given standard dose NRT for smoking cessation.[169,170] Comparatively little is known about the NMR and its influence on NRT effectiveness in pregnancy.

1.8.5. Nicotine metabolism in pregnancy

Dempsey *et al.* have demonstrated that nicotine and cotinine clearances are increased in pregnancy by up to 60% and 140% respectively.[124] Increased metabolism is hypothesised to reduce the blood concentration of free nicotine. Addicted smokers titrate the amount they smoke, or the amount of NRT they use, in order to maintain nicotine homeostasis.[171] Nicotine metabolism may affect efficacy of NRT because higher doses of nicotine might be needed to ameliorate withdrawal symptoms experienced by smokers who have faster rates of nicotine metabolism. Having a non-invasive biomarker, such as the NMR, that predicts whether a woman is likely to benefit from NRT patches is of potential interest; Women who are identified as slow metabolisers and might benefit more from standard dose nicotine patches could have optimised behavioural therapy and NRT regimes given to them.[170]

In a small study of 16 pregnant smokers (eight light and eight heavy smokers), Kim *et al.* used saliva samples to measure cotinine and the NMR.[172] Samples were collected throughout pregnancy and analysed by gas chromatography. Mass spectrometry suggested that a ratio of 0.41 was best to differentiate between light and heavy smokers,[172] i.e. a NMR of ≤0.41 was associated with being a lighter smoker (80% smoked half a pack, 7-10 cigarettes per day) and a NMR of >0.41 was associated with being a heavier smoker (94.3% smoked one

pack per day, 20-40 cigarettes per day).[172] Nicotine metabolism has also been found to increase with gestational age,[173] although, the point in gestation at which nicotine metabolism begins to change is not known. Two studies have attempted to address this; metabolism in pregnancy was measured using hair sample analysis, which investigated the rate of nicotine metabolism within different trimesters using the nicotine to cotinine ratio.[174,175] Koren *et al.* recruited 276 women at 24-26 weeks gestation and took hair samples for each of the trimesters.[175] The nicotine: cotinine ratio decreased significantly from the first to third trimester (13.4 ± 10.7 ng/g versus 10.3 ± 7.8 ng/g, p=0.001), which indicates an increasing rate of metabolism in pregnancy.[175] A smaller study by Klein *et al.* (N=28) also found increasing rate of metabolism from the first to third trimester.[174] Potential problems with these methods are that Kim's study[172] which used saliva cotinine study included only a small number of women (N=16) and the results may have arisen due to chance alone. In addition, hair cotinine analysis[175] represents long term tobacco smoke exposure and thus it is difficult to relate NMR taken from hair samples to a particular point in gestation. Furthermore, the nicotine: cotinine ratio is a less stable measure of metabolism than the 3HC: Cotinine ratio, due to the shorter half-life of nicotine.[124,163,176] Further study of nicotine metabolism via the NMR derived from a stable source using a larger sample of women is needed to overcome these limitations.

1.8.6. Summary: metabolism of nicotine in pregnancy

The NMR has been validated in non-pregnancy as a useful, non-invasive means of measuring the rate of nicotine metabolism. However, few studies have investigated the qualities of the NMR in pregnant smokers; there are no large studies that have measured the NMR from a stable source, i.e. saliva or plasma, or that have investigated their associations with validated cessation in pregnancy. The rate of nicotine metabolism in pregnancy should be investigated further to determine when any changes in nicotine metabolism might occur,

what factors are associated with this and if increases in NMR are responsible for the apparently reduced the effectiveness of NRT in pregnancy.

1.9. Summary of the current evidence

Smoking in pregnancy is a significant public health problem, but especially so since it affects the health and wellbeing of the mother and the developing child. Smoking cessation would result in reduced exposure to nicotine, CO and other toxic components implicated in poor maternal and foetal outcomes. Smoking prevalence in the UK has decreased in recent years but remains at 12%, proving a burden on health services as the leading preventable cause of poor health outcomes for both mother and child. In order to reduce this, smoking during pregnancy and its influences should be addressed and, from the existing evidence, use of NRT provides an eminently treatable way of doing this. However, at present, NRT has not been found to increase the odds of smoking cessation in pregnancy and its safety has not yet been proven despite this being done so in the general population.

Further investigation into factors associated with cessation (**Section 1.3** and **1.4**), the safety of NRT in pregnancy (**Section 1.6**), adherence to NRT in pregnancy (**Section 1.7**) and the characteristics of nicotine metabolism (**Section 1.8**) is required. This thesis will augment the current body of evidence for the understanding of treating smoking in pregnancy with NRT using data from the Smoking, Nicotine and Pregnancy (SNAP) trial to investigate these issues further.

Chapter 2

Aims and objectives

2.1. Aim

The aim of this thesis is to use data from a large cohort of women enrolled in the SNAP trial, to investigate influences on smoking cessation in pregnancy and relationships between factors affecting this, to investigate hypotheses (other than non-efficacy) explaining the absence of evidence for NRT efficacy in pregnancy thus far, and to raise hypotheses about ways in which NRT use in pregnancy might be changed such that it has greater potential to be effective. This aim was investigated through the objectives detailed below.

2.2. Objectives

Within pregnant women who were enrolled in the SNAP trial:

I. To investigate the associations between baseline maternal characteristics and validated cessation at one month after randomisation and at delivery.

II. To investigate whether or not the relationship between social disadvantage and smoking cessation in pregnancy is mediated by more proximal influences on behaviour.

III. To investigate any associations between NRT use and infants' birth outcomes.

IV. To investigate relationships between adherence with NRT and validated smoking cessation at one month after randomisation and delivery, including an assessment of whether or not 'reverse causality' might explain these.

V. To describe rates of nicotine metabolism amongst participants and to investigate associations between nicotine metabolism, maternal characteristics and smoking cessation outcomes.

Chapter 3

The Smoking, Nicotine and Pregnancy (SNAP) trial

3.1. Introduction

The aim of this chapter is to provide an overview of the dataset used for analyses throughout this thesis. As outlined in **Chapter 2**, the aims and objectives of this thesis involve investigating which factors are associated with NRT use and which may influence cessation, including adherence to NRT and the rate of nicotine metabolism. As a result, when investigating these hypotheses, prospective data with a high percentage of outcome ascertainment available for individuals completing follow-up, that was from a large sample, and, that validated the positive response of abstinence among participants would be ideal. The data collected from the SNAP trial fulfils these requirements and thus provides a reliable dataset in which to investigate the aforementioned aims and objectives.

3.2. Study setting and population

The data for this thesis were obtained from the SNAP trial, a NHS Health Technology Assessment (HTA) Programme funded study to investigate the efficacy, cost effectiveness and safety of nicotine replacement therapy for smoking cessation in pregnant women. The study was a placebo randomised controlled trial (RCT) and recruited 1,050 pregnant women between 12 and 24 weeks gestation who smoked ≥10 cigarettes per day prior to pregnancy and smoked ≥5 cigarettes per day at the time of recruitment. The SNAP trial is currently the largest randomised control trial of NRT in pregnancy.[116] Pregnant women were recruited from seven hospital antenatal clinics between May 2007 and February 2010. These hospitals were located in the East Midlands, Stoke and Cheshire and served patients from Nottinghamshire and the surrounding areas.

3.3. Eligibility criteria

At baseline, pregnant smokers eligible for inclusion in the study had the following characteristics:

- 16-50 years of age
- 12-24 weeks pregnant
- smoked ≥10 cigarettes per day prior to pregnancy
- smoked ≥5 cigarettes per day at recruitment
- had an exhaled CO reading of ≥8ppm

Women who had enrolled once before were not allowed to enrol for a second pregnancy.

3.4. Sample size and randomisation

In less than three years, 1,050 pregnant women were recruited to the study. The study aimed to recruit 525 women into each arm. A power calculation estimated that a trial which recruited 500 women to each arm would have 93% power to detect an absolute difference of 9% in smoking cessation rates, or 80% power to detect a 7% change in rates,[177] when analysing by randomisation grouping. Randomisation was carried out using a computer generated pseudo-randomised block method, using blocks of varying size.

3.5. Intervention and control groups

Eight weeks' worth of transdermal patches was administered in four week batches. Pregnant women who were randomised to the treatment group (n=521) received a total of eight weeks' worth of treatment with a standard dose (15mg/16hr) transdermal nicotine patches. Alongside this, behavioural support was provided by the research midwife at initial recruitment, and details were provided for participants to contact the research midwife if extra support sessions were needed. Women were also made aware of the extra behavioural support sessions available, and these were provided by the local NHS stop

smoking services. In the control group (n=529), eight weeks' worth of placebo transdermal patches, that were indistinguishable from the nicotine treatment patches, were given alongside behavioural support in a similar method to those in the treatment group. Participants and administration staff were all blinded to treatment allocations.

3.6. Data collected

At the initial support session before randomisation, all women received a behavioural support session of approximately one hour, from the research midwife after giving consent for entry into the trial. Participants were followed up at one month and delivery (primary end point), as well as six months and one and two years postpartum (data not currently available). Patients received brief information about the trial in the form of patient information sheets (PIS) and data collection was carried out by the research midwife. The initial trial protocol outlined telephone calls at three days post-quit date and eight weeks, although these were for reporting of adverse events. However, no other data were collected at these time points. A complete list of data collected at baseline, one month post-quit date and at delivery is detailed in **Appendix A**.

At baseline, 91.1% of women had complete data for the variables listed. The outcome data in the SNAP trial had a high level of completeness at one month and delivery. At one month, 81.5% and at delivery, 93.4% of women provided outcome data.[116] Women who did not provide outcome data were assumed to not have achieved cessation.

Data was accessed via the Stata trial database. These data was kept on a secure hard drive and patient and practice data was anonymised to avoid deductive disclosure of patient identity. Access to the database was restricted and password protected. In addition to the data provided in the trial database, these

data were augmented with socio-economic information, in the form of the Index of Multiple Deprivation (IMD), and with metabolic data derived from plasma samples of participants included in the SNAP trial. Measures of free and total cotinine and 3'hydroxycotinine from plasma samples were used to derive the nicotine metabolic ratio (NMR). All analyses conducted in the thesis using these data were original and planned for this thesis by Luis Vaz, in consultation with supervisors Tim Coleman, Jo Leonardi-Bee and Paul Aveyard.

3.7. Outcome criteria and follow-up points

Follow-up for validation was carried out at one month post-quit date and at delivery.

At one month, participants were telephoned and were asked about their smoking status. They were questioned about whether they had smoked since the quit date and if so how often they had smoked and whether they had smoked in the previous 24 hours. Additionally, participants were asked about whether they had received any extra behavioural support via telephone, text message or face-to-face. Women who reported themselves as being abstinent were visited for collection of a saliva sample for cotinine estimation and CO validation also took place.

At the primary end point for the study (delivery) reported abstinence from the agreed quit date until delivery was validated using saliva cotinine measurements with or without exhaled CO readings. Women who were unable to be contacted whilst in hospital (i.e. at delivery) were telephoned by one of the study midwives to arrange validation within four weeks of delivery. Furthermore, maternal and infant outcomes were also collected at this follow-up point.

3.8. Summary

The SNAP trial provides data on a large number of pregnant women, who had validated measures of shorter and longer term smoking cessation. The trial data was collected prospectively with high levels of data completeness for both exposure and outcome variables. As a result, the SNAP trial provides an ideal cohort in which to investigate the use of medicinal NRT for smoking cessation in pregnancy.

Chapter 4

Factors associated with cessation in pregnancy

4.1. Introduction

Smoking whilst pregnant is a significant public health problem. Data from the UK Infant Feeding Survey found that, in 2010, 26% of pregnant women had smoked at some point before or during pregnancy and that 12% smoked throughout their complete gestation.[5] The prevalence of smoking in pregnancy in the USA, Finland, Germany, France and UK were 13%, 14%, 15%, 18% and 27% respectively.[97] As a preventable cause of poor health outcomes for mothers and their babies, quitting smoking before or during pregnancy is important. However there are few evidence-based cessation interventions that are proven to work for cessation in pregnancy. In this chapter I will investigate which factors are associated with shorter and longer term cessation in pregnancy.

A previous investigation of the predictors of successful quit attempts made by non-pregnant smokers found that having made fewer attempts to quit and having a higher level of nicotine dependence were both inversely associated with the odds of cessation.[95] Age of the individual and duration of smoking were also factors that were significantly and inversely associated with smoking cessation.[96] A systematic review of factors associated with achieving smoking cessation in pregnancy also found that nicotine dependence was inversely associated with cessation, and that multiparous women had decreased odds of cessation.[97] Additionally, the socio-economic factors of increased income and higher educational levels of the mother and partner have been shown to be positively associated with cessation in pregnancy.[28,97,99] Survey studies, conducted in the UK and Spain have shown similar associations between decreased levels of education and socio-economic status, and having lower odds of cessation.[98] In addition, these studies also found that smoking fewer cigarettes, beginning smoking when older, having a partner who did not smoke and being primiparous increased odds of achieving cessation.[98] However, as these factors are all related to economic deprivation, observed associations may also be explained by higher

rates of smoking and slower declines in smoking rates which have been found in women from lower socio-economic groups.[37]

While many studies have investigated factors which might influence cessation by pregnant[96,97] and non-pregnant smokers[95] in both supported and unsupported quit attempts, there is almost no evidence about which factors might influence women's success when using NRT in cessation attempts made during pregnancy. Although generally, continued smoking is hypothesised to be largely reliant on an individual's dependence on nicotine, attempts to promote cessation in pregnancy by addressing this with NRT have thus far been unsuccessful.[112] As a result, factors associated with cessation in pregnancy from trials where women have been treated for nicotine dependence are investigated in this chapter.

4.2. Aim

The aim of this chapter was to use a cohort of participants from the SNAP trial to investigate independent associations between baseline maternal characteristics and smoking cessation. This was investigated at early (one month post-quit date) and late (delivery) follow-up time points.

4.3. Methods

4.3.1. Baseline explanatory variables included in analysis

A full list of variables collected in the SNAP trial is listed in **Appendix A**. When considering which baseline variables to include in the modelling process, candidate variables were assessed for potential collinearity prior to the modelling process. In particular, multicollinearity was assessed between the heaviness of smoking index, which is derived from the time to first cigarette and the number of cigarettes smoked, and baseline cotinine level. No collinearity was found between any of the baseline exploratory variables included in this analysis (VIF [Variance inflation factor]: 1.09, r^2: 0.083). Baseline variables collected from

participants that were included in this exploratory analysis included: date of birth, ethnicity, age on completion of full time education, partner's smoking status, parity, gestational age, heaviness of smoking index (HSI), body mass index (BMI) and previous use of NRT during their current pregnancy. Cotinine estimation was derived from saliva and plasma samples, and carbon monoxide (CO) readings were also obtained; these measured nicotine and cigarette smoke exposure respectively. The trial recruitment site and participants' treatment assignment, either to NRT or placebo transdermal patches, were also available from baseline data collected prior to randomisation.

4.3.2. Baseline explanatory variables excluded from analysis

The number of additional behavioural support sessions provided by the National Health Service (NHS) Stop Smoking Services (SSS), either by face-to-face contact, telephone or text message was excluded from the analysis. The rationale for excluding this variable was that women would only be likely to attend NHS SSS for behavioural support sessions if they were not smoking; attendance at behavioural support sessions would be very strongly related to cessation and measuring a very similar quality. It would not have been appropriate to include this variable in a model that attempts to determine associations between baseline maternal factors and the odds of cessation because this would potentially mask the relationship between other baseline variables and cessation.

4.3.3. Outcome variables

For analyses in this study, validated cessation at one month post-quit date and at delivery were used as outcome variables. At one month, cessation was defined as reported continuous abstinence from quit date to one month, validated by an exhaled CO reading of <8ppm; and, at delivery, cessation was defined as reported continuous abstinence from a quit date until delivery,

validated by an exhaled CO reading of <8ppm and/or a saliva cotinine level of <10ng/ml. Cessation was defined in accordance with the Russell Standard, which allows participants classified as abstinent if they have smoked up to five cigarettes in total between quit date and a validated follow-up point. It is likely that women who did not report smoking status continued smoking, and they were, therefore, coded as not having achieved cessation.

4.3.4. Analysis strategy

Initially, associations between the previously mentioned baseline characteristics and cessation at one month after beginning treatment i.e. one month post-quit date, and also at delivery were investigated. A multivariable model was built for each time point.

Firstly, significant univariable associations ($p \leq 0.05$) were identified between these variables and validated cessation at each time point. Secondly, the variables were entered into a multivariable logistic regression model using a stepwise backwards elimination method, removing variables that subsequently had non-significant associations with outcomes ($p > 0.05$). Thirdly, variables that originally showed no association with validated cessation were added back into models individually to determine whether or not they then became significantly associated with outcomes. Two multivariable models were built for validated cessation at each time point and treatment assignment was considered an *a-priori* confounder in both models. This was because women who received NRT rather than placebo transdermal patches were hypothesised to be more likely to achieve cessation.[112]

To maximise the number of participants included in the analysis, a 'missing' category was created for categorical variables with missing data where possible, and imputation was planned for continuous variables (baseline cotinine) where

more than 10% of cases had missing data. Where all missing values for an exposure occurred among women in the same outcome category (i.e. continuing smokers), or a small percentage of data was missing for a continuous variable, a univariable sensitivity analysis comparing all participants against those with complete data was conducted, to verify that they did not differ in their baseline characteristics.

All analyses were conducted using Stata 13.1 (College Station, TX).

4.4. Results

4.4.1. Descriptive analysis

In the one month and delivery multivariable analyses, missing data for the categorical variable, 'age full time education finished' could not be included as all those with missing data were smokers at follow-up and inclusion as an extra category would perfectly predict the outcome. Furthermore, imputation for the continuous variable 'baseline cotinine level' was not carried out due to data being missing for only 80 participants (7.6%). As a result, analysis was undertaken on 957/1050 participants (91.1%), for whom complete exposure data were available, and the characteristics of women included are detailed in **Table 2**. At one month 167 (17.5%), and at delivery 84 (8.8%) of the participants in this sample of the SNAP trial participants, achieved validated cessation. Including the 93 participants for whom there was some data missing did not alter the multivariable model.

Table 2 Participant characteristics for cessation at one month and delivery

Variable	One month		Delivery	
	Smoking (n=790)	Abstinent (n=167)	Smoking (n=873)	Abstinent (n=84)
Age [median (IQR)] (years)	25 (21-30)	25 (21-31)	25 (21-30)	25 (21-31)
Primiparous	36.8%	37.1%	36.3%	42.9%
Age full time education finished (years)				
≤16	80.5%	68.9%	79.6%	66.7%
>16	19.5%	31.1%	20.4%	33.3%
Ethnicity				
British - White	97.3%	96.4%	97.4%	95.2%
Other	2.7%	3.6%	2.6%	4.8%
Gestational age [median (IQR)] (weeks)	15 (13-18)	15 (13-18)	15 (13-18)	15 (13-20)
Baseline cotinine [median (IQR)] (ng x 10^{-1}/ml)	12.7 (8.7-18.2)	9.1 (5.8-13.8)	12.4 (8.1-17.8)	10.6 (6.0-15.3)

4.4.2. Univariable and multivariable associations with validated cessation at one month

At one month, in the univariable analysis, women who finished full time education when they were >16 years had greater odds of cessation; women who had a higher baseline cotinine levels, HSI, and those who were recruited from trial site 4, were found to have lower odds of cessation (**Table 3**). The final multivariable model shows which baseline variables were independently associated with validated cessation at one month. Women who were aged >16 years when they finished full time education (OR: 1.82, 95%CI: 1.24-2.67, p=0.002) had significantly increased odds of achieving validated cessation. Participants who had a higher baseline cotinine (OR: 0.94, 95%CI: 0.91-0.96, p<0.001 for a 10ng/ml increase) and higher HSI score (OR: 0.50, 95%CI: 0.32-0.76, p=0.001) had significantly lower odds of cessation at one month after quit date. The effect of trial recruitment site 4 did not remain significant when added to the multivariable model (OR: 0.69, 95%CI: 0.36-1.34, p=0.277).

Table 3 Univariable and multivariable associations with validated cessation at one month

Variable	N	Smoking at 1m (%)	Cessation at 1m (%)	Crude OR	95% CI		P-value	Adjusted OR	95% CI		LRT P-value
					Lower	Upper			Lower	Upper	
Age (years)											
<20	147	129 (87.8)	18 (12.2)	0.64	0.36	1.12		-	-	-	
20-24	322	264 (82.0)	58 (18.0)	1.00	-	-		-	-	-	
25-29	228	183 (80.3)	45 (19.7)	1.12	0.73	1.73	0.250*	-	-	-	-
30-34	158	134 (84.8)	24 (15.2)	0.82	0.49	1.37		-	-	-	
35-39	78	63 (80.8)	15 (19.2)	1.08	0.58	2.04		-	-	-	
≥40	24	17 (70.8)	7 (29.2)	1.87	0.74	4.73		-	-	-	
Ethnicity											
Other	27	21 (77.8)	6 (22.2)	1.00	-	-	0.521	-	-	-	-
British - white	930	769 (82.7)	161 (17.3)	0.73	0.29	1.84		-	-	-	
Age full time education finished											
≤16	751	636 (84.7)	115 (15.3)	1.00	-	-	0.001	1.00	-	-	0.008
>16	206	154 (74.8)	52 (25.2)	1.87	1.29	2.71		1.82	1.24	2.67	
Partners' smoking status											
No	225	186 (82.7)	39 (17.3)	1.00	-	-		-	-	-	
Yes	654	544 (83.2)	110 (16.8)	0.96	0.65	1.44	0.412	-	-	-	-
Not applicable	78	60 (76.9)	18 (23.1)	1.43	0.76	2.69		-	-	-	
Parity											
≤1	649	535 (82.4)	114 (17.6)	1.00	-	-		-	-	-	
2 or 3	256	211 (82.4)	45 (17.6)	1.00	0.68	1.46	0.919*	-	-	-	
≥4	52	44 (84.6)	8 (15.4)	0.85	0.39	1.86		-	-	-	
Baseline cotinine (ng x 10^{-1}/ml)											
12.1 (7.8-17.6), median (IQR)	957	790 (82.6)	167 (17.4)	0.93	0.90	0.95	<0.001	0.94	0.91	0.96	<0.001
BMI											
<18.5	28	27 (96.4)	1 (3.6)	0.18	0.02	1.34		-	-	-	
18.5-24.9	362	300 (82.9)	62 (17.1)	1.00	-	-		-	-	-	
25-29.9	267	213 (79.8)	54 (20.2)	1.23	0.82	1.84	0.987*	-	-	-	-
>30	254	212 (83.5)	42 (16.5)	0.96	0.62	1.47		-	-	-	
Missing	46	38 (82.6)	8 (17.4)	1.02	0.45	2.29		-	-	-	

Table 3 (continued)

Length of first behavioural support session											
16-30	143	123 (86.0)	20 (14.0)	1.00	-	-		-	-	-	
31-45	791	648 (81.9)	143 (18.1)	1.36	0.82	2.25	0.478	-	-	-	-
>60	23	19 (82.6)	4 (17.4)	1.29	0.40	4.20		-	-	-	
Previous preterm births											
0	872	718 (82.3)	154 (17.7)	1.00	-	-	0.577	-	-	-	-
≥1	85	72 (84.7)	13 (15.3)	0.84	0.45	1.56		-	-	-	
Use of NRT since pregnancy began											
No	914	756 (82.7)	158 (17.3)	1.00	-	-	0.548	-	-	-	-
Yes	43	34 (79.1)	9 (20.9)	1.27	0.60	2.69		-	-	-	
Trial recruitment site											
Site 1	102	81 (79.4)	21 (20.6)	1.00	-	-		-	-	-	
Site 2	113	97 (85.8)	16 (14.2)	0.64	0.31	1.30		-	-	-	
Site 3	188	151 (80.3)	37 (19.7)	0.95	0.52	1.72		-	-	-	
Site 4	238	212 (89.1)	26 (10.9)	0.47	0.25	0.89	0.032	-	-	-	-
Site 5	165	127 (77.0)	38 (23.0)	1.15	0.63	2.11		-	-	-	
Site 6	77	63 (81.8)	14 (18.2)	0.86	0.40	1.82		-	-	-	
Site 7	74	59 (79.7)	15 (20.3)	0.98	0.47	2.06		-	-	-	
Gestational age (weeks)											
12-19	766	637 (83.2)	129 (16.8)	1.00	-	-	0.326	-	-	-	-
20-24	191	153 (80.1)	38 (19.9)	1.23	0.82	1.83		-	-	-	
Heaviness of smoking index											
0-3	631	495 (78.5)	136 (21.5)	1.00	-	-	<0.001	1.00	-	-	0.001
4-6	326	295 (90.5)	31 (9.5)	0.38	0.25	0.58		0.50	0.32	0.76	

*p value - test for trend

†age included as continuous variable in multivariable model to derive p value, equivalent to test for trend adjusting for covariates

4.4.3. Univariable and multivariable associations with validated cessation at delivery

Table 4 shows the univariable and multivariable associations with validated cessation at delivery; the univariable results found women who finished full time education at >16 years and had lower baseline cotinine levels had increased odds of cessation, and those with higher HSI scores had lower odds of cessation. In the final multivariable model women who continued school beyond the compulsory minimum age (16 years) were more likely to stop smoking (OR: 1.89, 95%CI: 1.16-3.07, p=0.010), those with a higher HSI score (OR: 0.43, 95%CI: 0.23-0.79, p=0.006) and women with a higher baseline cotinine level were less likely to achieve cessation (OR: 0.96, 95%CI: 0.92-0.99, p=0.010).

Table 4 Univariable and multivariable associations with validated cessation at Delivery

Variable	N	Smoking at Del (%)	Cessation at Del (%)	Crude OR	95% CI		P-value	Adjusted OR	95% CI		LRT P-value
					Lower	Upper			Lower	Upper	
Age											
<20	147	135 (91.8)	12 (8.2)	0.93	0.46	1.89		-	-	-	
20-24	322	294 (91.3)	28 (8.7)	1.00	-	-		-	-	-	
25-29	228	206 (90.4)	22 (9.7)	1.12	0.62	2.02	0.403*	-	-	-	-
30-34	158	145 (91.8)	13 (8.2)	0.94	0.47	1.87		-	-	-	
35-39	78	74 (94.9)	4 (5.1)	0.57	0.19	1.67		-	-	-	
≥40	24	19 (79.2)	5 (20.8)	2.76	0.96	7.96		-	-	-	
Ethnicity											
Other	27	23 (85.2)	4 (14.8)	1.00	-	-	0.300	-	-	-	-
British - white	930	850 (91.4)	80 (8.6)	0.54	0.18	1.60		-	-	-	
Age full time education finished											
≤16	751	695 (92.5)	56 (7.5)	1.00	-	-	0.009	1.00	-	-	0.026
>16	206	178 (86.4)	28 (13.6)	1.95	1.20	3.16		1.74	1.07	2.84	
Partners' smoking status											
No	78	199 (88.4)	26 (11.6)	1.00	-	-		-	-	-	
Yes	225	604 (92.4)	50 (7.6)	0.63	0.38	1.04	0.193	-	-	-	-
Not applicable	654	70 (89.7)	8 (10.3)	0.87	0.38	2.02		-	-	-	
Parity											
≤1	649	588 (90.6)	61 (9.4)	1.00	-	-		-	-	-	
2 or 3	256	235 (91.8)	21 (8.2)	0.86	0.51	1.45	0.304*	-	-	-	-
≥4	52	50 (96.2)	2 (3.8)	0.39	0.09	1.62		-	-	-	
Baseline cotinine (ng x 10^{-1}/ml)											
12.1 (7.8-17.6), median (IQR)	957	873 (91.2)	84 (8.8)	0.95	0.92	0.98	0.002	0.96	0.93	0.99	0.050
Length of first behavioural support session											
16-30	143	135 (94.4)	8 (5.6)	1.00	-	-		-	-	-	
31-45	791	718 (90.8)	73 (9.2)	1.72	0.81	3.64	0.258	-	-	-	-
>60	23	20 (87.0)	3 (13.0)	2.53	0.62	10.34		-	-	-	

Table 4 (continued)

BMI											
<18.5	28	27 (96.4)	1 (3.6)	0.44	0.06	3.37		-	-	-	
18.5-24.9	362	334 (92.3)	28 (7.7)	1.00	-	-		-	-	-	
25-29.9	267	243 (91.0)	24 (9.0)	1.18	0.67	2.08	0.273*	-	-	-	-
>30	254	225 (88.6)	29 (11.4)	1.54	0.89	2.65		-	-	-	
Missing	46	44 (95.7)	2 (4.3)	0.54	0.12	2.35		-	-	-	
Previous preterm births											
0	872	795 (91.2)	77 (8.8)	1.00	-	-	0.852	-	-	-	-
≥1	85	78 (91.8)	7 (8.2)	0.93	0.41	2.08		-	-	-	
Use of NRT since pregnancy began											
No	914	837 (91.6)	77 (8.4)	1.00	-	-	0.106	-	-	-	-
Yes	43	36 (83.7)	7 (16.3)	2.11	0.91	4.91		-	-	-	
Trial recruitment site											
Site 1	102	90 (88.2)	12 (11.8)	1.00	-	-		-	-	-	
Site 2	113	106 (93.8)	7 (6.2)	0.50	0.19	1.31		-	-	-	
Site 3	188	172 (91.5)	16 (8.5)	0.70	0.32	1.54		-	-	-	
Site 4	238	221 (92.9)	17 (7.1)	0.58	0.26	1.26	0.397	-	-	-	-
Site 5	165	152 (92.1)	13 (7.9)	0.64	0.28	1.47		-	-	-	
Site 6	77	69 (89.6)	8 (10.4)	0.87	0.34	2.24		-	-	-	
Site 7	74	63 (85.1)	11 (14.9)	1.31	0.54	3.15		-	-	-	
Gestational age (weeks)											
12-19	766	704 (91.9)	62 (8.1)	1.00	-	-	0.147	-	-	-	-
20-24	191	169 (88.5)	22 (11.5)	1.48	0.88	2.47		-	-	-	
Heaviness of smoking index											
0-3	631	561 (88.9)	70 (11.1)	1.00	-	-	0.001	1.00	-	-	0.006
4-6	326	312 (95.7)	14 (4.3)	0.72	0.60	0.87		0.43	0.23	0.79	

*p value - test for trend

4.5. Discussion

4.5.1. Main findings

The main finding from this study was that, amongst participants in a trial of transdermal nicotine patches, smoking cessation for a duration of one month after a quit date and also until delivery was positively associated with finishing full time education above the compulsory age of 16 years, and negatively associated with baseline cotinine levels and the heaviness of smoking index. Baseline cotinine levels and HSI are a markers of tobacco smoke exposure and cigarette dependence, and having left school at a younger age is a marker of social disadvantage, therefore, findings suggest that having a lower socio-economic status and increased nicotine dependence is related to having decreased probability of stopping smoking.

4.5.2. Strengths and limitations of analysis

The main strengths and limitations of this study lie in the type of data that were used. These data were from a trial of NRT and not from a study implemented specially to investigate the research question posed by this chapter's aims and objectives. Consequently, relatively few variables were collected about participants' behavioural and socio-economic characteristics, which previous work has found to influence quitting smoking.[97] Therefore, it follows that, in this non-randomised cohort study, the relationships observed between variables and cessation may have occurred because of unmeasured confounding. However, the study employed multivariable analysis techniques to determine which variables were associated with cessation so, at least within those variables which were actually measured, confounding should not have unduly affected findings. Additionally, the number of significance tests performed may lead to a type 1 error, i.e. that a false positive occurs. This is because, as more than 20 univariable tests were carried out at the 95% significance level, for every 20

tests, by chance, one might be positive and this may result in spurious associations. However, the observed findings are both plausible, consistent with previous findings, and do not appear to be chance occurrences. Furthermore, the study sample size was large and high levels of follow-up permitted the inclusion of 91% of the participants originally randomised to the SNAP trial, increasing the chance that weak associations between the baseline variables and validated cessation in the study were discovered. Perhaps the main advantage to the use of trial data however, is that participants reported cessation status was validated biochemically, via saliva cotinine or CO, or both. This is advantageous as the accuracy of self-reported smoking status can be low due to the perceived social unacceptability of pregnant smoking which could make pregnant women more likely to falsely report abstinence.[178]

4.5.3. Findings in the context of previous work

The associations investigated in this study have, up to this point, only been investigated in either population-based studies with non-validated smoking outcomes or in trials which used validated cessation outcomes but which were small. Two previous systematic reviews, in non-pregnant[95] and pregnant smokers,[97] respectively, have investigated predictors of smoking cessation. Both found that nicotine dependence is an important predictor of quit attempt success, with higher levels reducing the likelihood of a successful quit attempt. The analyses in this chapter provide complementary data showing that lower cotinine concentration was associated with increased odds of cessation amongst women in both early and late pregnancy, in the context of an NRT trial. Cotinine levels are a marker of tobacco smoke exposure rather than being a measure of nicotine dependence. However, within these trial participants, both plasma and saliva measures of cotinine have been found to be highly correlated with a validated measure of nicotine dependence in pregnancy;[179] it is likely that lower

levels of nicotine dependence would also increase the odds of women achieving cessation.

The previous reviews also investigated associations between socio-economic factors and smoking cessation and found strong evidence for a positive association between higher socio-economic status or, higher income levels, and cessation in pregnancy;[97] little evidence was found for an association between income, level of education or employment status and cessation in non-pregnant smokers.[95] The present study concurs with the previous findings that higher levels of social disadvantage, as reflected by less maternal education, are associated with worse outcomes in pregnant women who attempted cessation as part of an NRT trial.

All studies of cessation in pregnancy included in the previous review, used self-reported cessation measures.[97] As mentioned earlier, these may be prone to underreporting of smoking behaviour due to social desirability bias and, so, the validity of findings from empirical studies included in this review could be questioned. As non-smokers are unlikely to report themselves as smokers, misclassification of women currently smoking as non-smokers could bias the strength of associations between factors relating to smoking behaviour and addiction, and those measuring social disadvantage and cessation, towards the null. In our dataset, misclassification of smoking status due to such biases in self-reported data cannot have occurred; consequently, our demonstration of the importance of nicotine dependence and social disadvantage to smoking cessation is likely to be valid.

Only one previous report has used validated cessation data in analyses investigating factors associated with successful cessation in pregnancy.[146] This was the US 'Baby Steps' trial[121] which found that women who were primiparous

and who used more NRT were more likely to report cessation at 38 weeks gestation;[146] however the study sample was small (104 women) and only univariable associations were reported, so these findings are difficult to interpret. In comparison, this much larger study had greater power and, used a multivariable analysis which will have eliminated at least that confounding which might have arisen from those variables which were included in the dataset.

The analyses presented in this chapter suggest that, in pregnant women who use NRT to attempt cessation, higher levels of social disadvantage and higher pre-treatment cotinine levels are associated with worse cessation outcomes. It is possible that both associations are causal, though a mechanism for cotinine concentration affecting cessation through its contribution to nicotine dependence is more immediately obvious. Outside of pregnancy, nicotine dependence is also more easily remedied, for example by NRT, which has been shown to reduce the strength of craving and be effective for smoking cessation.[102] These findings have research implications; further work investigating how socio-economic status may influence success in quit attempts could uncover factors that are amenable to intervention. Similarly, further research into the treatment of nicotine dependence may be indicated; there is currently no evidence that NRT is effective in pregnancy,[112] but future studies using higher doses of nicotine than those which have been trialled could be undertaken. Nicotine metabolism is faster in pregnancy[124] and the standard doses of NRT which have been investigated may be too low to be effective. The finding that increasing cotinine concentration, which is strongly correlated with nicotine dependence,[179] is also strongly associated with cessation failure, should provide a spur to investigate this possibility.

4.5.4. Conclusions

Amongst women in a trial of NRT for smoking cessation during pregnancy, those who did not continue education beyond the minimum age for leaving school and those who had higher pre-treatment cotinine concentrations, and were more nicotine dependent, were less likely to quit smoking. Women with these characteristics may require a different kind of smoking cessation support in pregnancy than others; however, from the data it is not possible to determine the nature of support that these women would find most helpful. Further research into the mechanism of the relationship between these characteristics and cessation is likely to be useful to determine the causal framework of how these factors are connected. Doing so, may lead to insights into how pregnant women with these characteristics may be helped to stop smoking more effectively. The following chapter investigates this issue in more detail.

Chapter 5

Investigating the association between social disadvantage and smoking cessation in pregnancy: mediation via nicotine dependence and partners' smoking status

5.1.Introduction

In **Chapter 4**, maternal factors associated with validated cessation were investigated and it was found that pregnant women with higher levels of social disadvantage (completing full time education at <16 years) and greater nicotine dependence (higher baseline cotinine level) had lower odds of quitting smoking one month after a quit date or at delivery. In this chapter, these factors are further investigated to discern how they relate to each other and whether or not mediation pathways proposed later in this chapter might explain the relationships between these factors and with quitting.

5.1.1. Are socially disadvantaged women less likely to quit?

In non-pregnant smokers, studies using observational data have shown that higher levels of social disadvantage are independently associated with reduced likelihood of achieving smoking cessation.[180] However, non-pregnant smokers' characteristics which are known to be associated with social disadvantage, such as education level, gender and marital status have not been found to be consistently related to cessation.[95] Nicotine dependence appears to be more consistently related to quitting behaviour; a systematic review of factors associated with cessation in non-pregnant smokers after a quit attempt was made, found that nicotine dependence was the only consistent predictor of smoking cessation.[95] The relationship between social disadvantage and cessation is likely to be a complex one and may not be directly causal; there may be the potential for other variables to mediate this association and in this chapter, this is investigated from the perspective of pregnant smokers.

In pregnant women, many factors that measure aspects of disadvantage have also been found to be inversely associated with cessation.[98,99] For example, women who smoke throughout pregnancy, and do not stop, are more likely to work in manual occupations,[5] have a lower socio-economic status (SES), have

completed full time education at a younger age and become pregnant at a younger age.[4] These are all markers of social disadvantage; however, in pregnancy as outside of pregnancy, it seems unlikely, that social disadvantage would have a direct causal impact which hinders smokers' ability to quit. Rather, it is hypothesised that social disadvantage is related to more proximal factors which are also associated with smoking cessation but which also directly influence a woman's propensity to quit. The potential proximal factors that 'mediate' the association social disadvantage and cessation will be investigated using a mediation analysis. Mediation analysis seeks to make explicit the underlying causal pathways between an exposure and an outcome of interest. To this end, the following sections also briefly review potential mediating factors that could be associated with either social disadvantage or cessation.

5.1.2. Are nicotine dependence and partners' smoking associated with social disadvantage?

Some studies investigating smoking cessation in pregnancy have found relationships between both social disadvantage, or factors measuring this, and nicotine dependence.[97,166,181] The following section details the arguments for investigating whether or not nicotine dependence or partners' smoking status might mediate the relationship between social disadvantage and cessation, explaining the observed inverse relationship between these two qualities.

For one variable to mediate the relationship between an exposure and an outcome, it needs to have an association with both. In this section the evidence for relationships between social disadvantage, nicotine dependence and partners' smoking is assessed. Women from more affluent backgrounds often defer parenthood to study and in turn have higher levels of education,[182] which may explain why fewer women with higher levels of education smoke compared to those with lower levels of education.[183,184] As the overall prevalence of

cigarette use in developed nations decreases, it is increasingly those who are less affluent who smoke in these countries.[182] Additionally, those more socially-disadvantaged individuals who smoke are more likely to be heavier smokers than lighter smokers;[185] thus, socially disadvantaged women are likely to show higher levels of nicotine dependence.

It is hypothesised that partners' smoking can influence pregnant women and might influence their smoking behaviour; this factor might be related to women's social disadvantage. In male smokers, there is evidence to suggest that those who have a skilled job make more quit attempts, have higher quit rates and smoke their first cigarette later in the day; this latter fact indicates potentially, lower nicotine dependence.[186] Additionally, male smokers who are less socially disadvantaged may be more likely to quit smoking.[186] Since being less socially disadvantaged improves the odds of cessation amongst men, and qualitative evidence suggests women are likely to be pressured by their partners to quit smoking when pregnant,[187] partners' smoking appears to be a potentially important factor during a quit attempt. The intensity of these changes are likely to vary with whether partners were disengaged, conflictual or accommodating to women's decision to stop smoking,[187] which in turn are likely to change with partners' smoking status. It is possible, therefore, that pregnant women's partners' smoking status, which is related to their social disadvantage, might also affect their chances of smoking cessation and should, therefore, be investigated as a potential mediator of a social disadvantage-cessation association.

5.1.3. Are nicotine dependence and partners' smoking associated with failure to quit?

Nicotine dependence has been shown to be inversely associated with cessation in the cohort of pregnant women enrolled in the SNAP trial (**Chapter 4**).

Furthermore, systematic reviews from both smokers in the general population and in pregnancy have found nicotine dependence to be inversely associated with cessation.[95,97] Since pregnant smokers are more likely to be socially disadvantaged, it is possible to hypothesise that nicotine dependence could mediate the association between social disadvantage and smoking cessation.

The effect of partners' or cohabiting relatives' smoking behaviour may influence a pregnant woman's smoking. For example, the presence of another smoker in the home could increase the availability of cigarettes or reduce a woman's will to quit.[188] There is evidence suggesting that women who achieve cessation tend to have partners that are non-smokers[97] and that those who have smoking partners are less likely to quit spontaneously.[189] An Irish study examining women's perceptions of support from partners, family members and close friends for smoking cessation during pregnancy found that while women may have attempted to reduce smoking, and that partners supported this and influenced their smoking behaviour, there was little evidence that the partner's smoking behaviour changed.[188] An inference from this might be that women see their partner's smoking as an important factor in supporting their quit attempt, but as many partners do not change their smoking behaviour, this could negatively influence whether women achieve their target of cessation. Partners' smoking is more likely to impact on women's smoking which could increase the nicotine levels pregnant women expose the foetus to. While a systematic review revealed that there are few effective interventions that target partners' smoking to help pregnant women stop smoking,[190] there is evidence that smoking behaviour change amongst women's partners is associated with a reduction in women's smoking and increased odds of their cessation during pregnancy.[191]

5.1.4. Why use mediation analysis?

Previous sections show that social disadvantage, nicotine dependence and partners' smoking behaviour have all been found to be associated with smoking cessation by pregnant women in previous studies. However, the extent to which observed associations might be causal is not known and it is particularly difficult to hypothesise how social disadvantage, might have a direct causal influence on pregnant women's smoking. Analyses investigating whether or not, in pregnant smokers, the association between social disadvantage and cessation might be mediated by other factors such as nicotine dependence or partners' smoking, have not previously been undertaken. Such a longitudinal analysis could have several advantages over previous cross-sectional analyses including these factors.

Firstly, an advantage of mediation analyses is that these take into consideration the temporal order in which associations occur, rather than the linear associations that have investigated social disadvantages influence on cessation in pregnancy thus far. Additionally, where there are numerous markers of social disadvantage and nicotine dependence, and these have missing data, potential associations between these factors and cessation may be missed. Secondly, mediation analysis's use of latent variables i.e. variables not directly observed but which are inferred, are potentially more accurate due to accounting for missing data and multiple observed variables measuring similar factors. A third advantage of mediation analyses is that a proposed theoretical model for how social disadvantage relates to cessation must be specified *a-priori* and then tested, so the approach is not data driven. These models implicitly allow several association pathways between a dependent variable and an outcome. Using these alternative or 'indirect' pathways, it is possible to test important causal pathways by which social disadvantage and cessation may be connected.

Applying mediation analysis methods to the SNAP data, would allow one to test how much of the effect of social disadvantage is mediated by nicotine dependence or partners' smoking. To this end, Confirmatory Factor Analysis (CFA), Direct Acyclic Graph (DAG) and Structural Equation Modelling (SEM) techniques were employed in a mediation analysis and these are detailed in the following sections.

5.1.5. Justification of the methods used

CFA works by testing whether measured variables fit with a hypothesised measurement model, as opposed to exploratory factor analysis (EFA) where such a model is specified automatically by a statistical software program based on the fit of the variables. As an index is produced from the CFA, this reduces the chance of measurement error, which may arise from using a single measure, affecting the association. CFA allows the use of multiple factors to create a measurement model for a latent variable as well. Inclusion of many factors in a measurement model may create a more accurate index of the desired latent variable. Another advantage of the CFA modelling process is that collinear variables can be included together, though these must be included sparingly as high levels of multicollinearity can lead to a model from which definitive conclusions cannot be drawn.

In some circumstances, when considering the causal nature of associations, classical methods for selection of confounders are not successful at eliminating bias. Using standard multivariable methods to fit a model, all proposed confounding variables found to be statistically significantly associated with both the outcome and exposure are included in the final adjusted model. This ignores the nature of the relationships between the variables e.g. social disadvantage is associated directly with smoking cessation when it is probable that other intermediary variables are on this pathway. Over adjustment or confounding via

non-biasing pathways is an additional factor which might occur when adjustments are made for every potential confounding variable, irrespective of underlying relationships between explanatory variables.[192] Using DAG methodology for confounder selection involves considering how variables are linked, and all pathways which may connect two variables, rather than just including variables based on their association with the exposure or outcomes under investigation. Furthermore, it documents the rationale for potential confounder selection which is particularly important in analyses that investigate causal and biasing pathways. If links between variables are shown to occur via associations through paths not under investigation in the model of interest, these should be adjusted for to reduce bias and gain a more accurate estimate of effect in the mediation model.[192]

SEM allows for exposure, mediator and outcome variables to be specified, so a theoretical model of relationships between these can be tested.[193] Furthermore, if a single indicator variable is inadequate, SEM allows a variable to be measured by multiple indicators.[193] SEM can be used for testing model fit, but having a good fit in a structural equation model does not infer causality of effect. The estimate of effect is the strength of the association between two factors in either direction. Thus building a prior plausible structural model is of primary importance when assessing relationships between these variables by SEM.

It has been put forth that SEM is a class of methodologies that measures observed data means and variances in terms of smaller structural parameters, e.g. latent variables, in a hypothesised model.[194] SEM methods allow for a system of single or multiple linear regressions to be estimated from a proposed path diagram. Traditionally, the building of structural models with separate linear regressions is concerned with the measurement of direct and indirect (mediated) effects, but the addition of SEM techniques to this, allows for the

inclusion of latent variables. Latent variables reduce the possibility of bias due to measurement error and, as mentioned above, also allow for the use of indicators that are collinear.[195] SEM then tests the hypothesised model against the observed data using summary fit statistics as a guide; these include the Chi-squared test (χ^2), Root Mean Square Error of Approximation (RMSEA), Comparative Fit Index (CFI) and Standardised Root Mean Square Residual (SRMR).

Using these methods, an investigation was conducted of how factors indicative of social disadvantage and also associated with cessation, might be mediated by nicotine dependence or having a partner that also smokes.

5.2. Aim

To use a cohort of pregnant smokers, described in **Chapter 3**, to investigate whether or not partners' smoking behaviour or a woman's nicotine dependence mediated the association between social disadvantage and failure to stop smoking in pregnancy.

5.3. Methods

5.3.1. The proposed structural model

Figure 6 illustrates the proposed model that will be used to investigate the stated aim: to test whether the effect of social disadvantage on validated smoking cessation is mediated by either partners' smoking status or nicotine dependence. The 'structural model' is depicted in the red box. The blue boxes highlight 'measurement models' for social disadvantage and nicotine dependence which are informed by variables from the SNAP trial. Square markers denote directly measured variables and oval markers denote latent variables that will be derived from the measurement models.

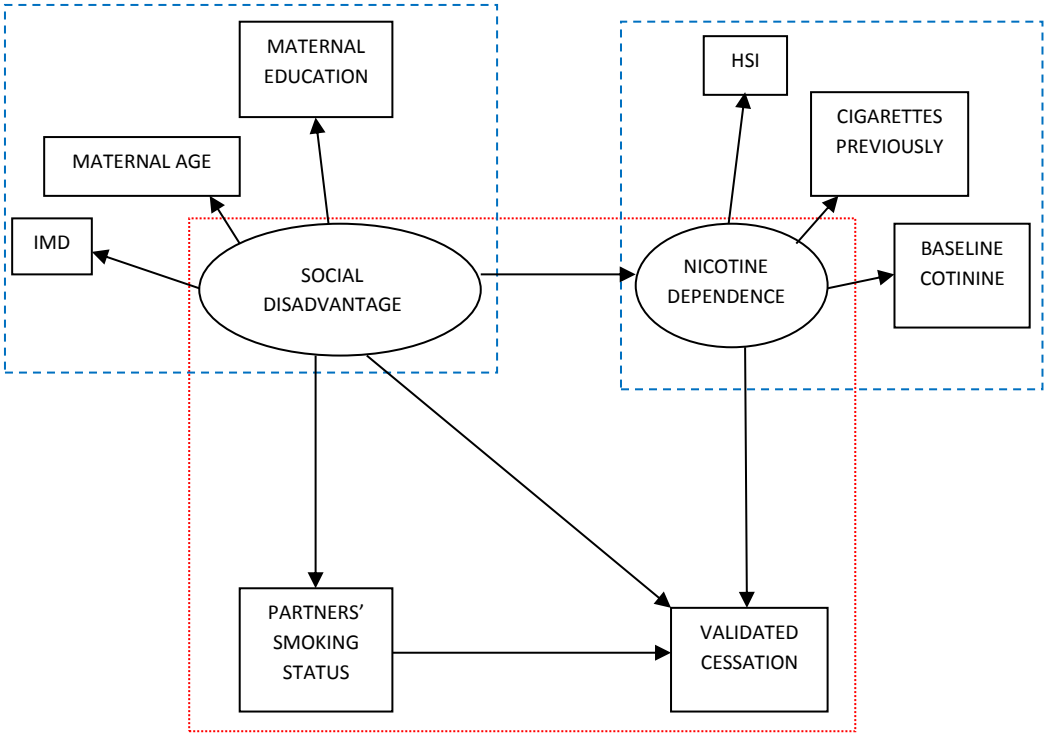


Figure 6 Hypothesised mediation of social disadvantage by partners' smoking status and nicotine dependence. IMD= index of multiple deprivation, HSI= heaviness of smoking index. Blue boxes indicate which factors were eventually used to measure the latent variables, social disadvantage and nicotine dependence. Red box indicates how these latent variables were hypothesised to be related in the structural model investigated.

5.3.1.1. Measurement models

Nicotine dependence and social disadvantage are latent variables created from factors measured in the SNAP trial. Validated smoking cessation was measured at one month and at delivery. At one month, cessation was defined as reported continuous abstinence from quit date to one month, validated by an exhaled CO reading of <8ppm. At delivery, cessation was defined as reported continuous abstinence from a quit date until delivery, validated by an exhaled CO reading of <8ppm and/or a saliva cotinine level of <10ng/ml. The Russell Standard was followed,[196] under which up to five cigarettes between quit date and cessation at delivery were permitted. Participants who were lost to follow-up were counted as continuing smokers.

5.3.1.2. Mediators

Partners' smoking status and nicotine dependence were hypothesised as mediators of this relationship. Partners' smoking data were re-categorised as a binary variable for this analysis. Women with partners who did not smoke and women who did not have a partner were combined as one variable category as neither group had partners that smoked and the number of women with no partners was small (n=87, 8%). This was thought to be a logical merging of data as the availability of cigarettes, a key factor in partners' smoking status influencing maternal smoking, is reduced and similar in both groups. Inclusion of partners' smoking status as a binary variable allowed Full Information Maximum Likelihood (FIML) methods to be used and avoids list-wise deletion of participants that had one or more other missing items of data; FIML method SEM's with categorical mediators are not currently possible.

5.3.1.3. Missing values

There were missing IMD values for 70 trial participants because postcodes could not be linked to area level IMD data. This resulted in a total of 980 women being included in the model measuring social disadvantage. However, other measures of social disadvantage measured in the SNAP trial had less missing data[116] and it was possible to use maximum likelihood estimation for the structural model. This was especially important for assessing nicotine dependence because also allowed the use of all the information as many participants (n=80) did not have a cotinine concentration measured. As a result, all 1,050 women from the trial cohort were included in this analysis. No other data were missing for the indicators of social disadvantage, partners' smoking status or measures of validated cessation.

5.3.2. How CFA, DAG and SEM methodology was applied to the mediation analysis

This section details how various methods were employed to assess whether or not the relationship between social disadvantage and validated cessation was mediated by nicotine dependence and partners' smoking status; it describes how the proposed theoretical model **Figure 6** was built and was interpreted.

5.3.2.1. Derivation of latent variable measurement models using CFA

Both confirmatory factor analyses used standardised estimates so that associations used for measuring latent variables could be compared with one another. A standardisation method was used for the results of the CFA, with the results based on the variances of latent and observed variables that were included in the CFA models.[193] This allowed comparison of the variables predicting the latent variable in the CFA. The effect estimates of the CFA represent the amount of change in either social disadvantage or nicotine dependence per standard deviation of a predictor variable. Skewness of covariates in the model was handled through the use of maximum likelihood estimation using robust standard errors.

5.3.2.1.1. Social disadvantage measurement model

A measurement model/latent variable was created using CFA to assess social disadvantage. The index of multiple deprivation (IMD), maternal age and the age at which full time education was completed were used to create this latent variable. Since variables assessed in the CFA measurement models will be included in the SEM, and this methodology allows for the inclusion of potentially collinear variables, IMD and age full time education was completed were able to be used together to measure social disadvantage. IMD is a composite measure based on 38 variables that measure factors related to income, employment, health and disability, education, skills and training, barriers to housing and other services, and crime and living environment. Participants in the SNAP trial were asked for their postcode and this was linked to the IMD score, representing the

area in which they lived. IMD values were assigned based on the 2011 output area classification, using GeoConvert to find the measure of deprivation for the lower super output area. Lower super output areas correspond to between 400 and 1,200 households.

These variables were chosen *a-priori* because they were considered to reflect social disadvantage and because including area and individual level variables would theoretically provide a maximal potential use of data available for measuring social disadvantage. Research into measurement of SES, indicates that single measurements such as level of education and income are not interchangeable and it is considered better to measure disadvantage using as much socio-economic information as possible.[197] This study accomplished this by using individual and area level data combined to measure social disadvantage.

5.3.2.1.2. Nicotine dependence measurement model

A second measurement model was created using CFA to measure participants' nicotine dependence; the number of cigarettes smoked prior to pregnancy, the heaviness of smoking index (HSI)[179] measured in pregnancy at baseline and cotinine concentration from either blood or saliva at baseline were used to create a latent variable. These variables were chosen because they have previously been used or have been associated with nicotine dependence.[52,124,179,198] Although variables included were potentially collinear, factor analysis allows for this and we hypothesised that inclusion of a number of indicators of nicotine dependence should measure this more accurately than using only one.

5.3.2.2. Direct acyclic graph (DAG) for confounder selection

Direct acyclic graph (DAG) techniques were used instead of classical selection of confounders.[192] The DAG explored confounders of the relationship between social disadvantage and cessation. A DAG was made of potential confounders between

social disadvantage and cessation, and variables that were not already included in the structural model were also included as confounders. After using DAG methods, only variables which were established as being on biasing causal pathways were included in the final structural equation model. A biasing pathway is one that remains between social disadvantage (exposure) and cessation (outcome) after the 'six step' DAG method, detailed below has been conducted. It represents an indirect path by which a change in social disadvantage might alter the odds of cessation, although this path may not have a plausible mechanism (non-causal) or be of interest to the current investigation. The six stage DAG process used to identify biasing pathways[192] was verified using the DAGitty tool.[199] A DAG including the model to be tested and those confounding factors that may need to be controlled for was devised (i.e. involving both variables for which we had data and also for which we did not).

The following six step method was carried out with the aim of identifying potentially biasing pathways between social disadvantage and validated cessation by variables that may need to be adjusted for.[192]

Step 1: Covariates not considered 'descendants' of the exposure variable were included in the model (**Figure 7**).

Step 2: Variables considered 'non-ancestors' of the exposure, non-ancestors of the outcome and non-ancestors of the covariates that were to be tested for biasing pathways, were removed from the DAG diagram (**Figure 8**).

Step 3: All associations emanating from the exposure variable were deleted from the DAG diagram (**Figure 9**).

Step 4: Any two variables that were associated with a 'common child' variable were connected (**Figure 10**).

Step 5: The arrows heads that infer causality from the DAG were removed (**Figure 11**).

Step 6: The lines between the covariates in the model to be tested and any other covariates in the DAG were deleted (**Figure 12**).

The reason for following this sequence was to transparently demonstrate the rationale behind the selection of confounders which should be adjusted for when investigating mediation of the relationship between social disadvantage and cessation. After these steps had been taken, if the exposure variable in the DAG was dissociated from the outcome, then the model chosen should have the potential to produce an estimate of association with the outcome, and an estimate of the amount of mediation with as little bias as possible.[192] If the exposure remains connected to the outcome, the variables on the biasing pathway should be included in the model.

The initial structure of the DAG model (i.e. that hypothesised at the start of the six step process outlined above) was constructed from consideration of the literature on factors associated with both social disadvantage and smoking cessation and also from the findings of previous analyses in this thesis (**Chapter 4**). These variables were fitted around the model under investigation as stated in the hypothesis. The six step process outlined above is illustrated in **Figure 7** to **Figure 12**. The following section explains the rationale for the structure of the DAG used.

5.3.2.2.1. Rationale for relationships between variables shown in DAG diagrams

The underlying association that the DAG was based on was that of social disadvantage being inversely associated with smoking cessation. This was a necessary choice as the SEM model aimed to investigate the mediation of this association.

Women who live in working class families or are unemployed, and have lower levels of education are at increased odds of smoking.[200] Being married has been found to significantly reduce the odds of smoking in pregnancy.[200] In a large prospective cohort study of infants, Pickett *et al.* found that a number of psychosocial factors that are correlated with socio-economic disadvantage are prevalent amongst pregnant heavy smokers, including having worse interpersonal relationships, adaptive functioning and other worse health related behaviours (drug use, alcohol dependency).[201] Many indicators of social disadvantage have been found to be associated with cessation, and from this evidence it is possible to construct a causal diagram, in the form of a DAG. This was used to investigate possible confounders of the main association in the structural model, between social disadvantage and smoking cessation.

Maternal age was hypothesised to be associated with the age at which full time education was completed and also with parity. This was because either younger smoking mothers have less education and are more likely to be disadvantaged than older smoking mothers, or because older mothers have higher levels of education and are less deprived.[2] Maternal age is also likely to be associated with parity as the older a woman becomes, the more likely it is that she will have more children. It was also hypothesised that the age at which full time education was completed would be inversely associated with social disadvantage and maternal nicotine dependence. This was because level of education, along

with income, are strong indicators of SES,[197] which is associated with social disadvantage. Women who have lower levels of education are also more likely to have started smoking at a younger age[5,19,20] and length of time smoking is thought to contribute to the level of maternal nicotine dependence. Parity was associated with partners' smoking status as women and cohabiting partners might reduce the amount they smoke with a first child,[18] but if they have had more than one child whilst smoking without any adverse events, they might be more likely to smoke. Nicotine metabolism was associated directly with maternal nicotine dependence as the rate of nicotine metabolism is hypothesised to be strongly associated with the strength of maternal nicotine dependence.[166] Finally, maternal nicotine dependence was associated with odds of cessation as higher dependence, measured either via number of cigarettes or by indexes such as HSI, as they have been found to be inversely associated with odds of cessation.[179,202]

5.3.2.2.2. Direct acyclic graph (DAG) process used to determine confounding variables of the social disadvantage – cessation association

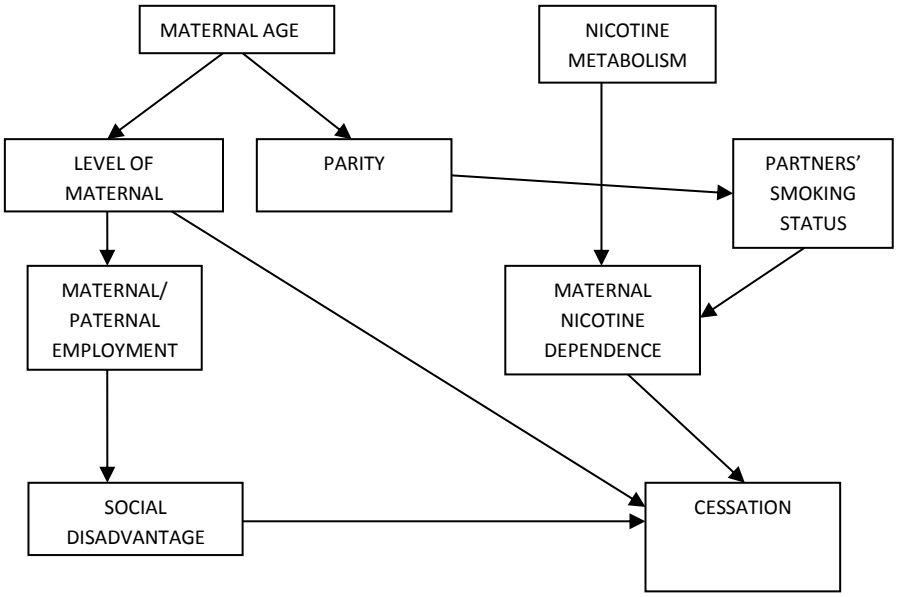


Figure 7 Step 1: Covariates not considered descendants of the exposure variable were included in the model.

In this step, a DAG was created in which variables on the causal pathway to be mediated were excluded.

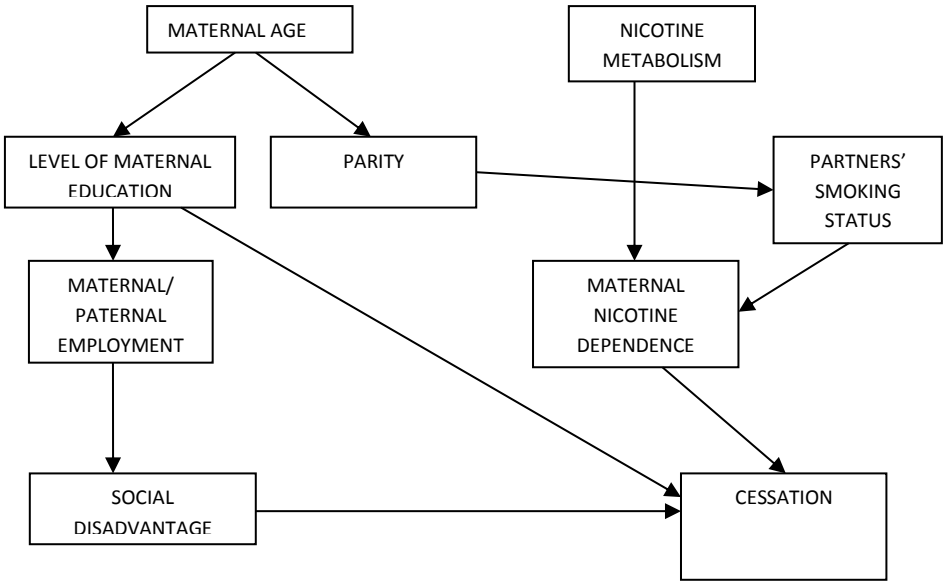


Figure 8 Step 2: Variables considered non-ancestors of the exposure, non-ancestors of the outcome and non-ancestors of the covariates that were to be tested for biasing pathways, were removed from the DAG diagram.

This step involved removing variables that were not related to the structural or measurement models.

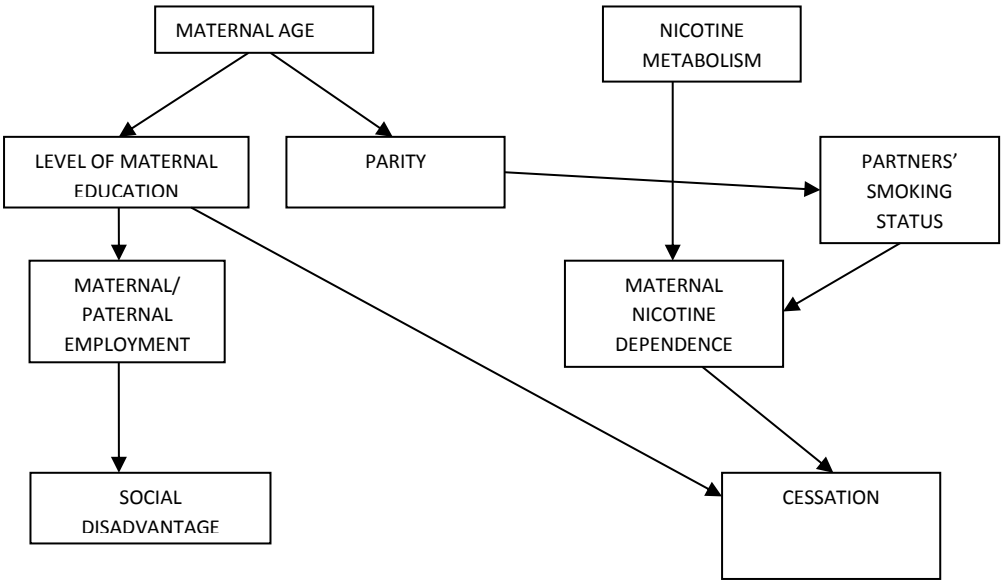


Figure 9 Step 3: All associations emanating from the exposure variable were deleted from the DAG diagram.

In this step, the pathway that is being mediated was deleted, so only potentially biasing pathways remained.

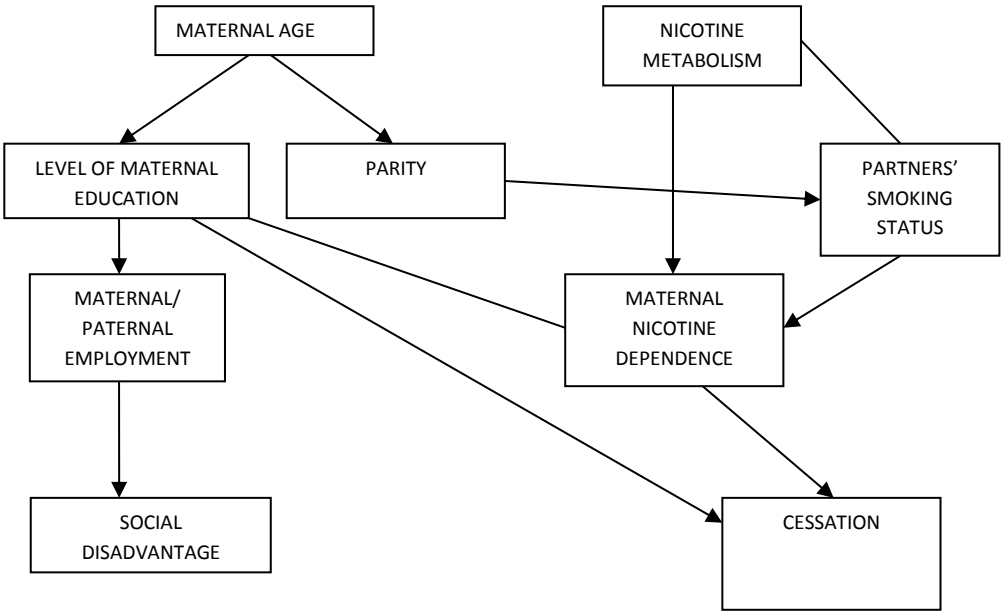


Figure 10 Step 4: Any two variables that were associated with a common child variable were connected.

This step involved variables that might change in tandem, but that were not necessarily causally connected. These were joined as these changes might create a non-causal biasing pathway.

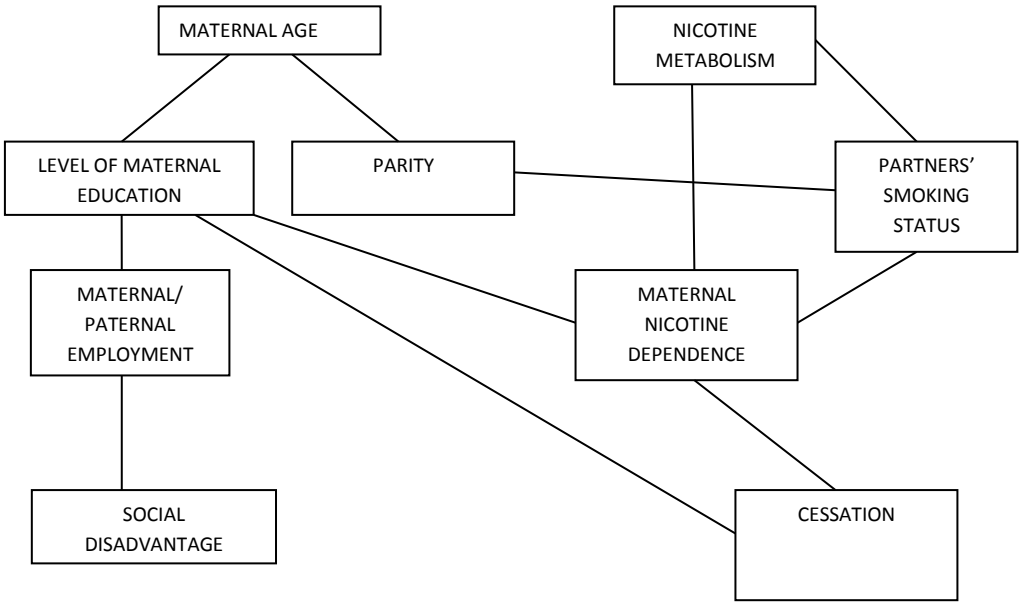


Figure 11 Step 5: The arrows heads that infer causality from the DAG were removed.

This step removed all arrow heads, since not all connected variables represented causal associations.

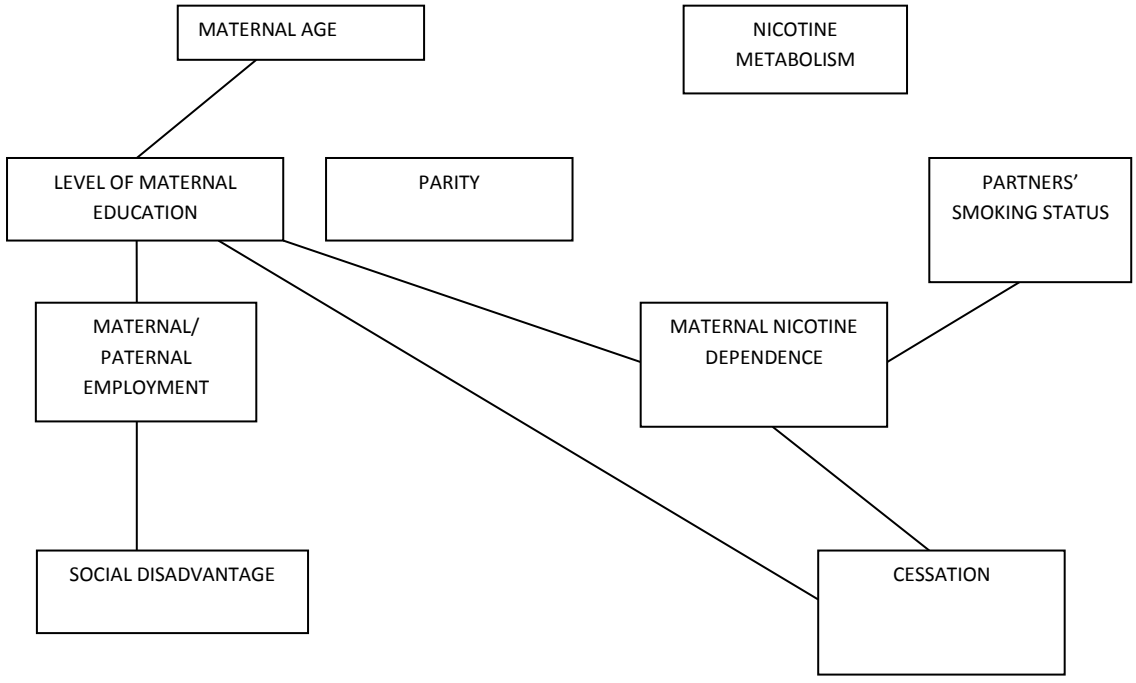


Figure 12 Step 6: The lines between the covariates in the model to be tested and any other covariates in the DAG were deleted.

This step represents the graphical equivalent of standard regression. As including covariates masks other pathways going through them, it is possible to delete variables not collected in the SNAP trial, as they would be masked.

5.3.2.2.3. Implementation of the DAG results in the SEM

The results of the DAG in **Figure 12** were incorporated into the SEM, by ensuring that variables on the remaining biasing pathway between social disadvantage and cessation were included in the SEM model. The SEM model, its methods and criteria for how it will be used to analyse the overall model in **Figure 6**, is described in the following section.

5.3.2.3. Structural equation modelling (SEM)

SEM was used to link the two CFA measurement models (**Section 5.3.2.1**) and to simultaneously measure the degree to which social disadvantage was mediated via either partners' smoking status or women's nicotine dependence. When modelling the proposed theoretical model (**Figure 6**), the factor loadings of the measurement models for social disadvantage and nicotine dependence were fixed so that they were on the same scale and comparable with each other. A model was considered to have a reasonable fit where the χ (chi) value divided by degrees of freedom (χ/DF) is <5, RMSEA is <0.06, CFI is >0.90 and SRMR is <0.08.[203] The output from the structural equation model used standardised results to allow comparison of the associations of covariates predicting latent variables.[193] FIML estimation was used to handle missing data.

The proportion of the association between social disadvantage and cessation which was mediated by either partners' smoking or nicotine dependence was calculated by dividing the estimate of the specified indirect pathways by the direct pathway from social disadvantage to smoking cessation.

Although the proportion mediated was calculated, this may be compromised by the fact that mediation can occur without satisfying the Baron and Kenny criteria for causal mediation.[204] These criteria state that the independent variable must

be associated with the outcome and with the mediating variable, and that the mediating variable is associated with the outcome. As such, despite careful model specification and adjustment for covariates, calculating the proportion may not be an ideal method of measuring the magnitude of mediation. Therefore, the kappa-squared (κ^2) statistic was also used to interpret the size of effect.[205] The κ^2 uses the variance, covariance's and β values of the direct and indirect effect to calculate the size of mediation; thresholds for small, medium and large effect sizes are defined as 0.01, 0.09 and 0.25 respectively.[206]

5.3.2.3.1. Bootstrapped confidence intervals and approximate odds ratios

Although the variables included in the CFA's and SEM had normally distributed continuous variables, standardised estimates with robust standard errors were used, because when two normal distributions like this are combined in an indirect effect (**Figure 6**), the distribution of the product is not always symmetrical. By bootstrapping 10,000 draws for the model estimates, it was possible to quantify the confidence intervals of the mediation effect, (i.e. of the indirect pathways via nicotine dependence and partners' smoking) where these were non-symmetrically distributed. The estimates produced by SEM techniques produced probit coefficients and these were transformed to an approximate odds ratio value using the following formula, where x is the probit coefficient:

$$OR \sim \exp^{1.81x}$$

5.3.2.3.2. Selection of an appropriate SEM smoking cessation outcome measure

One necessary criterion for conducting a SEM analysis is that there is an association between the exposure and outcome of interest. Therefore a mediation analysis for cessation at delivery was not conducted as there was no

evidence of a significant association between social disadvantage and smoking cessation at delivery when included in a SEM model (Approx. OR: 1.00, 95%CI: 0.99-1.01).

All mediation analyses were undertaken for smoking cessation at one month post-quit date, and analyses were conducted using Stata 13.1 (College Station, TX) and Mplus 6.12 (Muthen & Muthen).[193]

5.4. Results

The results in this section are those for the fit of the social disadvantage and nicotine dependence measurement models, and the overall SEM model fit. The latter was informed by the hypothesised structural model in **Figure 6** and the results of the DAG in **Section 5.3.2.2.2**.

5.4.1. Measurement model for social disadvantage

The measurement model for social disadvantage (**Section 5.3.2.1.1**) used maternal age, age full time education was completed and the IMD score as factors entered into the CFA. **Table 5** illustrates the estimates of the measurement model for social disadvantage and this appears to be a weak fit (χ/DF: 34.4, RMSEA: 0.18, CFI: 0.07, SRMR: 0.13). This implies that maternal age, age full time education was completed and IMD are amenable to combination and can be used together as a proxy measure for the latent variable, social disadvantage. Findings show that higher IMD was associated with greater social disadvantage and social disadvantage was inversely associated with maternal age at birth and at completion of their education. These results are plausible and suggest that the constructed measure is valid.

Table 5 Social disadvantage CFA model results

				95% CI		
	Estimate	S.E.	p-value	Lower	Upper	Approx. OR
Social disadvantage by						
Index of Multiple Deprivation	0.447	0.073	<0.001	1.73	2.91	2.25
Maternal Age	-0.369	0.063	<0.001	0.41	0.64	0.51
Age full time education completed	-0.365	0.062	<0.001	0.41	0.64	0.52

5.4.2. Measurement model for nicotine dependence

The measurement model for nicotine dependence (**Section 5.3.2.1.2**) used baseline cotinine level, the number of cigarettes smoked prior to pregnancy and the HSI as factors entered into the CFA. **Table 6** illustrates the estimates of the measurement model for nicotine dependence. The model for this latent variable suggested that there was a weak fit for the variables in the measurement model for nicotine dependence (χ/DF: 8.6, RMSEA: 0.09, CFI: 0.79, SRMR: 0.08), but indicates that data from all three variables should be used together to measure this; all three variables were positively associated with the latent variable, nicotine dependence, which was a plausible result.

Table 6 Nicotine dependence CFA model results

				95% CI		
	Estimate	S.E.	p-value	Lower	Upper	Approx. OR
Nicotine dependence by						
Baseline cotinine level	0.228	0.038	<0.001	1.32	1.73	1.51
Heaviness of smoking index	1.268	0.129	<0.001	6.28	15.69	9.93
Cigarettes smoked prior to pregnancy	0.526	0.057	<0.001	2.12	3.17	2.59

5.4.3. Structural model regression coefficients

Table 7 details the simultaneously calculated regression coefficients from the SEM model described in **Figure 6**, the latent variables for which were informed by social disadvantage and nicotine dependence CFA measurement models. Approximate odds ratios and 95% CI for the regression coefficients are also included. **Table 7** shows strong associations between cessation at one month,

social disadvantage and nicotine dependence. There were also strong associations between social disadvantage, nicotine dependence and partners' smoking status, but partners' smoking status was not associated with cessation. Since having no association between mediator and dependent variable does not preclude mediation, this was still tested for. The overall fit of the SEM (i.e. structural model and the measurement models) indicated that they were in agreement with the data and a good fit (χ/DF: 4.9, RMSEA: 0.06, CFI: 0.93, SRMR: 0.05). The structural model and approximate odds ratios are depicted in **Figure 13**.

Table 7 Standardised structural equation model results

Effects from IMD to One month validated cessation	Estimate	S.E.	p-value	95% CI Lower	95% CI Upper	Approx. OR
Nicotine dependence by						
Baseline cotinine level	0.247	0.033	<0.001	1.39	1.76	1.56
Heaviness of smoking index	1.170	0.078	<0.001	6.30	10.96	8.31
Cigarettes smoked prior to pregnancy	0.571	0.042	<0.001	2.42	3.26	2.81
Social disadvantage by						
Index of multiple deprivation	0.468	0.067	<0.001	1.84	2.96	2.33
Maternal age	-0.298	0.054	<0.001	0.48	0.71	0.58
Age full time education finished	-0.408	0.062	<0.001	0.38	0.60	0.48
Nicotine dependence on						
Social disadvantage	0.193	0.047	<0.001	1.20	1.68	1.42
Partners' smoking status on						
Social disadvantage	0.182	0.053	0.001	1.15	1.68	1.39
One month validated cessation on						
Social disadvantage	-0.123	0.054	0.024	0.66	0.97	0.80
Nicotine dependence	-0.109	0.031	0.001	0.74	0.92	0.82
Partners' smoking status	0.014	0.034	0.690	0.91	1.16	1.03

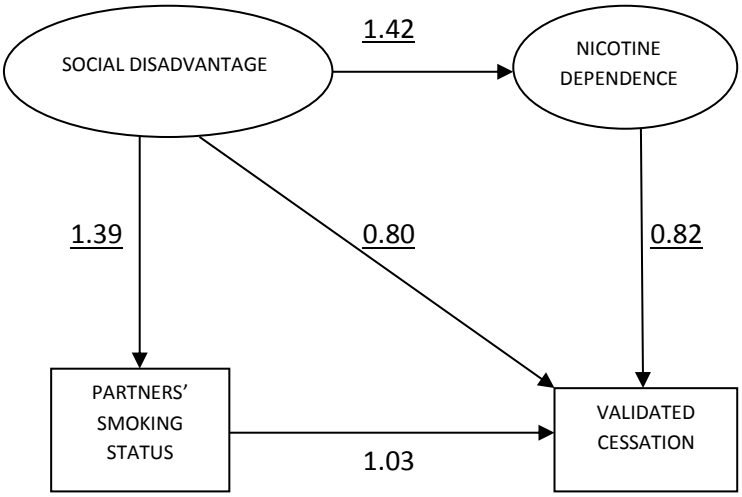


Figure 13 The proportion of mediation of social disadvantage by either nicotine dependence or partners' smoking status. Coefficients are approximate odds ratios listed in Table 7. Significant associations are underlined.

5.4.4. Proportion of the effect of social disadvantage that is mediated by dependence and partner smoking

The specific indirect effect estimates of mediation via either partners' smoking status or nicotine dependence, of social disadvantage on validated smoking cessation at one month, are listed in **Table 8**. These were calculated by the sum of the indirect effects and represent a measure of whether or not either pathway significantly mediates the effect of social disadvantage.

Table 8 Standardised indirect effects from structural equation model

Indirect Effects from IMD to			95% CI*		p-	95% CI		Approx.
One month validated cessation	Estimate	S.E.	Lower	Upper	value	Lower	Upper	OR
Specific indirect effect								
Via Nicotine dependence	-0.021	0.008	-0.040	-0.002	0.010	0.94	0.99	0.96
Via Partners' smoking status	0.002	0.006	-0.011	0.016	0.696	0.98	1.03	1.00

*95%CI generated from bootstrapped estimates

The proportion of mediation was calculated by dividing the specific indirect effect estimate (nicotine dependence: -0.021; partners' smoking status: 0.002) (**Table 8**) by the direct effect estimate, that of social disadvantage on validated cessation (-0.123) (**Table 7**). There was a very small amount of mediation by partners' smoking status, of 1.6% (p=0.696) which was not statistically

significant. However, 17.1% of the association between social disadvantage and cessation was mediated by the effect of nicotine dependence and this was statistically significant, (p=0.010). While a significant proportion of the direct effect of social disadvantage was mediated, the overall size of this association, as determined from the size of the effect estimate and the approximate odds ratio and 95%CI (OR: 0.96, 95%CI: 0.94-0.99), was considered a small to medium effect;[206] Nicotine dependence: (κ^2: 0.021). Bootstrapped confidence intervals for specific indirect effects (**Table 8**) were used to help quantify significance of the mediation effect, due to the non-symmetric distribution of the products of combined regression coefficients in the indirect pathways. These agreed with the approximate odds ratio and 95%CI that a small amount of mediation occurs via nicotine dependence, but little to none occurred via partners' smoking status.

5.5. Discussion

5.5.1. Main findings

In this cohort of pregnant smokers, a proportion (17.1%) of the relationship between social disadvantage and smoking cessation at one month after enrolment in the SNAP trial was mediated by their nicotine dependence. This equates to a small to medium effect, although a significant one. There was no evidence for any similar mediation through the influence of living with a partner who also smoked. Higher levels of social disadvantage were associated with pregnant women having greater difficulty in quitting and this appears to be partially explained by their nicotine dependence.

5.5.2. Strengths and limitations

There are several strengths associated with using SEM methods. The most significant strength is that it allowed flexible assumptions to be made about the structure of the model, as opposed to a logistic regression model which only

tests the significance of associations against a null model. The CFA indexes that have been used to measure the social disadvantage and nicotine dependence may have reduced the chance that measurement error of an individual variable contributed to biasing the association or mediation path being tested. Another advantage of using SEM methods is that it enables the measurement of direct and indirect effects, thereby allowing mediation modelling. The final methodological advantage of SEM is that each of the individual regressions is estimated simultaneously using an iterative process to find the optimal solution of estimated relationships. The simultaneous nature of SEM accounts for other variables in the model by constructing the model to fit the sample covariance. The fit of the overall structural model suggests that it supports the data.

A further advantage of the study lies in its use of validated smoking cessation data. Pregnant women may hide their smoking status due to the perceived social unacceptability of smoking during pregnancy but validation overcomes this problem. However, using baseline cotinine level (i.e. an objective variable) in the measurement model for nicotine dependence reduces the possibility of this occurring.

Furthermore, this is the first analysis to use mediation to examine the causal nature of the association between social disadvantage and validated smoking cessation in pregnancy; therefore, the findings are novel. They suggest that, although social disadvantage and smoking cessation in pregnancy are inversely related, around one fifth of this relationship may be explained by higher levels of nicotine dependence seen in materially deprived pregnant smokers. A clinical benefit of this greater understanding of the relationship between these variables is that addressing nicotine dependence is likely to be an effective strategy for helping socially disadvantaged smokers to achieve cessation. Still, there remains a significant inverse association between level of social disadvantage and odds of

smoking cessation. There may be other unmeasured factors that could mediate this association such as stress or measures of self-efficacy,[207] or cognitive characteristics like impulsivity or risk-taking attitudes. These variables were not included as they were not recorded in the SNAP trial.

Perhaps the main limitation of the study is that there was relatively limited data on social disadvantage, which was measured using maternal age, age at completion of education and IMD. Social disadvantage has many different facets, and it will manifest in a number of ways. For example, there was no data on income as this was not measured in the SNAP trial. One approach that has been suggested for measuring social disadvantage more accurately is to include as many predictors of social disadvantage as possible to capture the complexity of social disadvantage.[197] As a result the measurement models derived from CFA were kept despite their limited fit, as previous research and hypotheses about which factors reflect social disadvantage[99,182] were considered alongside the fit indices of the CFA's. An advantage of this approach though, is that the measurement and structural model associations are more theoretically sound, than those produced simply from exploratory factor analysis or CFA and classical adjustment for confounder methods, i.e. than those using simply data driven models. Increased IMD score, lower maternal age and decreased level of maternal education are hypothesised to indicate greater levels of social disadvantage. The results from the structural model supported their combination to produce the latent variable (**Table 7**). The inclusion of area level and individual level measures make it more likely that this latent variable will capture familial and community aspects of social disadvantage. Baseline cotinine level, HSI and the number of cigarettes smoked prior to pregnancy were included for the nicotine dependence measurement model on the same basis.

Information on other household members smoking that could influence women's smoking status might have created a more sensitive latent variable that would better represent household influences on pregnant smoking than using partners' smoking status alone. The majority of women in SNAP had partners who were smokers (68.2%), but the remainder might have had other smoking members, which might influence smoking behaviour during pregnancy. However, as most women consider their partner's smoking status a stronger influence than other family or friends,[188] this may not be a major limitation.

5.5.3. Findings in the context of previous work

No similar studies have been conducted in pregnant smokers. However, Hiscock *et al*. elucidated the association between social gradient and smoking cessation using similar methods,[149] concluding that non-pregnant smokers who used cessation services were more likely to be successful if they were more affluent. The study included 2,397 participants from Nottingham, Glasgow and North Cumbria and found that after 52 weeks, 14.3% of affluent smokers remained quit as opposed to 5.3% of the most disadvantaged.[149] This provided evidence to support the basis of the mediation analysis conducted, which was to determine whether increased levels of disadvantage result in fewer pregnant smokers achieving cessation and whether nicotine dependence or partners' smoking could explain this association. Businelle *et al*. investigated the mediators of the association between socio-economic status (SES) and cessation in non-pregnant smokers. In a sample of 424 participants, five latent variables measuring social support, neighbourhood disadvantage, negative affect/stress and agency (self-efficacy) were found to be key mediators of the inverse association between SES and smoking status.[207] Specifically, the pathways SES → Neighbourhood disadvantage → Social support → Negative affect → Agency (Coef.= -0.005, p=0.02) and SES → Social support → Negative affect → Agency → 4 week cessation (Coef.= -0.011, p=0.04) were both significant.[207] While this

study does not use the same mediators measured from the SNAP trial, social support or perceived support with quitting is likely to be related to partners' smoking status when making a quit attempt and the fact that this was a significant mediator for non-pregnant smokers[207] supports the rationale for inclusion of partners' smoking status the proposed structural model (**Figure 6**). However, the present study did not find partners' smoking status significantly influenced the effect of social disadvantage on smoking cessation.

Businelle *et al.* did not include nicotine dependence in their analysis as it was not associated with smoking status in their study.[207] Also, although they found mediation by agency and craving which were hypothesised to measure aspects of nicotine dependence, these were measured using self-reported questionnaires and not objective measures, making the present study's findings more reliable. Findings from the studies above, which were conducted in non-pregnant smokers,[149,207] provide evidence which supports the hypothesis that the association between social disadvantage and cessation may be mediated by similar factors in pregnancy as outside of pregnancy.

The present study provides new evidence in the context of pregnancy, suggesting that the association between social disadvantage and smoking behaviour is partially mediated through the more proximal factor of nicotine dependence. Previously, factors that represent social disadvantage have been investigated to see if they are associated with likelihood of cessation[97,208]; this study goes further by examining how these factors may be associated with each other and a possible simple pathway by which social disadvantage may be associated with smoking cessation is highlighted. Evidence that has looked at factors that are independently associated has paid no attention to temporality. With a multiple linear regression approach it is not possible to answer the questions of whether inclusion of baseline cotinine levels, the number of

cigarettes smoked previously, or the HSI increases accuracy of estimates of nicotine dependence, or if the indirect effect of social disadvantage on cessation via this latent variable is significant, and therefore important. Addressing statistically significant indirect mediation pathways, such as that of nicotine dependence, may attenuate the effect of social disadvantage on smoking cessation in pregnancy.

These results imply that at least some of the adverse impact of disadvantage on cessation could be ameliorated through better treatment of nicotine dependence. Many women in this cohort were able to maintain prolonged cessation from a quit date up until the one month follow-up, yet, by delivery, many more of these women had relapsed back to smoking.[116] Hence, it was not possible to investigate this model with cessation by delivery. This result suggests that the nicotine patch seemed to work in the short term, however, since many women relapsed, treatment for relapse prevention beyond this point may be necessary. Relapse to smoking could either be due to lack of adherence to treatment or an increased rate of nicotine metabolism in pregnancy, reducing the effectiveness of any NRT patches used. Both of these possibilities are further investigated in **Chapter 7** and **Chapter 8** respectively. Partners' smoking status did not significantly mediate the effect of social disadvantage on cessation despite being perceived as an important factor to women when make a quit attempt. Addressing the smoking of women's partners does not appear to ameliorate the negative effect that social disadvantage has on propensity to quit smoking in pregnancy.

5.5.4. Conclusions

In pregnant smokers who were recruited to a trial of nicotine replacement therapy, the relationship between social disadvantage and smoking cessation was partially-mediated by their nicotine dependence but not by partner's

smoking. There appears to be a complex relationship between social disadvantage and smoking cessation and this is the first study in any group of smokers to show that mediation by nicotine dependence occurs. Findings imply that pregnant smokers who are more disadvantaged may be more successful in cessation attempts if their nicotine dependence is addressed. However, more data from studies with wider ranges of potential mediators are needed, both to validate findings and to explore other possible explanations.

Chapter 6

The relationship between NRT use and birth outcomes: does greater exposure to NRT, with and without concurrent smoking, lead to better birth outcomes?

6.1. Introduction

In the previous chapters, nicotine dependence was found to be inversely associated with cessation and to be a key mediator of the association between social disadvantage and cessation; nicotine dependence is therefore an important factor in the propensity of pregnant women to quit smoking. NRT aims to address nicotine dependence in smokers, but evidence on its safety in pregnancy is lacking. In this chapter, I will investigate the association between increased exposure to nicotine and birth outcomes.

Compared with not smoking in pregnancy, maternal smoking results in more frequent occurrence of asthma, sudden infant death syndrome (SIDS), obesity, behavioural difficulties and brain damage in children.[48] Several of the poor maternal and birth outcomes caused by maternal smoking, such as ectopic pregnancy,[66] preterm delivery,[4] low birth weight,[70] and miscarriage,[75] have been shown to exhibit a dose response relationship with the number of cigarettes smoked per day. A recent Cochrane review of psychosocial interventions for smoking cessation by Chamberlain *et al.* found that stopping smoking leads to an increase in mean birth weight of 40.8g and reduced number of preterm births.[3] A second meta-analysis of birth outcomes found a 37% reduction in placenta praevia and placental abruption, 44% reduced odds of ectopic pregnancy and 41% reduction in preterm premature rupture of membranes (PPROM) when smoking abstinence occurred during early pregnancy.[209] However, later tobacco exposure in the third trimester of pregnancy is still strongly associated with significantly reduced infant growth measured at birth,[210] and quitting smoking earlier in pregnancy is associated with lower risk of low birth weight.[211,212] Indeed, it is hypothesised that earlier quitting is associated with better birth outcomes in general; some estimates suggest quitting cigarette use may be as important for improving birth outcomes as quitting illicit drug use.[213]

Cigarette smoke contains many potentially harmful products including pesticides, herbicides, tar, ink, nickel, arsenic, lead and free radicals,[40] as well as nicotine and carbon monoxide (CO).[214] Most research thus far has focussed on the harmful effects of nicotine and CO[48] in pregnancy, and this chapter focusses on nicotine. Nicotine has been hypothesised to be responsible for foetal growth restriction which leads to the development of low birth weight infants. There is some evidence that nicotine is a vasoconstrictor and reduces blood flow to the foetus.[215] It has been suggested that this vasoconstriction, may cause reduced foetal growth due to hypoxia,[4] though the magnitude of any effect may be minimal. Furthermore, nicotine has its own associated dangers, as it is metabolised to nitrosamine 4-(methylnitrosamine)-1-(3-pyridyl)-1-butanone (NNK),[216] a carcinogenic compound implicated in the development of tumours. Current evidence suggests that nicotine plays a small role in disease mechanisms, but nicotine addiction is the proximal cause of disease caused by tobacco smoke. Tobacco smoke has been found to be associated with respiratory tract and other infections, osteoporosis, reproductive disorders, delayed wound healing and adverse postoperative events, duodenal and gastric ulcers and diabetes.[54]

NRT, which provides a therapeutic dose of 'clean' nicotine, is the only medication not contraindicated for use in pregnancy for smoking cessation.[107] A population study based in the UK using data from 2001 to 2012 found that NRT is prescribed for women more frequently during pregnancy than before or after pregnancy and, that during this 11 year period, there has been an increase in prescribing NRT from 0.7% to 11.4% of pregnant women.[217] Use of NRT varies in pregnant women,[218] but it is possible that when women make greater use of NRT, they simultaneously smoke less and, hence may experience lower overall tobacco exposure in pregnancy than they would have done if they had smoked

and not used NRT. The effect of reduced tobacco smoke exposure would be a reduction in exposure to chemicals other than nicotine which are also contained in cigarette smoke.[216] If this does occur, one would expect pregnant women who use more NRT to have better birth outcomes than those who do not adhere to treatment regimens so closely and use less NRT. Concern about nicotine exposure to the foetus exists as well though, and may be warranted if smoking is concurrent with NRT use, as treatment may then increase the level of nicotine women are exposed to. When NRT is used alone in pregnancy, however, it is very likely that the levels of nicotine derived from NRT are substantially lower than those gained from continued smoking.[128] Consequently, investigating whether or not nicotine exposure from NRT use is associated with adverse birth outcomes or whether this association is due to other tobacco smoke constituents from concurrent smoking is important. This chapter will investigate the associations between increased exposure to nicotine and birth outcomes. It will also investigate the evidence that any associations may have been caused by concurrent smoking.

Thus far, much of the evidence on the dangers of nicotine exposure has come from animal studies. Nicotine and cotinine have both been found to cross the placenta and have been shown to have neurotoxic properties in animal studies.[126] Dose dependent inverse associations between increased nicotine exposure and brain weight have been found in animal models.[219] A literature review of longer term foetal and neonatal exposure to nicotine in animal studies has shown associations between nicotine exposure and adverse outcomes.[216] Hyperactivity, cognitive impairment, increased anxiety, somatosensory deficits, neurochemical alterations, changes to the sensitivity and thus self-administration of nicotine, altered patterns of neural cell survival and synaptogenesis have all been observed with prenatal exposure to nicotine in rodents.[216] Furthermore it is possible that there is reduced fertility in female

animal subjects exposed to nicotine and it is possible that promotion of tumour growth occurs by nicotine's transformation to the carcinogenic tobacco specific nitrosamine, NNK.[216] This chapter aims to investigate whether nicotine exposure may be a possible cause of the equivalent poor birth outcomes in humans.

In humans, concurrent exposure to the aforementioned tobacco smoke constituents, some of which are also carcinogens (nickel, arsenic, lead, free radicals), means it is not clear whether nicotine exposure causes foetal harms or if this is due to other tobacco smoke constituents and nicotine is actually harmless. There is some evidence that nicotine administration in pregnant women has negative short term effects on the cardiovascular systems of both the mother and the foetus.[60] Nicotine increases the heart rate, constricts blood vessels, causes transient increases in blood pressure and leads to uteroplacental insufficiency (lack of blood supply to placenta).[60] However, relevant data from trials investigating the use of nicotine (as NRT) by smokers in pregnancy has been summarised in a Cochrane review of pharmacological interventions for smoking cessation in pregnancy.[112] This found there were no statistically significant differences between miscarriage, stillbirth, birth weight or low birth weight in NRT and control groups; preterm births, neonatal intensive care unit admissions and neonatal deaths were all lower in the NRT groups, although non-significantly so.[112] A recent RCT (N=476) also found that nicotine patches did not alter birth weights when adjusting nicotine doses derived from treatment to match those from smoking.[220] A further review of smoking cessation interventions for pregnancy and postpartum by the Agency for Healthcare Research and Quality (AHRQ) identified 13 studies that reported birth outcomes,[221] four of which used NRT as an intervention.[116,119,121,222] In trials in pregnancy that have investigated the use of NRT versus placebo with birth outcomes,[116,117,119,121] no significant differences in the rate of miscarriages, spontaneous abortions, and stillbirths were noted. The outcomes of these

reviews are reassuring as trials conducted in pregnant smokers provide some evidence that nicotine is not harmful when used alone as NRT.

The only longer term RCT comparisons of human data on the effects of nicotine administration in pregnancy comes from two year follow-up of infants born in the SNAP trial.[223] This found that infants of women who were randomised to NRT were more likely to be developmentally unimpaired at two years than those randomised to placebo (73% versus 65%, p=0.023).[223] Smoking rates at the end of pregnancy did not differ between trial groups but there was a statistically significant doubling of quit rates in NRT group women observed at one month after their quit dates when they were still pregnant.[223] Additionally, in this study there was a dose-response relationship between the reported use of NRT patches and infants surviving to two years old without any developmental problems.[223] Outcomes of infants in women who reported having used between one and ten NRT patches in total were not statistically different to those in women who reported using no NRT patches, but women using between 11 and 56 patches (i.e. for 11 to 56 days) were more likely to have infants with no impairment two years after delivery (OR: 1.72, 95%CI: 1.22-2.57, p=0.004).[223] It is possible, therefore, to hypothesise that those better developmental outcomes in NRT group infants resulted from the temporary reduction in smoking noted at one month post-quit during pregnancy. If this hypothesis is correct it would follow that harm to the foetus from smoking is probably not caused by nicotine but by other constituents of tobacco smoke.

It is possible that NRT might have had a direct protective effect on child development; however, given findings from animal studies, summarized above, this seems unlikely as nicotine appears to be neurotoxic. Rather, greater use of NRT in pregnancy when making a quit attempt may be associated with less smoking and reduced exposure to cigarette smoke which benefits early

development in children. There is currently no evidence on the association between the level of exposure to nicotine and birth outcomes using prospective data. Overall, it is unclear whether or not the level of nicotine exposure alters risk of adverse birth outcomes in children of mothers who smoke during pregnancy. Decreasing the exposure of pregnant women to cigarette smoke is likely to be important for improving birth outcomes and so any method by which that is achieved is welcome. Such evidence would also provide a stronger underpinning of the rationale for the findings at two years in the SNAP trial.[223] Therefore, the hypothesis that increased use of NRT is associated with better outcomes is investigated in this chapter.

6.2. Aim

This study aimed to investigate whether, within a cohort of pregnant women recruited in the SNAP trial, greater exposure to nicotine from transdermal nicotine patches was associated with better or worse birth outcomes.

6.3. Objectives

I. To investigate the association between use of NRT and foetal growth/prematurity as measured by birth weight and being born small for gestation age (SGA).

II. To perform an exploratory analysis investigating associations between use of NRT and all other birth outcomes measured in the SNAP trial.

6.4. Methods

The stated aim and objectives were achieved using the analysis strategies outlined below and variables defined in the following sections. All analyses were undertaken using data from the SNAP trial.[116]

6.4.1. Baseline variables

A full list of the baseline variables from the SNAP trial is provided in **Appendix A**. The main exposure variable used in this chapter was use of NRT, as measured by the number of days on which women reported using a trial patch. Maternal age, ethnicity, age full time education was completed, the number of cigarettes smoked before pregnancy and currently, time after waking until first cigarette of the day, partners' smoking status, parity, baseline cotinine level, previous use of NRT, achieving abstinence at either one month post-quit date or at delivery, and treatment assignment were used to characterise the sample of women used in this analysis.

6.4.2. NRT use definition

Participants were initially provided with four weeks' worth of daily placebo or 15mg/16hr NRT patches. A further four weeks' worth of either placebo or NRT patches was provided upon confirmation that the participant was abstinent by CO validation at one month after randomisation. When providing patches, participants were informed of the importance of not using NRT whilst also smoking. At one month women were asked how many days' worth of patches they had used within the first 28 days since randomisation (potential range = 0-28 days, referred to as 'shorter term NRT use') and at delivery they were asked how many trial patches were used in total (potential range = 0-56 days, referred to as 'longer term NRT use'); both shorter and longer term use were included as continuous predictor variables in all analyses.

Where data regarding patch use was missing, women were assigned a value of zero days' use rather than imputing an average NRT use value. This was unlikely to introduce bias as missing data was equally distributed between NRT and placebo patch users. If a mean value had been imputed, this would have potentially overestimated the usage of NRT because most women who did not report NRT use would be expected to have stopped using NRT. A study of

misclassification of smoking status found that mothers who did not manage to stop smoking during pregnancy may be more likely to avoid trial follow-up and questions about smoking.[224] Assigning a value of zero days NRT use for participants who did not provide information gives a conservative estimate of NRT use as, at least some women who were lost to follow-up might have been expected to use NRT for a period. Women who were given placebo patches were also coded as having zero days' NRT use because these patches contained no nicotine, hence there was no physiological process by which use of these patches might influence birth outcomes. This gave greater power to detect associations between use of NRT and birth outcomes and adjust for baseline maternal characteristics by allowing for these participants' inclusion. A complete case analysis for use of NRT data was carried out as a sensitivity analysis to assess the impact of using data on NRT use in this manner.

6.4.3. Outcome variables: birth outcomes

Birth weight and being born SGA were chosen *a-priori* as the main birth outcomes of interest. These outcomes were chosen because of their ubiquity in comparable analyses,[117,119,121] their association with smoking and cotinine levels in pregnant women in previous analyses[48] and their demonstrable importance for association with future wellbeing. Low birth weight, usually classified as babies born weighing <2,500g, is strongly associated with infant mortality, as is gestational age and foetal growth.[225] Analyses presented in this chapter investigate the association between nicotine exposure with birth weight and SGA, as well as exploratory associations with other birth outcomes measured in the SNAP trial. The derivation of the SGA is detailed in **Section 6.4.3.1**. The associations between the use of NRT and birth weight as well as SGA are presented for qualitative comparison.

The other birth outcomes in SNAP, which were collected at birth or shortly afterwards, were analysed in an exploratory analysis. These included miscarriage between randomisation and 24 weeks gestation, whether the mother was admitted to the antenatal unit, Apgar score, cord blood pH, intraventricular haemorrhage, neonatal convulsions, congenital abnormalities, necrotising enterocolitis, whether the infant was ventilated for longer than 24 hours and the delivery method. Many of these were rare outcomes and as their low incidence did not permit more complex analyses, univariable regressions were performed. It was not possible to assess the association with stillbirth because this analysis was conducted for women who had singleton births only, and stillbirths occurred in twin births in the SNAP trial cohort.

6.4.3.1. Calculation of birth weight centiles for SGA derivation

Birth weight centiles adjusted for gestational age were created.[226] These were used as there is evidence that adjusted centiles identify foetuses at higher risk of stillbirth, neonatal death and having an Apgar score <7 at five minutes, due to better identification of foetal growth restriction.[227] The birth weight centiles adjusted the normal range of foetal size based upon gestation of the mother at birth, from which a birth weight $\leq 10^{th}$ centile was considered to be small for gestational age. This calculation yielded expected birth weights for a given gestational age. Hadlock's formula[226] was used to calculate birth weight adjusted for gestational age:

$$\text{Birth weight adjusted for gestational age} = \exp(0.578 + 0.332\ GA - 0.00354\ GA^2)$$

GA: gestational age in weeks.

Hadlock's formula predicted a median adjusted birth weight of 3,619g at 40 weeks gestation. The unadjusted average birth weight in the SNAP trial for the singleton infants born to women in this sample (n=1,010) at 40 weeks gestation

was 3,284g. This is a lower birth weight than Hadlock's formula predicts. However, use of Hadlock's formula and the derived birth weight adjusted for gestation more accurately identifies risk of perinatal morbidity than use of unadjusted data,[228] by better identifying babies that are born small for gestational age. Adjusting for gestational age in this way allows for infants born at later gestations, which are generally heavier, to potentially be classified as being SGA. These are babies that are likely to have experienced foetal growth restriction; using crude birth weights, some cases of foetal growth restriction may be missed. The following diagram illustrates birth weight at delivery and the expected foetal weights adjusted for gestational age using Hadlock's formula, with 10th and 90th percentile confidence intervals for each of the 1,010 infants included in the analysis (**Figure 14**):

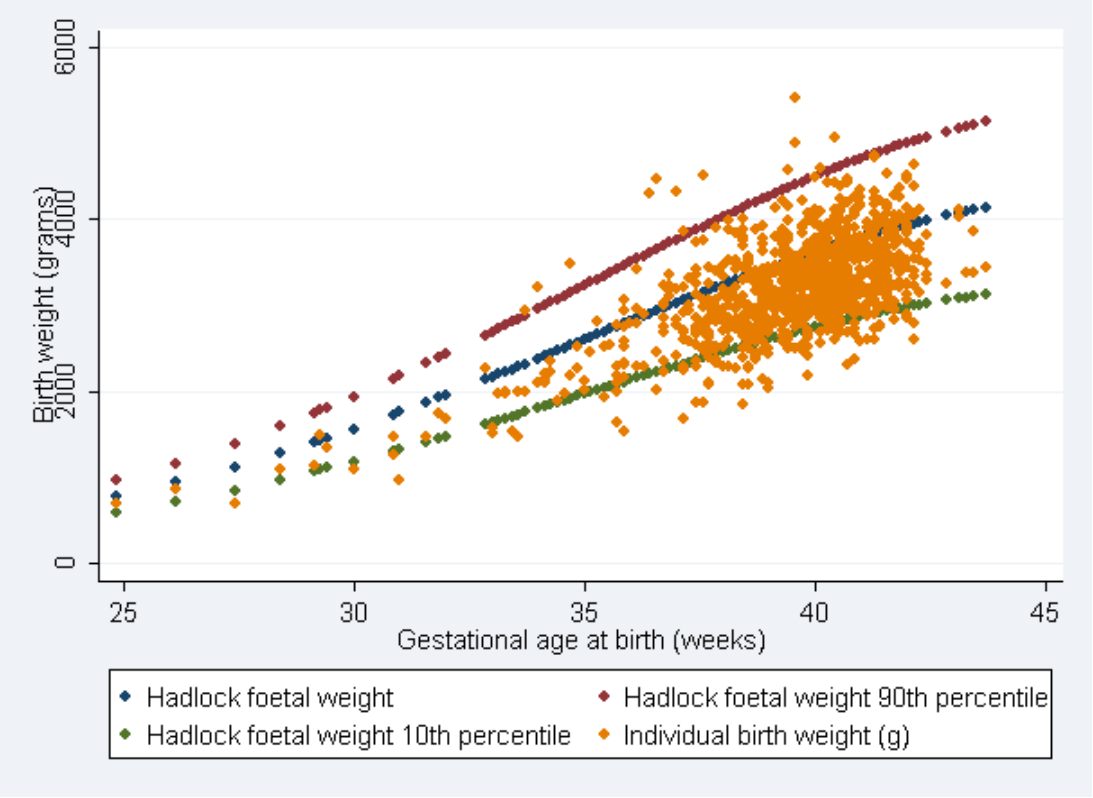


Figure 14 Birth weight and predicted values of birth weight adjusted for gestational age (n=1,010)

Infants who weighed less than the 10th percentile predicted by Hadlock's formula were categorised in a binary variable as being born SGA.

6.4.4. Analysis strategy

6.4.4.1. Descriptive statistics: Baseline characteristics of women who provided birth outcomes data

Descriptive statistics were used to report the characteristics of women with a singleton pregnancy that provided birth outcome data. Twin and multiple births were excluded from this analysis because of the higher risk for low birth weight and preterm birth.[229] Means and standard deviations, or numbers and percentages, were tabulated for all factors investigated to characterise this group of pregnant women. The mean birth weight of babies born and the percentage of babies born SGA were tabulated with level of NRT use to discern if a trend between these factors existed. There were a large number of women who were categorised as having used 'zero days' worth of patches', due to placebo patches containing no nicotine. As a result, the categories used for describing associations before model building were defined by a median split, with an additional category for 0 days' use, i.e. 0, 1-10 and 11-56 days of patches used.

6.4.4.2. Relationship between use of NRT and birth weight/SGA

An analysis was undertaken investigating use of NRT with unadjusted birth weight, and being born SGA; outcomes chosen allowed investigation into whether differences in birth weight, which might be attributed to NRT use, were in fact due to differences in gestational age. A multivariable linear regression model was built for birth weight and a multivariable logistic model for SGA. Exposure to nicotine, as measured by the number of day's trial patches were used, was included as a continuous variable. This association was adjusted for potential confounders listed in the baseline outcomes (**Section 6.4.1**) using a

systematic strategy for confounder selection. Significant univariable associations ($p \leq 0.05$) with birth weight or SGA were entered into a multivariable linear regression (birth weight) or logistic regression (SGA) model using the stepwise backwards elimination method. After entering all variables that were significant and the univariable level, variables that were non-significantly associated were removed from the model, and following this, variables which showed no association were tested one at a time to see if they became significantly associated with either outcome, in their respective models. *A-priori* confounders that were adjusted for were maternal age, ethnicity and partners' smoking status. Since smoking is strongly related to decreased birth weight and increased incidence of SGA, it was necessary to adjust for concurrent smoking when investigating the relationship between exposure to nicotine and birth weight/SGA. Adjusting for the amount smoked at one month or at delivery in a multivariable analysis may mask the effect of increased use of NRT as abstinence from smoking is positively associated with NRT use. However most women were recruited at approximately 16 weeks gestation and smoked up until this point. Smoking until this point must be factored into this analysis, and hence the number of cigarettes smoked per day prior to pregnancy was also adjusted for. A complete case analysis was performed for comparison, where NRT use was not imputed as zero days' patch use when originally recorded as missing.

In addition to the multivariable analysis, average birth weight and the number of women who had babies born SGA were tabulated, stratified by whether or not women achieved validated cessation at neither follow-up point, either one month or at delivery or at both one month and at delivery. This will help to provide evidence for an association between NRT use and these outcomes, if masking did occur in the multivariable analysis.

6.4.4.3. Univariable associations between use of NRT and exploratory birth outcomes

In addition to the main analysis between use of NRT and birth weight, an exploratory analysis was undertaken using other birth outcomes collected in SNAP. Univariable logistic regression was carried out to investigate associations between NRT use and the following birth outcomes: Apgar score <7 at five minutes, cord blood pH<7, intraventricular haemorrhage, neonatal convulsions, congenital abnormalities, necrotising enterocolitis, infant ventilation for >24 hours, caesarean or assisted birth, and whether any of these conditions was experienced. Some birth outcomes were very infrequent; 'separation', where a continuous predictor might perfectly predict a categorical outcome, might occur in these instances.[230] This is more likely in rare outcomes, since there are a large number of women who used zero days' worth of patches. Firth's method of penalised likelihood approach,[231] which used robust standard errors to calculate confidence intervals, was used to calculate odds ratio's in variables where <1% of women experienced the adverse birth outcome.[232] This was done as it is difficult to assign accurate odds ratios when outcomes are rare. A regression was also carried out between use of NRT and experiencing any of the adverse events. Finally, a Bonferroni correction was carried out to adjust the significance level for multiple testing of these nine birth outcomes. In addition to this analysis, a complete case analysis was also employed where missing values for the number of NRT patches used was not imputed as zero days' patch use, and instead was excluded.

All analyses were conducted using Stata 13.1 (College Station, TX).

6.5. Results

6.5.1. Descriptive statistics

Data on patch use was available for all participants in the SNAP trial, since those who had missing data (18% for shorter term patch use, 7% for longer term patch use) were categorised as using no NRT (zero days' NRT patches). For 'shorter term patch use' of up to four weeks duration and reported at four weeks after randomisation, 18.9% of placebo users and 16.3% of NRT users had missing data that was coded as zero days patch use. For 'longer term patch use' of up to eight weeks duration and reported at delivery, 6.2% of placebo users and 6.9% of NRT users had missing data which was coded as zero days patch use.

Mean use of patches among trial participants was 6.8 days' worth and complete pregnancy outcome data was available for 1,026/1,050 participants (97.7%). Mothers who experienced stillbirth and miscarriage could not provide birth outcome data and were not included in the 1,026 women who did provide full birth outcome data. The planned analyses were undertaken for all women who had live singleton births (n=1,010, 96.2%). Twin births were excluded from the analysis because of the worse birth outcomes associated with this type of pregnancy.[229] **Table 9** identifies the baseline characteristics in those women who had singleton births and provided birth outcome data.

Table 9 Characteristics of singleton women (n=1010)

Characteristics	Mean (SD) / n (%)
Maternal age (years)	26.3 (6.2)
Ethnicity	
White British	981 (97.1)
Other	29 (2.9)
Age full time education finished (years)	
≤16	792 (78.4)
>16	205 (20.3)
Missing	13 (1.3)
Cigarettes smoked prior to pregnancy	18.9 (6.9)
Cigarettes currently smoked	14.0 (6.6)
Time to first cigarette	
<15min	547 (54.2)
15-60min	382 (37.8)
>60mins	81 (8.0)
Partners' smoking status	
N/A	82 (8.1)
Non-smoking	233 (23.1)
Smoking	695 (68.8)
Parity	
0-1	694 (68.7)
2-3	258 (25.5)
>4	58 (5.7)
Baseline cotinine level (ng/ml) (n=970)	135.3 (79.9)
Use of NRT in pregnancy prior to randomisation	
No	964 (95.5)
Yes	46 (4.5)
Treatment assignment	
NRT	503 (49.8)
Placebo	507 (50.2)

Singleton mothers were on average 26 years old, predominantly white British, had mainly (78.4%) completed full time education at or before 16 years of age and had partners that smoked (68.8%). Furthermore, they smoked a mean average of 14 cigarettes per day and, most had their first cigarette within 15 minutes of waking. A scatter plot of birth weight and use of NRT (**Figure 15**) indicated non-linearity of association, which was compatible with the categorisation of NRT as previously mentioned, for describing the association between NRT, birth weight and SGA.

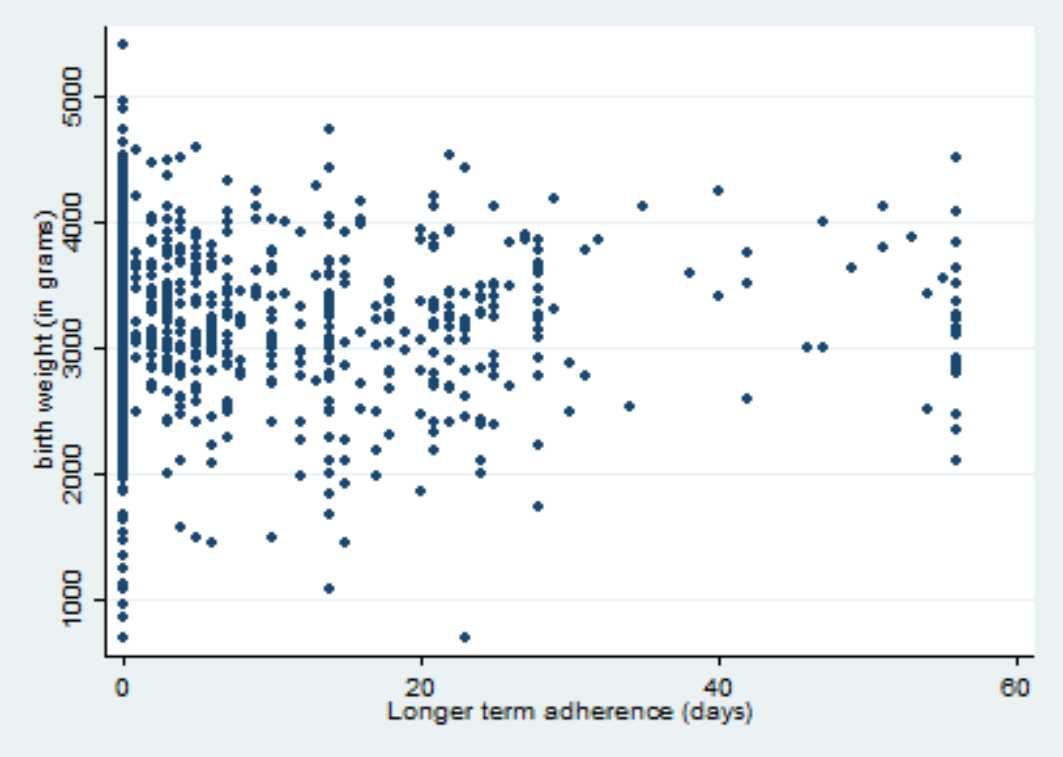


Figure 15 Scatter plot of the distribution of birth weight by the number of days trial patches were used

Table 10 lists average birth weight and the percentage of babies born SGA, with increasing level of NRT use. NRT use was included as a categorical variable in this table only for ease of presentation and was included as continuous in all subsequent analyses.

Table 10 Mean data for birth weight and SGA by NRT use reported at delivery

Variable	Birth weight (g) mean (SD)	p-value for trend	SGA N (%)	p-value for trend
Longer term NRT use (days)				
0	3170 (612)		52 / 521 (9.7)	
1-10	3240 (568)	0.560	24 / 214 (10.1)	0.149
11-56	3180 (645)		32 / 203 (13.6)	

Mean birth weight appears to change very little with an increase in the reported number of patches used. However, there is a suggestion that there might be a

trend with SGA. The proportion of babies born SGA with zero to ten days use of NRT was approximately 10% and this was 13.6% in women using 11-56 days' worth of NRT; the trend of increasing number of babies born SGA with longer use of NRT did not reach statistical significance.

6.5.2. Multivariable associations for use of NRT with birth weight and SGA

In this analysis, use of NRT was included as a continuous variable. These associations were adjusted for age, ethnicity, the number of cigarettes currently smoked and partners' smoking status. There was no association between shorter term NRT use and birth weight (β: -1.33, 95%CI: -5.68 to 3.01, p=0.547) or SGA (OR: 1.02, 95%CI: 0.99 to 1.04, p=0.162). In a complete case analysis the association between shorter term NRT use with birth weight (β: 0.08, 95%CI: -4.76 to 4.92, p=0.973) and SGA (OR: 1.01, 95%CI: 0.99 to 1.04, p=0.298) were also non-significant.

Similarly, when investigating longer term NRT use there was also no association with either birth weight (β: -0.46, 95%CI: -3.58 to 2.66, p=0.773) or SGA (OR: 1.01, 95%CI: 0.99 to 1.03, p=0.184). In the complete case analysis, NRT use was not associated with birth weight (β: 0.35, 95%CI: -2.59 to 3.29, p=0.815) or SGA (OR: 1.01, 95%CI: 0.99 to 1.02, p=0.312).

Table 11 describes the average birth weight and the number of children born SGA by whether mothers achieved cessation at neither, one or at both follow-up points at which smoking status was ascertained.

Table 11 Mean data for birth weight and SGA by abstinence

Variable	Birth weight (g) mean (SD)	p-value for trend	SGA N (%)	p-value for trend
Number of follow-up points where validated cessation was recorded				
Neither follow-up	3153 (592)		93 / 825 (11.2)	
Either at one month or delivery	3298 (590)	<0.001	10 / 114 (8.8)	0.027
Both one month and delivery	3461 (587)		1 / 71 (1.4)	

These results indicate that increased length of abstinence from smoking, as measured by the number of follow-up points cessation was achieved at, was significantly associated with increased mean birth weight and reduced incidence of SGA in this cohort.

6.5.3. Univariable associations between use of NRT and birth outcomes

Table 12 and **Table 13** show univariable associations between birth outcomes and 'shorter' and 'longer' term use of NRT. There were no associations found between shorter term NRT use and Apgar score <7 at five minutes, cord blood pH, intraventricular haemorrhage, neonatal convulsions, congenital abnormalities, necrotising enterocolitis, infant ventilation for >24 hours, caesarean or assisted birth, or experiencing any of these conditions. Similarly, using data on longer term NRT use, there were no associations with adverse birth outcomes for the majority of outcomes tested. However, **Table 13** shows there were small, borderline-significant increases in the number of babies born with an Apgar score <7 at five minutes (OR: 1.02, 95%CI: 1.00-1.05) and in the odds of having cord blood pH<7 if trial patches were adhered to for longer (OR: 1.04, 95%CI: 1.01-1.07, p=0.014).

Table 12 Univariable associations between use of NRT reported at 4 weeks and infant birth outcomes (n=1010)

			95% CI		
Characteristics	N (%)	OR*	Lower	Upper	p-value
Apgar score <7 at five minutes	37 (3.6)	1.03	1.00	1.07	0.074
Cord blood pH<7	11 (1.1)	1.02	0.96	1.09	0.534
Intraventricular haemorrhage*	5 (0.5)	0.96	0.85	1.09	0.577
Neonatal convulsions*	10 (1.0)	0.93	0.82	1.05	0.243
Congenital abnormalities	21 (2.1)	0.99	0.95	1.05	0.743
Necrotising enterocolitis*	9 (0.9)	0.94	0.84	1.06	0.317
Infant ventilated >24hrs	23 (2.2)	1.00	0.95	1.05	0.904
Caesarean or assisted birth	278 (27.1)	1.01	0.99	1.03	0.180
Any adverse birth outcome	326 (32.3)	1.01	0.99	1.02	0.223

*Penalised log likelihood: Firth's method of logistic regression

Table 13 Univariable associations between use of NRT reported at 8 weeks and infant birth outcomes (n=1010)

			95% CI		
Characteristics	N (%)	OR	Lower	Upper	p-value
Apgar score <7 at five minutes	37 (3.6)	1.02	1.00	1.05	0.044
Cord blood pH<7	11 (1.1)	1.04	1.01	1.07	0.014
Intraventricular haemorrhage*	5 (0.5)	1.02	0.96	1.08	0.528
Neonatal convulsions*	10 (1.0)	0.97	0.90	1.05	0.492
Congenital abnormalities	21 (2.1)	0.99	0.96	1.03	0.798
Necrotising enterocolitis*	9 (0.9)	0.99	0.93	1.06	0.769
Infant ventilated >24hrs	23 (2.2)	1.00	0.96	1.04	0.885
Caesarean or assisted birth	278 (27.1)	1.01	0.99	1.02	0.338
Any adverse birth outcome	326 (32.3)	1.01	1.00	1.02	0.281

*Penalised log likelihood: Firth's method of logistic regression

However, these apparently significant associations may have arisen by chance. Many of the birth outcomes analysed in the univariable analyses were infrequent in the SNAP trial. The capability to assign more accurate odds to these may be diminished, despite having used a penalised log likelihood method for outcomes that occurred in <1% of participants. After applying a Bonferroni correction to the significance level ($\alpha = 0.05/9 = 0.006$), use of NRT patches was not associated with cord blood pH<7 or Apgar score <7 at five minutes. Data from

larger population based studies with more detailed data on use of NRT may be required to further investigate rare birth outcomes.

6.6. Discussion

6.6.1. Main findings

This study found no significant relationship between shorter or longer term use of NRT patches in pregnancy and birth weight, or birth weight adjusted for gestational age (SGA). Furthermore shorter and longer term use of NRT was not associated with any of the other exploratory birth outcomes investigated from the SNAP trial. It did, however, find a significant trend with increasing birth weight and decreasing numbers of SGA births with achieving validated cessation at more points in pregnancy.

6.6.2. Strengths and limitations

The main strength of this analysis is that few studies have investigated the effect of randomisation to NRT on birth outcomes,[48] and to our knowledge, this is the only study that has had the power to investigate the impact of NRT use on the birth outcomes, birth weight and SGA. This study had greater power to detect associations, if they existed, than those previously conducted.[22,23,26] The correct identification of SGA infants used in this study helped to better capture this population, and thus any associations, more accurately than previous investigations of NRT on birth outcomes in pregnancy have before. Previously, research on NRT and birth outcomes has focused on whether women had or had not been prescribed NRT and birth outcomes,[130,233,234] as opposed to the actual reported use of NRT. This study failed to find an association between increasing reported use of NRT and birth weight, SGA or other adverse pregnancy related outcomes and, therefore, provides no evidence to suggest that exposure to nicotine at levels present when using NRT either increases or decreases the risks of adverse birth outcomes. Furthermore, as mean birth weight births were least

frequent, and the number of children born SGA was highest in women who did not achieve cessation at any point in pregnancy this indicates that smoking has a greater impact on birth outcomes than nicotine does. Given these findings, it is logical to hypothesise that poorer birth outcomes in smokers are caused by tobacco smoke constituents other than nicotine, but there is no direct evidence for this. A study with more detailed data on nicotine exposure from NRT and also more detailed data on any concomitant smoking behaviour could provide stronger evidence for or against this hypothesis. It remains possible that some associations between NRT use and the exploratory outcomes investigated may have been missed by chance because they are rare (e.g. intraventricular haemorrhage, neonatal convulsions, necrotising enterocolitis); the SNAP trial may not be large enough to detect these associations if they exist.

A potential limitation is that nicotine exposure, as measured by use of NRT, was calculated from the total number of days trial patches were used; this is a self-reported measure. There are three potential implications associated with the use of this variable. Firstly, women may have provided an incorrect estimate of the number of trial patches used. Secondly, trial patches may not have been used continuously. Thirdly, trial patches may have been used whilst also smoking and so it is difficult to disentangle whether poor birth outcomes are due to NRT or concurrent smoking. To address the first implication, while there is evidence that pregnant women underestimate the amount they smoke,[235] and thereby may have overestimated the number of days on which they used NRT only, there is no way to verify this without measuring smoking concurrently e.g. using daily diaries' measurement of daily smoking or NRT use. These data were unavailable from the SNAP trial. This is also a limitation of the second implication, as it is assumed that there is continuous use of trial patches by pregnant women. Although, it seems unlikely that women, having lapsed to smoking, would quit smoking again and re-start using NRT for any significant period of time. It is

therefore most probable that the use of trial patches was continuous from the beginning of the quit date until either relapse to smoking occurred or the participant was abstinent and chose to stop using the patches. Non-continuous use of NRT might affect birth outcomes differently based upon when during gestation smoke exposure from cigarettes was higher; there is some evidence that reduced exposure at earlier gestation is associated with increased birth weight.[211,212] From this study, it is unclear when NRT patches were used, although this study was conducted under the assumption that they were used continuously from the beginning of the quit attempt. With respect to the third implication, one cannot be certain that reduced exposure to tobacco smoke was perfectly inversely related to greater use of NRT, due to the lack of concurrently collected smoking behaviour data. Poor birth outcomes that might have been demonstrated to be associated with NRT use would have been difficult to explain as they may in fact be due to smoking whilst making a quit attempt. However, though it was not possible to adjust for concurrent smoking, it was possible to tabulate mean birth weight and number of SGA infants born stratified by the length of abstinence from smoking that was achieved by mothers. This provided a means of assessing the impact of concurrent smoking on birth outcomes and this impact does seem to have been a lot stronger than any impact from NRT. As pregnant women in the SNAP trial were only instructed to use NRT as part of a cessation attempt and to stop using NRT if they re-started smoking, it is likely that the observed association between cessation and birth outcomes is primarily due to women's smoking and the impact of concurrently using NRT should be minimal. To determine the relative extent to which poorer birth outcomes might be due to smoking or NRT, better quality prospective data on both women's smoking behaviour and NRT use would be necessary. However, despite having to make some assumptions about women's smoking behaviour and NRT use, we used data from the largest trial of NRT to date,[116] and this is likely the most robust which is currently available. Studies assessing rare birth outcomes or

congenital abnormalities necessitate population based sample sizes with similar data on NRT use. This would be prohibitively expensive and not practical, which is why population based studies have not done this. The results of this chapter are compared with those from population based studies carried out thus far, in the following section.

6.6.3. Findings in the context of previous studies

Only large epidemiological population based cohort studies have explicitly investigated the association between use of NRT with birth outcomes. Prospective trials of NRT for cessation, that have secondarily investigated whether assignment to NRT or not is associated with birth outcomes, have thus far been constrained by their sample sizes as many birth outcomes are infrequent.[117,119,121] Here, the results of the epidemiological cohort studies that have sample size to detect rare outcomes are compared with the findings from the largest analysis using prospective data.

Lassen *et al.* investigated use of NRT and associations with birth weight in 72,761 pregnant smokers in the Danish National Birth Cohort (DNBC).[236] There was no significant association between the number of weeks' worth of NRT use and birth weight (β=0.25g per week, 95%CI: -2.31 – 2.81).[236] Furthermore, using more than one form of NRT simultaneously was not associated with birth weight (β=-10.73g per week, 95%CI: -26.51 – 5.05).[236] This study also found birth weight was not associated with whether NRT was received as patch, gum or inhaler. Another cohort study including 5,716 women from the Pregnancy Risk And Monitoring System (PRAMS) found that women prescribed NRT had approximately twice the odds of low birth weight (OR: 1.95, 95%CI: 1.10-3.46) compared with non-smokers.[233] This study also found that the risk of preterm birth was also twice as likely in women who used NRT.[233] However, as the comparator was women who did not smoke, it is not possible to interpret

whether the increased odds of low birth weight were due to NRT or this was due to NRT being prescribed to heavier smokers. The findings of the present analyses are consistent with those of the larger DNBC population based study by Lassen and colleagues.[236] This study also included women who were smoking though, presenting the same problem as that from the PRAMS based study. Indeed, there is some evidence that, in pregnancy, heavier smokers who are more nicotine dependent might be more likely to be given NRT;[233] e.g. a cohort study of nicotine use from 1989 to 1998 found only 30% of NRT users achieved cessation as opposed to 39% of non-users.[234]

Morales-Suarez-Varela *et al.* investigated the use of nicotine gum, patches or inhalers during the first 12 weeks of pregnancy, in 76,768 women in the DNBC, on the incidence of congenital malformations.[130] Children born to former smokers who used NRT had a 60% increased prevalence of congenital malformations (RR: 1.61, 95%CI: 1.01-2.58).[130] When this was restricted to musculoskeletal malformations, the prevalence rate ratio was 2.63 (95%CI: 1.53-4.52).[130] However, these RR were based on 19 individuals who experienced any malformations, out of 250 non-smoking mothers who used NRT. Again, this higher level of congenital malformations in NRT users may be due to heavier smokers being more likely to be given NRT. In contrast, the present study of SNAP data in 1,010 singleton births from pregnant smokers making a quit date, using prospective data, found no significant association between up to four weeks' worth of NRT patches (OR: 0.99, 95%CI: 0.95-1.05) or up to eight weeks' (OR: 0.96, 95%CI: 0.96-1.03) worth of patches and congenital abnormalities. This study's findings are more reliable since pregnant women rarely use more than eight weeks' worth if patches and the SNAP data are prospective and used validated cessation. This concurs with a more recent study conducted by Dhalwani *et al*. amongst 192,498 children born in the UK between 2001 and 2012.[237] This study found that while maternal congenital anomalies

were more common in NRT users versus smokers (35% versus 27%), there were no significant associations between NRT and system-specific anomalies, except for respiratory anomalies (OR: 4.65, 95%CI: 1.76-12.25), of which there were only 10 cases.[237]

Strandberg-Larsen *et al.* investigated the use of NRT and its associations with stillbirth.[132] Participants either used transdermal patch, gum, inhaled substance or were categorised as various, for women who used more than one type of NRT.[132] 495 pregnancies out of 87,032 singleton pregnancies included in the study resulted in stillbirth (Hazard Ratio (HR): 0.57, 95%CI: 0.28-1.16).[132] However, whilst smoking versus not smoking conferred an increased rate of stillbirth (HR: 1.46, 95%CI: 1.17-1.82), women who used both NRT and smoked did not experience significantly different rates of stillbirth compared to not smoking (HR: 0.83, 95%CI: 0.34-2.00).[132] This indicates that in the case of stillbirth, using NRT may reduce the harmful effects of smoking, by reducing exposure to chemicals other than nicotine, found in cigarette smoke.

Although stillbirth was not able to be investigated in this study, as it was restricted to singleton births, evidence from previous studies appears to show that use of NRT is associated with no greater odds of poor birth outcomes. These results complement the findings of the present study, which found that greater exposure to nicotine from NRT was not associated with either an increase or decrease in poor birth outcomes.

6.6.4. Conclusions

Reported use of more NRT whilst abstinent during pregnancy was not associated with significantly different birth weight or being born small for gestational age. There is no evidence that greater use of NRT in pregnancy is associated with either harm or benefit with respect to birth outcomes measured in the SNAP

trial. However, longer periods of abstinence from smoking were associated with greater mean birth weights and reduced numbers of SGA births. The next chapter continues my investigation into how use of NRT might affect outcomes and investigates the strength of, and explanations for, any relationship between adherence with NRT and smoking cessation in pregnancy.

Chapter 7

Adherence to Nicotine Replacement Therapy in pregnancy

7.1. Introduction

Nicotine replacement therapy (NRT) has been proven to be an effective medication for smoking cessation in the non-pregnant population,[102] but not in pregnancy.[112] This may be because of lack of adherence seen in previous RCT's of NRT (**Section 1.7**). Non-adherence with treatment for the recommended duration in pregnancy may limit the efficacy of NRT,[123] but equally, NRT may not be effective given complete adherence; if the former is the case, high adherers are hypothesised to be more likely to achieve cessation and if the latter is the case, high adherers might not be more likely to achieve cessation. The rationale for this is that pregnant smokers will provide themselves with a steady supply of nicotine and therefore be less likely to experience the craving to smoke. No study has investigated adherence to NRT in pregnancy sufficiently to allow us to assess the degree to which non-adherence may or may not reduce efficacy. This chapter aims to address this.

Until this point, baseline maternal characteristics associated with greater adherence to NRT have scarcely been investigated (**Section 1.7.4**).[146] The influences on adherence are key to understand the underlying reasons for why pregnant women opt to discontinue treatment. Investigating factors thought to promote or reduce adherence may help target those who are less likely to do so. The SNAP trial provides data on a large number of pregnant smokers with verified abstinence data in which to investigate adherence to NRT,[116] but in particular, whether increased adherence to NRT results in greater odds of cessation.

In the general population, adherence to NRT is associated with increased odds of cessation,[139-142] despite adherence decreasing severely after six weeks of use.[138] This association between adherence and cessation has not been examined in pregnancy. Thus far, the most valid NRT trials in pregnancy have reported the

average number of patches used overall and a systematic review of these summarised that NRT use was low in pregnancy (only 7-30% of women adhered to the complete course offered).[112] Summary estimates of adherence were not able to be produced from this review due to the heterogeneity in the ways adherence was reported. A secondary analysis of one of the trials included in the systematic review conducted a univariable analysis on a small number of women (n=104) and found that use of NRT within the first 48 hours of a quit attempt was significantly related to longer term NRT use.[146] In turn, those women who used NRT for longer were more likely to report cessation at 38 weeks gestation.[146] This provides evidence to suggest that adherence may be an important factor in achieving cessation. However, an analysis adjusting for confounders and investigating each extra piece of NRT used is required before a dose-response association between adherence and cessation can be proposed in pregnancy. Since adherence to NRT has been found to increase the odds of cessation outside of pregnancy, this study investigates whether adherence to NRT increases the odds of cessation during pregnancy.

Determining whether an association exists between adherence to NRT and cessation is important for providing evidence of its effectiveness, which could encourage more pregnant women to use it. However, if such an association were to be found, one would need to eliminate alternate explanations for this before a causal relationship could be seriously considered. An association may arise because a lapse to smoking causes reduced patch use i.e. reverse causation. An analysis that associates adherence with cessation in a restricted group of completely abstinent smokers during their quit attempt, should eliminate this possibility. Outside of pregnancy, an informed review of analyses that have controlled for reverse causation found that a positive association between adherence and cessation remained.[140] The chance of reverse causation occurring in pregnancy is hypothesised to be greater in women whose quit attempts fail as

they are more likely to give up NRT than other smoking women since their perceptions regarding NRT's potential harm may alter use during pregnancy; pregnant women may view using NRT whilst smoking as especially harmful to their baby. Therefore the relationship between NRT and cessation may differ in pregnancy compared to non-pregnancy. Here, any association between adherence to standard dose NRT's and cessation in pregnancy will be tested to see if it is consistent with reverse causation.

There is a second possible cause of an association between adherence and cessation. It is possible that individuals with high motivation to abstain from smoking will also have high motivation to adhere to medication when asked to do so by a midwife or health professional. If this is true, then adherence to placebo patches would be associated with odds of cessation similar to that of adherence to NRT. However, if it is nicotine itself that improves outcomes, then adherence to active nicotine patches will be associated with greater odds of achieving cessation than placebo patches. To investigate this, an interaction test between adherence and active/placebo NRT medication will be carried out. This analytical approach has been used in a study conducted in a population of non-pregnant smokers. Shiffman *et al.* conducted an interaction test between active and placebo lozenges and found that higher use of active lozenges during the first two weeks was associated with greater odds of cessation at six weeks post-quit date whereas adherence to placebo patches was not.[142] Furthermore, another double blind RCT of 'over the counter' NRT found a significant interaction between active/placebo patches and adherence.[139] These results therefore suggest that, in the general population, nicotine is responsible for some of the effect on the association between adherence and cessation in addition to any effect particular to the characteristics of highly adherent people; this hypothesis is tested within pregnant smokers in this chapter.

Chapter 1 reviewed possible factors associated with lower adherence, including women feeling that the medication is no longer needed or having low motivation to continue, possibly due to partner or familial smoking influencing this decision. **Chapter 4** and **Chapter 5** in this thesis have also attempted to quantify the importance of some factors such as nicotine dependence and social disadvantage, but no studies have yet investigated the relationship between pregnant smokers' characteristics' and adherence to medication or whether these might be confounders of a hypothesised adherence-cessation association in pregnancy. In this chapter, data collected during the SNAP trial[116] was used to investigate adherence to standard dose 15mg/16h NRT transdermal patches in pregnancy for smoking cessation and alternative hypotheses for this association. By using data from the SNAP trial it is possible to investigate factors associated with adherence in pregnancy and its association with cessation, including controlling for reverse causation.

7.2. Aim

The aim of this chapter is to investigate the characteristics of pregnant women which are associated with adherence to NRT and the nature of any relationship between adherence to treatment and smoking cessation.

7.3. Objectives

The stated aim was investigated through the following objectives:

I. To investigate the characteristics which were associated with adherence to treatment.
II. To investigate any association between adherence to transdermal patch treatment and validated cessation, adjusting for confounders.
III. To investigate whether or not any observed association between adherence and cessation might be caused by 'reverse causation'. Reverse

causation is defined as a lapse in smoking that causes reduced use of patches.

IV. To investigate whether or not adherence to NRT patches was more strongly associated with smoking cessation than greater adherence to placebo patches.

7.4. Methods

At baseline, prior to randomisation, data was collected on: age, ethnicity, age full time education completed, cigarettes smoked before pregnancy and currently, time after waking until first cigarette of the day, partners' smoking status, parity, gestational age, body mass index (BMI) and previous use of NRT. Saliva samples for cotinine estimation and exhaled CO readings were also taken. A full list of variables is detailed in **Appendix A**. The baseline variables in this chapter's analyses were tested for collinearity, in particular between the heaviness of smoking index and baseline cotinine, however no association was found between these (**Section 4.3.1.**), or any two variables.

7.4.1. Adherence and cessation definitions

Women were asked the number of days on which they had used trial patches. Trial participants were informed of the potential importance, with regards to foetal health, of not using trial patches at the same time as smoking, making it unlikely that women used patches if smoking had resumed. Trial patches were given in four week batches: first at the beginning of their quit date, with women receiving a second batch if they were abstinent at one month and wished to receive them. At one month and delivery, participants were asked about their smoking behaviour and use of trial patches. At one month participants could report using patches for a maximum of 28 days and, at delivery for a maximum of 56 days; adherence was measured with respect to these values. Women who

had data missing for the reported number of days trial patches were used were considered to have zero days' adherence.

Acceptance of a second batch of patches was also used as an alternative measure for adherence beyond the one month's worth of patches allocated to all SNAP trial participants. This was used as it represented more closely how NRT is provided to smokers in clinical practice. This second measure of adherence was used in addition to the number of days of trial patches used, in the analysis of objectives I and III only.

At one month, participants were asked about their smoking status in the last 24 hours and also since the quit date which had been set shortly after randomisation. If they reported abstinence from their quit date to one month post-quit, this was validated using an exhaled CO reading (<8ppm). At delivery, participants who reported abstinence between their quit date and delivery had this validated using exhaled CO measurement (<8ppm), and saliva samples were taken for cotinine estimation. Those who reported prolonged abstinence from quit date to respective follow-up and were validated as non-smoking were considered to have achieved validated cessation.

7.4.2. Transformation of the measure of adherence to treatment

Adherence data reported at delivery was square root transformed to adjust for the positive skew of the data. The number of days that trial patches were used (range 0-56) is a count measure and transformation of this meant that adherence, when used as an outcome, better fitted the assumptions of linear regression by assuming a more normal distribution. The transformation was not carried out for adherence data up until one month post-quit date as the skewness and kurtosis values suggested that this was closer to normal when untransformed (i.e. closer to 0 and 3, respectively). The transformed variable

was only used when using adherence as an outcome variable, i.e. when investigating maternal characteristics associated with adherence between 0-56 days' worth of patches (Objective I). The results of the regression were then back-transformed.

7.4.3. Analysis strategies

The analysis strategies selected were intended to address the questions posed by each of the previously stated objectives.

7.4.3.1. Investigation of the factors associated with adherence to transdermal patch treatment

To assess which of the SNAP trial participants' characteristics were associated with adherence to treatment linear regression was used to assess univariable associations with cessation data collected at one month post-quit date and at delivery. Then using a manual stepwise, backward elimination method, two multivariable models were constructed. Firstly, all variables found to be significant after univariable analysis ($p \leq 0.05$) were included in a multivariable model. Those variables initially included, that were found to be non-significant within this model ($p > 0.05$) were then removed. Following this, variables which had not been found to have significant univariable associations with cessation were added one at a time to the multivariable models to determine if they were subsequently associated with adherence to treatment. Factors associated with adherence to patches were investigated in complete case analyses. In addition, using the same method of multivariable model building as previously described, a model for the second measure of adherence, whether women accepted a second four week batch of patches, was built.

7.4.3.2. Investigation of the relationship between adherence with treatment and cessation and whether potential confounding explains this

To investigate the relationship between adherence and smoking cessation in this sample, multivariable logistic regression for validated cessation at both one month and delivery was carried out using the same manual stepwise backwards method as detailed in the previous section, adjusting for the appropriate confounders. The association between adherence and cessation was also investigated using a complete case analysis.

Confounding factors may explain part of any association which exists between adherence and cessation. To investigate to what extent these might affect any observed association between adherence and cessation, we used variables identified from the analysis in **Chapter 4** or from the systematic review of factors associated with successful smoking cessation in the pregnant population as potential confounders;[97] the variables treated as potential confounders, if data were available, are as follows: age full time education was completed upon, baseline cotinine level, ethnicity, level of education, income, socio-economic status, employment status, partners' smoking status, cigarette consumption, craving and withdrawal symptoms and other measures of smoking behaviour and addiction. Treatment assignment was included as an *a-priori* confounder of this association, with the rationale being that an effect of adherence to treatment would only be expected in the group receiving nicotine patches. In addition, heaviness of smoking index (HSI) is known to be a marker of women's addiction to tobacco. The rate of cessation was found to be significantly lower in those with high HSI, and this factor was therefore included as an *a-priori* confounder.

7.4.3.3. Investigation of reverse causation

Reverse causation is the term given to the hypothesis that reduced use of NRT patches is caused by a lapse to smoking rather than *vice versa*. If reverse causation occurs, it would result in a potentially strong, positive association between use of NRT and smoking cessation being observed in analyses. To address the issue of reverse causation, the association between adherence and cessation at delivery was restricted to women who were abstinent for one month after their quit date. Therefore, because all women adherent and non-adherent to a second batch of patches, were abstinent during the first month, reverse causation cannot have occurred. This analysis assumes, however, that adherence during the first month predicts adherence during the second month of treatment and that non-adherence during that second month could cause relapse.

Logistic regression was used to compare the odds of cessation at delivery between women who were high and low 'adherers', ascertained by a median split in the number of days trial patches that women reported using in the first 28 days after quit date. This analysis used early adherence, whilst abstinent, as a proxy for later adherence and operated under the assumption that women with higher levels of earlier adherence would also have greater adherence after one month into the trial when their abstinence from smoking was measured for the first time.

The acceptance of second batch of patches was used as the measure for greater adherence between one month post-quit date and delivery i.e. was acceptance of a second batch of patches associated with greater odds of cessation? There was no direct measure of how much NRT was used during the second month of treatment. However, non-acceptance means women would have only a few patches left from the first batch dispensed. This method might more closely reflect the manner in which NRT is administered in clinical practice. This was

tested using a similar logistic regression approach, within the same subgroup of women abstinent at one month, for cessation at delivery.

7.4.3.4. Investigation of whether adherence with NRT is more strongly associated with cessation than placebo adherence

An interaction test was used to assess whether greater adherence was associated with increased odds of cessation in women assigned to receive NRT than those assigned to receive placebo. To investigate this hypothesis, high and low adherence to treatment patches, defined by a median split in the number of trial patches used within the first month, was investigated by assignment to NRT or placebo. A logistic regression for cessation at delivery was performed, with an interaction between treatment assignment and 'high' and 'low' adherence, within a sample of women who were abstinent at one month.

All analyses were conducted using Stata 13.1 (College Station, TX).

7.5. Results

Of 1,050 women randomised in the SNAP trial, 957 (91.1%) women who had no missing data for baseline characteristics were included in the complete case analysis, and a restricted group of 167 (15.9%) women who were abstinent up until one month post-quit date. As described in **Chapter 4**, women who did not report the age they left full time education all failed to achieve cessation, and so were excluded from the analysis. For 80 women, there was no data on cotinine concentration at baseline (7.6%); imputation was not carried out since this was <10% of the original sample. Adherence data was available for 865 women for 0-28 days', and 981 women for 0-56 days' worth of patches used. Missing data for adherence was imputed as zero days used as it was likely that women who did not report adherence had lapsed, and were not using patches (One month:

17.6%, Delivery: 6.6%). Consequently, there were no missing data for adherence.

7.5.1. Adherence

The following are adherence statistics for the restricted group of women that were abstinent for at least one month. Women were classified as highly adherent if they used ≥7 patches and used them for a mean of 20.5 (SD 5.7) days. Those who had lower adherence (≤6) used patches for a mean of 3.6 (SD 2.0) days. Similarly, by delivery (7 days median split), highly adherent women used patches on average for 31.5 (SD 16.4) days and less adherent women used these for an average of 3.6 (SD 2.0) days.

Baseline maternal characteristics of participants using either placebo or NRT with low and high adherence are detailed in **Table 14** and **Table 15**. **Table 14** shows the characteristics of the women included in the complete case analyses for objectives I and II. Maternal age, level of education, ethnicity, primiparity and gestational age amongst low and high adherers were similar. However, baseline cotinine concentration was non-significantly lower for both highly adherent placebo and NRT users, suggesting a trend towards lighter smokers being more likely to use more patches in pregnancy. **Table 15** shows the characteristics of the women who were abstinent for one month, i.e. the restricted group which was used in the analysis for objectives III and IV. Maternal age and gestation were similar in this subset to the 957 women analysed in objectives I and II. However, the median value for baseline cotinine was lower in the restricted subset, which might be expected because these women achieved abstinence for at least one month. The restricted sample also had a higher percentage of primiparous women assigned to placebo patches and marginal differences in the level of education and ethnicity.

Evidence from likelihood ratio tests indicated that the adherence data were consistent with a linear trend, that decreased use over time at one month (LRT p=0.121) and at delivery (LRT p=0.999) and were therefore included as continuous variables for the proposed analyses.

Table 14 Baseline participant characteristics (n=957): complete case analysis of women with complete baseline data

	Adherence*			
	Low		High	
Variable	Placebo (n=289)	NRT (n=235)	Placebo (n=194)	NRT (n=239)
Age (years) [median (IQR)]	24.8 (21.2-30.7)	24.8 (21.5-28.7)	25.9 (21.7-30.9)	25.6 (21.4-31.0)
Primiparous	36.3%	35.2%	35.7%	39.9%
Age full time education finished				
≤16	77.6%	79.6%	79.0%	77.9%
>16	22.4%	20.4%	21.0%	22.1%
Ethnicity				
British - White	96.5%	96.8%	98.7%	96.9%
Other	3.5%	3.2%	1.3%	3.1%
Gestational age (weeks) [median (IQR)]	15.6 (13.7-18.7)	15.0 (13.4-18.1)	14.9 (13.4-18.0)	15.0 (13.3-18.3)
Baseline cotinine (ng/ml) [median (IQR)]	124.0 (74.5-180.8)	123.8 (87.1-178.4)	114.4 (79.6-167.6)	118.5 (73.5-179.8)

*High adherence based on median split of adherence at 1m follow-up

Table 15 Baseline participant characteristics (n=167): complete case analysis in restricted sample of women abstinent for at least one month

	Adherence*			
	Low		High	
Variable	Placebo (n=24)	NRT (n=17)	Placebo (n=35)	NRT (n=91)
Age (years) [median (IQR)]	27.6 (16.7-43.3)	24.6 (18.5-40.0)	26.3 (17.8-39.8)	27.4 (16.8-41.1)
Primiparous	50.0%	23.5%	40.0%	35.2%
Age full time education finished				
≤16	62.5%	76.5%	74.3%	67.0%
>16	37.5%	23.5%	25.7%	33.0%
Ethnicity				
British - White	95.8%	100.0%	94.3%	96.7%
Other	4.2%	0.0%	5.7%	3.3%
Gestational age (weeks) [median (IQR)]	16.7 (14.6-20.7)	14.0 (12.7-15.9)	14.4 (13.1-17.0)	14.9 (13.3-19.0)
Baseline cotinine (ng/ml) [median (IQR)]	75.0 (58.9-130.0)	68.3 (45.4-116.3)	80.2 (39.2-124.8)	99.9 (62.0-163.2)

*High adherence based on median split of adherence at 1m follow-up

7.5.2. Factors associated with adherence to trial patches

Factors associated with adherence are detailed in **Table 16** and **Table 17**. At one month, a univariable analysis showed that HSI score and baseline cotinine level were inversely associated with adherence, whilst treatment assignment to NRT and remaining in full time education beyond the age of 16 years were positively associated with adherence. In a multivariable analysis, only the inverse association with baseline cotinine (β: -0.08, 95%CI: -0.15- -0.01, p=0.020) and treatment assignment to receive NRT (β: 2.59, 95%CI: 1.50-3.68, p<0.001) remained significant (**Table 16**).

At delivery, univariable analysis showed that treatment assignment to NRT and age full time education was completed were both associated with increased odds of adherence. A higher HSI score was also found to be inversely associated with adherence. A multivariable analysis of these factors found that HSI's inverse association (β: -0.27, 95%CI: -0.50- -0.05, p<0.001) and treatment assignment to NRT (β: 0.51, 95%CI: 0.29-0.72, p<0.001) again, remained significant (**Table 17**).

In a multivariable analysis that investigated the factors associated with increased odds of acceptance of the second batch of patches, assignment to NRT (OR: 2.83, 95%CI: 1.76-4.55 p<0.001) and finishing full time education beyond the age of 16 years (OR: 1.65, 95%CI: 1.02-2.68, p=0.043) were associated with higher adherence to treatment. Baseline cotinine level was inversely associated with acceptance of second batch of patches (OR: 0.96, 95%CI: 0.93-0.99, p=0.028).

Table 16 Univariable and multivariable associations with adherence at one month

Variable		N	Crude	95% CI		P- value	Adjusted	95% CI		LRT
			β	Lower	Upper		β	Lower	Upper	P- value
Age (years)		957	0.07	-0.02	0.16	0.144	-	-	-	-
Ethnicity										
	Other	27	-1.65	-5.00	1.70	0.333	-	-	-	-
	British - white	930	ref	-	-		-	-	-	
Age full time education finished										
	≤16	751	ref	-	-	0.044	-	-	-	-
	>16	206	1.38	0.03	2.73		-	-	-	
Heaviness of smoking index (HSI)										
	0-3	631	ref	-	-	0.033	-	-	-	-
	4-6	326	-1.27	-2.44	-0.11		-	-	-	
Partners' smoking status										
	Non-smoking	225	ref	-	-	0.627	-	-	-	-
	Smoking	654	0.25	-1.08	1.57		-	-	-	
	Not applicable	78	1.11	-1.15	3.36		-	-	-	
Parity										
	≤1	649	ref	-	-	0.933	-	-	-	-
	2 or 3	256	-0.19	-1.46	1.08		-	-	-	
	≥4	52	-0.34	-2.81	2.14		-	-	-	
Baseline cotinine (ng x 10^{-1}/ml)										
	12.1 (7.8-17.6), median (IQR)	957	-0.08	-0.15	-0.01	0.021	-0.08	-0.15	-0.01	0.020
BMI										
	<18.5	28	1.43	-1.93	4.79	0.324	-	-	-	-
	18.5-24.9	362	ref	-	-		-	-	-	
	25-29.9	267	1.17	-0.22	2.55		-	-	-	
	>30	254	0.60	-0.80	2.00		-	-	-	
	Missing	46	2.23	-0.45	4.91		-	-	-	

Table 16 (continued)

Length of first behavioural support session									
16-30	143	ref	-	-		-	-	-	
31-45	791	-0.06	-1.61	1.50	0.585	-	-	-	-
>60	23	-1.95	-5.81	1.90		-	-	-	
Previous preterm births									
0	872	ref	-	-	0.608	-	-	-	-
≥1	85	0.51	-1.44	2.46		-	-	-	
Previous use of NRT									
No	914	ref	-	-	0.136	-	-	-	-
Yes	43	2.03	-0.64	4.71		-	-	-	
Gestational age (weeks)									
12-19	766	ref	-	-	0.572	-	-	-	-
20-24	191	-0.40	-1.78	0.99		-	-	-	
Treatment assignment									
Placebo	483	ref	-	-	<0.001	ref	-	-	<0.001
NRT	474	2.67	1.58	3.77		2.59	1.50	3.68	

Table 17 Univariable and multivariable associations with adherence at delivery

Variable		N	Crude	95% CI		P- value	Adjusted	95% CI		LRT
			β*	Lower	Upper		β*	Lower	Upper	P- value
Age (years)		957	0.01	-0.01	0.03	0.154	-	-	-	-
Ethnicity										
	Other	27	-0.41	-1.07	0.25	0.224	-	-	-	-
	British - white	930	ref	-	-		-	-	-	
Age full time education finished										
	≤16	751	ref	-	-	0.048	-	-	-	-
	>16	206	0.27	0.00	0.53		-	-	-	
Heaviness of smoking index (HSI)										
	0-3	631	ref	-	-	0.026	ref	-	-	<0.001
	4-6	326	-0.26	-0.49	-0.03		-0.27	-0.50	-0.05	
Partners' smoking status										
	Non-smoking	225	ref	-	-	0.811	-	-	-	-
	Smoking	654	0.08	-0.18	0.34		-	-	-	
	Not applicable	78	0.11	-0.34	0.55		-	-	-	
Parity										
	≤1	649	ref	-	-	0.654	-	-	-	-
	2 or 3	256	-0.08	-0.33	0.17		-	-	-	
	≥4	52	0.14	-0.34	0.63		-	-	-	
Baseline cotinine (ng x 10^{-1}/ml)										
	12.1 (7.8-17.6), median (IQR)	957	-0.01	-0.03	0.00	0.053	-	-	-	-
BMI										
	<18.5	28	0.10	-0.56	0.77	0.228	-	-	-	-
	18.5-24.9	362	ref	-	-		-	-	-	
	25-29.9	267	0.32	0.05	0.60		-	-	-	
	>30	254	0.10	-0.18	0.37		-	-	-	
	Missing	46	0.22	-0.31	0.75		-	-	-	

Table 17 (continued)

Length of first behavioural support session									
16-30	143	ref	-	-		-	-	-	
31-45	791	0.06	-0.25	0.36	0.471	-	-	-	-
>60	23	-0.38	-1.14	0.38		-	-	-	
Previous preterm births									
0	872	ref	-	-	0.496	-	-	-	-
≥1	85	0.13	-0.25	0.52		-	-	-	
Previous use of NRT									
No	914	ref	-	-	0.282	-	-	-	-
Yes	43	0.29	-0.24	0.82		-	-	-	
Gestational age (weeks)									
12-19	766	ref	-	-	0.963	-	-	-	-
20-24	191	0.01	-0.27	0.28		-	-	-	
Treatment assignment									
Placebo	483	ref	-	-	<0.001	ref	-	-	<0.001
NRT	474	0.50	0.29	0.72		0.51	0.29	0.72	

*β value and regression coefficients using square root transformed adherence

7.5.3. Investigation of confounders: reducing the bias of the adherence-cessation association estimate

At one month, a multivariable logistic regression model using adherence to trial patches for 0-28 days as a continuous variable found strong evidence to suggest that for each extra day trial patches were used, the odds of cessation at one month increased by 11% (Adjusted OR: 1.11, 95%CI: 1.08-1.13, p<0.001) (**Table 18**). For cessation at delivery, using adherence to trial patches for 0-56 days as a continuous variable, each extra day trial patches were used, the odds of cessation at one month increased by 6% (Adjusted OR: 1.06, 95%CI: 1.03-1.09, p<0.001) (**Table 19**). The associations at one month and delivery were both adjusted for potential confounders and variables known to be associated with adherence, which included: treatment assignment, HSI, maternal age, age at which full time education was finished, partners' smoking status, parity, baseline cotinine level, length of first behavioural support session, and gestational age. This approach attempted to reduce the bias of the estimated associations between adherence and cessation, but reverse causation may yet still explain the observed associations (**Table 18** and **Table 19**), and therefore associations may not be causal. The results of an analysis investigating this possibility are detailed in the following section.

Table 18 Multivariable associations with validated cessation at one month (n=957, 91.1%)

Variable	N	Adjusted	95% CI		LRT
		OR	Lower	Upper	P-value
Adherence 0-28 days	957	1.11	1.09	1.13	<0.001
Age full time education finished					
≤16	751	ref	-	-	0.044
>16	206	1.54	1.01	2.35	
Heaviness of smoking index (HSI)					
0-3	631	ref	-	-	0.001
4-6	326	0.75	0.63	0.88	
Baseline cotinine (ng x 10-1/ml)	957	0.94	0.91	0.97	<0.001
Treatment assignment					
Placebo	483	ref	-	-	0.004
NRT	474	1.77	1.20	2.61	

Table 19 Multivariable associations with validated cessation at Delivery (n=957, 91.1%)

Variable	N	Adjusted OR	95% Confidence Interval		LRT P-value
			Lower	Upper	
Adherence 0-56 days	957	1.06	1.03	1.09	<0.001
Heaviness of smoking index (HSI)					
0-3	631	ref	-	-	0.002
4-6	326	0.74	0.61	0.89	
Treatment assignment					
Placebo	483	ref	-	-	0.355
NRT	474	1.25	0.78	2.00	

7.5.4. Investigation of the reverse causation hypothesis as an explanation for the adherence-cessation association

167 women were abstinent from quit date until the one month follow-up. Within this restricted group, there was no statistically significant difference in the odds of cessation at delivery between women who adhered beyond the median number of patches used within the first 28 days compared to women who did not (OR: 0.63, 95%CI: 0.31-1.27, p=0.196).

When using the same restricted subgroup and acceptance of a second batch of patches as a proxy for subsequent adherence, there was also no evidence that acceptance was associated with greater odds of cessation (OR: 0.76, 95%CI: 0.34-1.67, p=0.494).

7.5.5. Investigation of the relationship between adherence to treatment, nature of treatment and cessation

The results from the interaction test between treatment assignment and adherence, within the restricted sample of women abstinent at one month, showed there was no significant increase in the odds of women achieving cessation at delivery amongst those who were more adherent to placebo patches (OR: 0.40, 95%CI: 0.14-1.16, p=0.093). There was also no significant difference in the odds of cessation at delivery in highly adherent NRT users (OR:

0.98, 95%CI: 0.44-2.18, p=0.964). A likelihood ratio test suggested that there was no evidence of an interaction between adherence to treatment and treatment assignment (LRT p=0.151).

7.6. Discussion

7.6.1. Main findings

Women who had been assigned to receive NRT were more likely to adhere to their treatment patches. HSI score and baseline cotinine level were also inversely associated with adherence to treatment. Similar factors, including treatment assignment and age full time maternal education was finished, were found to be associated with acceptance of a second batch of patches; baseline cotinine level was inversely associated with acceptance of a second batch of patches. After adjusting for possible confounding variables found to have an association with cessation, or those that have previously shown an association with adherence in pregnancy, there was still a strong relationship between adherence and cessation at one month and delivery. However, the association between adherence and cessation may be due to resumption of smoking causing reduced use of treatment patches. Finally, higher adherence to either placebo or NRT patches was not associated with significantly greater odds of cessation, indicating that where women are 'adherent types', there may be some characteristics intrinsic to them that simultaneously makes them both more adherent and also more likely to succeed in a cessation attempt.

7.6.2. Strengths and limitations

While several studies have investigated whether NRT may increase the odds of smoking cessation,[116-121] the analyses presented in this chapter are the first to suggest that adherence to NRT is associated with increased odds of cessation in pregnancy.

A strength of this study is that there is little evidence regarding adherence in pregnancy. This study contributes to the evidence base by showing which characteristics are associated with adherence, but also that adherence to the trial patches provides significantly improved odds of cessation irrespective of whether they contain nicotine; adherence in the NRT group did not lead to more cessation. A further strength was that the association between adherence and cessation was investigated at two time points: one month post-quit date and delivery, highlighting that adherence to each extra patch in early pregnancy had a greater effect than in late pregnancy. The analyses also provided evidence that the data was consistent with the possibility that women may lapse to smoking before reducing use of patches indicating that standard dose NRT may not be effective. Previous analyses of NRT in pregnancy have not analysed the number of pieces of NRT adhered to and thus may have underestimated standard dose NRT's effectiveness when used as directed.[146]

One limitation of this analysis is that patch use, used as a measure of adherence, was a self-reported measure. As a result, it is subject to recall bias and may have been over or under estimated. Another limitation is that some motivational factors, which have previously been found to predict treatment success,[97] were not collected in the SNAP trial. Therefore, as it was not possible to adjust for variables which had not been collected, residual confounding by non-collected motivational factors could have obscured the association between adherence and cessation. A further limitation of this study is the small number of women included in the restricted subgroup who were measured as completely abstinent between quit date and one month follow-up (n=167). This occurred because daily abstinence was not recorded in the SNAP trial and the self-reported number of days that trial patches were used, had to be used to define an abstinent subgroup. The findings in these analyses are predicated on the assumption that women did not smoke when they used patches. This is likely,

however, as it was impressed upon participants not to smoke and use patches at the same time in order to avoid unnecessary foetal exposure to nicotine. Furthermore, the associations in the interaction test are potentially underpowered; it is possible that adherence with NRT might have been associated with further increased odds of cessation in a larger abstinent subgroup. Still, the interaction test represents the first investigation of whether greater adherence to NRT is associated with increased odds of cessation in pregnancy, compared with greater adherence to placebo patches.

7.6.3. Findings in the context of previous work

There are many reasons for discontinuing the use of NRT in pregnancy. After beginning treatment, pregnant women may be more likely to stop adhering to NRT if they lapse, due to concern about avoiding unnecessary foetal exposure to nicotine.[126] Alternatively, they may be more motivated to keep their addiction under control and adhere to recommended NRT use.[123] Situational factors such as co-habiting with other smokers or being socially disadvantaged that may influence pregnant women's smoking and stop them from using smoking cessation medications. It is possible that individuals who feel that medication is not needed following a period of prolonged abstinence may be those with low levels of nicotine addiction and strong motivation to quit, e.g., women with support from non-smoking family members who therefore find it easier to stop smoking.[188] Additionally, pregnant women who experience adverse events, be they related or unrelated to NRT, could be more likely to discontinue treatment. Those who have low motivation to quit smoking due to the price of NRT (although it is approximate to that of smoking), taste, or running out of NRT, might also be less likely to adhere to a full course of treatment.[115]

Failure to adhere to medications is a primary cause of why some drug regimens are found to be efficacious but not effective.[123] It is well known that adherence

to all medications in pregnancy is typically low.[123] A recent systematic review conducted, which included randomised control trials and prospective studies, measured adherence to NRT prior to relapse and found that there was a lack of studies assessing the association between adherence to NRT and subsequent smoking cessation.[140] Studies that were eligible for inclusion in this systematic review of non-pregnant individuals had reported adherence to active NRT only and not to placebos, if indeed a placebo was used in the study design. Two secondary analyses from randomised RCT's,[141,142] two prospective observational studies[143,144] and one paper reporting original results[96] were identified. Studies that controlled for reverse causation, and were therefore valid, found that there was an association between adherence and cessation at 4-6 weeks follow-up.[140] However, the definitions of adherence were based on *post-hoc* criteria to analyse adherence, rather than any theoretical reason why individuals would adhere for a certain time or not, and so research into the minimum time a participant must adhere to NRT and why, must be undertaken. This may increase for more heavily dependent smokers or decrease for less heavily dependent smokers. Studies in pregnancy were excluded from this review and, there are no other studies have assessed the association between adherence to NRT and cessation in detail. As a result, the present study adds to our knowledge of adherence to NRT and cessation in pregnancy by providing evidence for the characteristics of women more likely to adhere, providing evidence for which factors might cause a reduction in adherence and therefore how it may be addressed, and evidence to show that longer adherence to NRT does increase odds of shorter term cessation i.e. one month post-quit date, but no more than adherence to placebo patches.

Findings indicate that women who had lower nicotine dependence, higher levels of education and received NRT patches had greater odds of adherence. The findings presented in this chapter are similar those of Fish *et al.,*[146] in that

increased adherence was associated with significantly increased odds of cessation. However, findings from analyses presented in this chapter go further and show that increased adherence confers greater odds of cessation earlier in pregnancy. The relationship was statistically greater at one month compared to delivery, which might partly account for the higher rate of cessation seen at one month follow-up in the original SNAP trial report.[116] This analysis provides evidence for an effect, as after adjustment for confounding factors, the relationship between adherence and cessation was still present. The strength of this effect probably does not reflect the true causal association of each extra day's worth of patch use because it may reflect adherence's association with lower abstinence after a lapse to smoking or residual confounding by unmeasured factors.

The data are compatible with the hypothesis that reverse causation may explain an association between adherence and cessation. Since there was no relationship between adherence and cessation soon after the quit date when women were still abstinent, women lapsing to smoking and stopping their nicotine patches cannot be ruled out as a cause for the association between adherence and cessation. This may be because of the relatively small sample of women included that were completely abstinent from quit date to one month (n=167). The reverse causal hypothesis might be able to be refuted with a larger sample size through inclusion of data from other trials.

While higher use of NRT may give women significantly greater odds of achieving cessation than lower adherence to NRT, women did not experience significantly higher odds of cessation than highly adherent placebo users, who also had greater odds of cessation than a placebo group that used fewer patches. This indicates that women who have the characteristics of someone likely to comply with the treatment regimen, 'adherent types', are more likely to achieve

cessation. The data suggests that greater amounts of nicotine derived by highly adherent standard dose NRT users, does not provide a significant benefit with respect to odds of cessation at one month post-quit date.

Collectively, these results raise questions about why, in the original SNAP trial report, the efficacy of NRT at one month post-quit date was significantly different from that seen at delivery. A possible explanation of this may be that the increased rate of nicotine and cotinine clearance that has been observed in pregnancy[124] may affect the efficacy of standard dose NRT and that this does not completely suppress the craving and withdrawal symptoms associated with quitting during pregnancy. Pregnant smokers are unable to titrate a fixed amount of nicotine derived from NRT patches. Alternatively, women may have concerns about the use of NRT during pregnancy and adverse effects on the child, however women's concerns about NRT use were not recorded in the SNAP trial and so this was unable to be investigated.

7.6.4. Conclusions

Adherence to treatment was found to be a characteristic that increased the odds of cessation in women trying to quit smoking. However, the evidence from this study suggests that being highly adherent to standard dose nicotine patches does not confer greater odds of cessation than high adherence to placebo patches. It may be that standard dose NRT is not adequate for fully alleviating symptoms of nicotine withdrawal in women who are more highly addicted to nicotine or by late pregnancy. In addition, since reverse causality could not be refuted in this analysis the evidence suggests that resumption of smoking may cause reduced patch use; efforts should be made to encourage adherence to NRT patches in pregnancy. Additional support sessions may prove useful to women who are more nicotine dependent. An alternative explanation for this result that was not investigated is that women have negative attitudes to NRT

use in pregnancy and concern for the foetus causes them to stop using NRT. This was not able to be investigated in the SNAP trial as such data were not collected. However, another explanation is that an increasing rate of nicotine metabolism in pregnancy causes reduced adherence to NRT; this hypothesis is investigated in the following chapter.

Chapter 8

The nicotine metabolite ratio in pregnancy measured by the *trans*-3'-Hydroxycotinine to Cotinine ratio: characteristics and relationship with smoking cessation

8.1.Introduction

Tobacco dependence is caused by nicotine, which is the addictive substance derived from smoking, and which regulates smoking behaviour.[54] The physical process of nicotine addiction occurs as follows: following sustained exposure, neuroadaptation occurs, in which tolerance to the effect of nicotine exposure develops. At normal smoking levels, nicotinic receptors are saturated and become desensitised, leading to more receptors being expressed in the brain.[60] An increase in smoking often follows as smokers become addicted to nicotine and this is hypothesised to be as a result of the smoker attempting to reduce withdrawal symptoms experienced. In the previous chapter, it was found that greater adherence to trial patches was associated with increased odds of cessation, but that odds of cessation were not greater in women assigned NRT compared to placebo. A possible explanation for this may be that the dose of NRT used was rendered ineffective due to an increased rate of nicotine metabolism in pregnancy. Hence, no greater effect on smoking cessation was seen in women who adhered to nicotine patches compared to those who adhered similarly to placebo patches. Understanding the role of nicotine metabolism in addressing nicotine dependence is hypothesised to provide insight into successfully reducing the amount smoked during pregnancy, or increasing the odds of cessation and thereby reducing exposure to the harmful constituents of tobacco smoke. This chapter investigates the importance of the rate of nicotine metabolism in pregnancy.

As described in **Chapter 1**, the primary metaboliser of nicotine has been identified as the cytochrome P450 enzyme CYP2A6, metabolising between 70-80% of the freely available nicotine to the non-psychoactive compound of cotinine and then to *trans*-3'hydroxycotinine (3HC).[52,152] During smoking, nicotine occupies the majority of enzyme sites with metabolism occurring at its maximal rate, and as a result, does not change in proportion to drug

concentration (zero order kinetics); a fixed amount of nicotine is metabolised over time.[153,238] Furthermore, binding of nicotine to plasma proteins is low, at <5%,[52] so the rate of nicotine metabolism is likely to be unaffected by free nicotine binding to these proteins in the blood. Changes in metabolic enzymes may occur as gestation progresses, but there is no evidence of induced expression of more of the major metabolising enzyme (CYP2A6) in extra-hepatic tissues by xenobiotic compounds in humans.[239] As nicotine is metabolised very quickly, the more direct measure of cotinine: nicotine ratio depends upon recent smoking. The 3HC: cotinine ratio (NMR - Nicotine Metabolite Ratio) is much more stable because the half-life of both compounds is much longer than nicotine's,[52,176] and is therefore little affected by recent smoking. The NMR is also a good phenotypic measure that accounts for both individual and environmental causes of smoking.[166] The NMR, therefore, provides an indirect measure of the speed of metabolism of nicotine because it reflects the efficiency of the system responsible for 70-80% of nicotine metabolism.[52,152] This chapter aims to provide descriptive data for the NMR in a population of pregnant smokers.

Tobacco smokers often titrate the amount they smoke to the level of nicotine they require from smoking.[240] Smoking behaviour is driven by the addictive properties of nicotine in cigarettes and nicotine is, in part, responsible for continued smoking. Furthermore, there is evidence of heritability of smoking persistence and rates of cessation.[241] Understanding the rate at which nicotine metabolism occurs and how much nicotine is freely available informs how acutely the individual will experience craving and withdrawal symptoms. The overall rate of nicotine metabolism is a key mediator in nicotine addiction. Those women who metabolise nicotine more quickly would be expected to smoke more cigarettes per day on average, and there is limited evidence of this in non-pregnant smokers.[155,166] Interindividual differences have been investigated in the

non-pregnant population,[166] and a weak association with the number of cigarettes smoked was found.[166] Faster metabolisers remove nicotine more quickly and therefore will experience nicotine withdrawal more rapidly after smoking than slower metabolisers; they may in effect receive a lower dose of nicotine. They might, therefore, be expected to smoke more often and, perhaps also to draw more deeply on their cigarettes to restore their nicotine concentration and ameliorate their withdrawal; the resulting increase in nicotine intake could lead smokers to develop more severe dependence, and this is strongly associated with a reduced ability to achieve abstinence when individuals try to quit smoking.[52,54] This chapter examines whether faster nicotine metabolism is associated with a reduced chance of cessation in pregnant women.

CYP2A6 increases its activity and metabolises nicotine to cotinine and then to 3HC more quickly during the second trimester of pregnancy.[124] The main SNAP trial reported evidence that NRT improved the rate of cessation four weeks into pregnancy,[116] but there was no evidence that NRT was effective for cessation by delivery. This finding was consistent with a prior meta-analysis[112] and a recent RCT of NRT conducted in pregnancy.[220] Since most women recruited in the SNAP trial attempted to stop smoking in the second trimester of pregnancy when the nicotine in the patch may have been more rapidly metabolised than would have been the case prior to pregnancy,[124,172,174,175] this could have undermined the effectiveness of NRT by providing an inadequate dose of nicotine. Findings confirming this would suggest that higher dose NRT may be needed to support cessation in pregnant women.

This chapter will use data from the SNAP trial to describe the characteristics of the NMR in pregnant smokers, investigate whether the NMR was associated with

increased odds of cessation and if increased rate of nicotine metabolism, measured by the NMR, was associated with decreased effectiveness of NRT.

8.2. Aim of the study

The aim of this study is to describe the NMR within a cohort of pregnant women who enrolled in the SNAP trial and to investigate relationships between nicotine metabolism and smoking cessation.

8.3. Objectives

The aim of the study was investigated through these stated objectives:

I. To describe the NMR within the pregnant population and provide normative data.

II. To investigate the hypothesised inverse relationship between NMR and odds of cessation at one month post-quit date and at delivery.

III. To evaluate whether increases in NMR reduce the effectiveness of NRT in pregnancy.

8.4. Methods

8.4.1. Baseline variables

Relevant baseline data for the investigation of NMR in pregnancy collected in the SNAP trial included: date of birth, ethnicity, age on completion of full time education, heaviness of smoking index (HSI),[242] partners' smoking status, parity, previous use of NRT, gestational age at baseline, trial recruitment site and participants' treatment assignment. In addition, blood samples were taken for cotinine estimation, and CO readings were taken to validate cessation in women who claimed abstinence. The NMR was derived from these blood samples. A full list of variables collected is described in **Appendix A**.

8.4.1.1. Derivation of the NMR

Analysis was carried out by ABS Laboratories Ltd, Welwyn Garden City, Hertfordshire, UK. The NMR was derived from whole blood samples that were taken at baseline. Blood samples were frozen at -20^{O}C on the day of collection in BD Vacutainer Gold top tubes. Samples were later transported from sites in insulated containers and further storage of the haemolysed samples was carried out at -80^{O}C until they underwent cotinine analysis. The stability of the cotinine levels was assured by the laboratory, and they assayed approximately 10% of samples twice to demonstrate robustness of the method. Liquid chromatography tandem mass spectrometry was used to derive sample measurements, which has been validated as the best measure to assess active or passive smoking.[243] The following plasma assays were recorded from the blood samples: free cotinine (ng/ml), free 3HC (ng/ml), total cotinine (ng/ml) and total 3HC (ng/ml). From this, free and total 3HC: Cotinine ratios were calculated.[167] Pregnant women who smoke more cigarettes have higher concentrations of 'free' and 'total' cotinine. The affinity of nicotine to nicotinic acetylcholine receptors is higher in smokers than non-smokers,[52] but since all those included in the SNAP trial were verified as smokers before inclusion, nicotine receptor affinity was not assumed to differ greatly between individuals and affect the NMR. A higher NMR (3HC divided by total cotinine) indicates faster nicotine metabolism, with more of the parent metabolite, cotinine, having being metabolised into the downstream metabolite, 3HC. The present analysis used a NMR derived from total cotinine and total 3HC as the analysis by ABS Laboratories indicated no significant differences between the 'free' and 'total' values when glucuronides were hydrolysed, i.e. free and total cotinine values were the same.[244] When investigated, the values were found to be highly correlated (r^2=0.865). This concurs with recent evidence that suggests variation in the glucuronidation of 3HC does not affect the NMR.[156] As a result, total cotinine was used in the analyses presented, as opposed to free cotinine, as this included glucuronide conjugated cotinine.

8.4.2. Outcome data: validated cessation and adherence with nicotine replacement therapy (NRT)

Cessation was defined as continuous abstinence from quit date to the respective follow-up point with up to five individual cigarettes allowed. At one month, a CO reading of <8ppm was used for validation, and at delivery, either a CO reading or a saliva cotinine sample of <10ng/ml, or both, was used for validation. Women who were lost to follow-up and had a missing value for whether they achieved cessation were defined as continuing smokers. These definitions were taken in accordance with the Russell standard criteria.[245]

8.4.3. Analysis strategy

8.4.3.1. Participant characteristics and normative NMR Data

To ascertain whether women who provided NMR data differed in any key characteristics to those who did not provide any NMR data, a sensitivity analysis was conducted comparing the baseline characteristics between these two groups. Following this, the distribution and characteristics of the NMR were studied to provide normative data on NMR in pregnancy.

8.4.3.2. Associations between baseline variables and the NMR

Using univariable linear or logistic regression, associations between selected baseline characteristics (i.e. baseline cotinine level, HSI, gestational age, maternal age and ethnicity) and NMR were examined. Since NMR was approximately normally distributed, it was included as a continuous outcome variable in this analysis.

8.4.3.3. Associations between the NMR and smoking cessation

The association between NMR and validated smoking cessation at one month and delivery was investigated using logistic regression. This association was

adjusted for the *a-priori* confounding factors. Treatment assignment and HSI were adjusted for where appropriate in the multivariable models.

Treatment assignment to either NRT or placebo patches was adjusted for because the initial SNAP trial investigation found an association between NRT and cessation at one month post-quit date,[116] and the data used in this study is from this trial. Individual HSI score was adjusted for because there are some studies that show weak associations between NMR and the number of cigarettes smoked, and some that show none.[166] When examining the association between NMR and cessation, markers of dependence were adjusted for (HSI and the number of cigarettes). This was done to permit comparison with and discussion of other studies.

8.4.3.4. Interaction between treatment assignment and NMR with respect to cessation

To investigate whether or not the relationship between nicotine metabolism and cessation was modified by using NRT, an interaction was fitted between treatment assignment and NMR in a logistic regression for cessation at one month. This test investigated whether the relationship between NMR and cessation was the same in both NRT and placebo groups. NMR was included as a continuous variable to maximise the power of the interaction test between NMR and treatment allocation.

All analyses were conducted using Stata 13.1 (College Station, TX).

8.5. Results

There were 1,050 participants that were originally enrolled in the SNAP trial.[116] NMR was measured from baseline blood samples taken in 673 women (64.1%); nine individuals were excluded due to blood samples not being received at either

Ninwells hospital, Dundee (n=6), or ABS Laboratories (n=3). Two participants were excluded due to previous use of NRT in the seven days before outcomes were measured, which could have affected cotinine levels. All analyses were undertaken in the remaining 662 (63.0%) participants. This sample of 662 women consisted of those who provided blood samples and received either placebo or NRT patches.

8.5.1. Normative NMR data

A sensitivity analysis listing the baseline characteristics of participants included and excluded from the analysis is detailed in **Table 20**. Women who provided samples for cotinine analysis and those who did not were similar in all baseline characteristics compared, apart from being less likely to have a partner that smoked and being less likely to be socially disadvantaged.

Table 20 Baseline characteristics of women included in the study sample (n=662)

Characteristics	Included (662)	Excluded (388)	p-value
Maternal age (years) [mean (SD)]	26.7 (6.2)	25.6 (6.0)	0.999
Ethnicity			
White British	644 (97.3%)	374 (96.4%)	0.418
Other background	18 (2.7%)	14 (3.6%)	
Age full time education finished (years) [mean (SD)]	16.3 (1.46)	16.2 (1.69)	0.913
Heaviness of smoking index			
0-3	439 (66.3%)	245 (63.1%)	0.298
4-6	223 (33.7%)	143 (36.9%)	
Gestational age at baseline [mean (SD)]	16.3 (3.5)	16.1 (3.5)	0.829
Partners smoking status			
N/A	49 (7.4%)	38 (9.8%)	0.006
Non-smoking	176 (26.6%)	71 (18.3%)	
Smoking	437 (66.0%)	279 (71.9%)	
Parity			
0-1	455 (68.7%)	264 (68.0%)	0.962
2-3	170 (25.7%)	101 (26.0%)	
>4	37 (5.6%)	23 (5.9%)	
Baseline cotinine level (ng/ml) [median (IQR)]	136.2 (80.7)	133.9 (79.4)	0.660
Treatment assignment			
NRT	341 (51.5%)	200 (51.5%)	0.339
Placebo	321 (48.5%)	188 (48.5%)	
Index of multiple deprivation [mean (SD)]	31.6 (17.1)	34.5 (16.1)	0.004

The distribution of the NMR ranged from 0 to 0.9 and was almost normally distributed, being only weakly positively skewed; mean: 0.44 (SD: 0.16). A histogram illustrating NMR distribution is depicted in **Figure 16**.

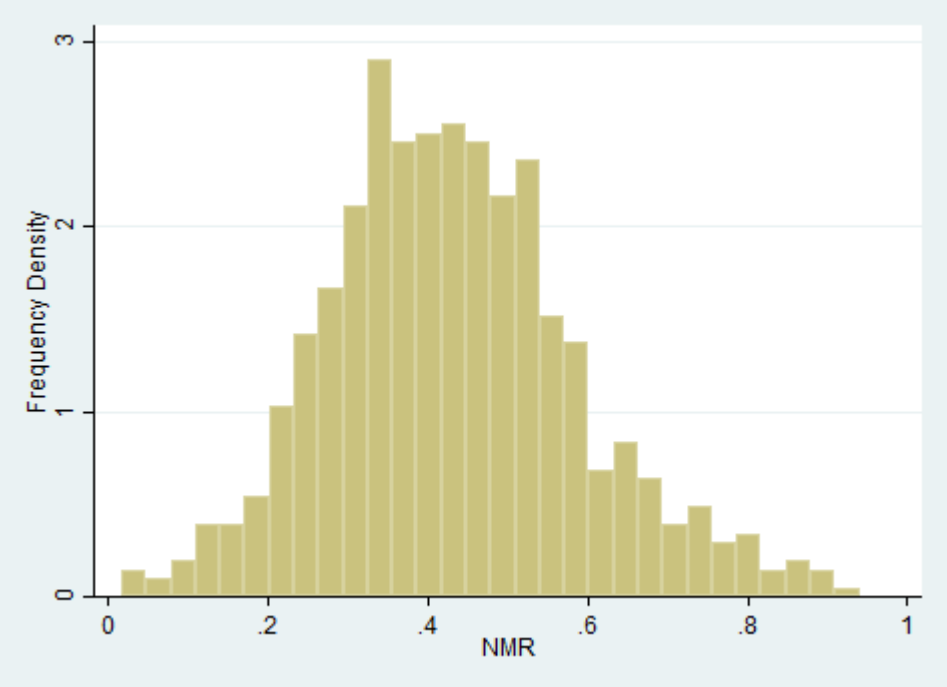


Figure 16 Histogram of NMR from total cotinine.

8.5.2. Associations between baseline variables and NMR

Regression analysis was used to predict associations using univariable linear tests between selected baseline variables and NMR, and the results are detailed in **Table 21**. There was a significant association between NMR and each additional cigarette smoked prior to pregnancy (β: 0.027, 95%CI: 0.008-0.047, p=0.006). No other variables measured at baseline that were hypothesised to influence NMR showed significant associations.

Table 21 Univariable associations for regression on 0.1 unit increase in NMR (n=662)

			95% CI		
Variable	N	β	Lower	Upper	p-value
Maternal age (years)	662	-0.013	-0.032	0.006	0.190
Ethnicity					
Other	18	-0.340	-1.082	0.402	0.369
British - white	644	ref	-	-	
Cigarettes smoked prior to pregnancy	662	0.027	0.008	0.046	0.006
Heaviness of smoking index					
0-3	439	ref	-	-	0.560
4-6	223	0.076	-0.180	0.332	
Gestational age (weeks) at baseline	662	0.026	-0.009	0.060	0.143

A multivariable analysis was not carried out for these associations as only one variable remained significant, and none of the non-significant variables approached even a weak association.

8.5.3. Associations between NMR and smoking cessation

The univariable associations between NMR and validated cessation at both one month and delivery were strong. At one month, there was a 14% decrease in the odds of achieving validated cessation for every 0.1 unit increase in NMR (OR: 0.86, 95%CI: 0.76-0.98, p=0.026). When adjusted for treatment assignment and HSI the association changed minimally (OR: 0.87, 95%CI: 0.76-0.99, p=0.043). Adjusting for the number of cigarettes smoked resulted in a non-significant association between NMR and cessation (OR: 0.88, 95%CI: 0.77-1.00, p=0.057), which was expected since it is on the causal pathway between NMR and cessation.

At delivery, there was a 22% decrease in the odds of achieving validated cessation for every 0.1 unit increase in NMR (OR: 0.78, 95%CI: 0.65-0.93, p=0.005) and after adjustment for treatment assignment and HSI there remained a strong and significant negative association (OR: 0.79, 95%CI: 0.66-0.94, p=0.008). When adjusting for the number of cigarettes smoked, the odds

of cessation did not change significantly (OR: 0.79, 95%CI: 0.66-0.95, p=0.010).

8.5.4. Interaction between treatment assignment and NMR with respect to cessation

An interaction was fitted between NMR and treatment assignment on the odds of cessation at one month post-quit date, adjusted for HSI. There was no evidence for an interaction between NMR and treatment assignment on the odds of cessation at one month post-quit date (LRT p=0.556); the chance of successful cessation with NRT does not vary with NMR. If increasing NMR does undermine the odds of cessation in pregnant women between 16 and 28 weeks' gestation, then the effect is very slight. An interaction test was not carried out for longer term cessation at delivery, as this was likely to lack power due to the small number of women who maintained cessation until delivery that also provided NMR data (n=62).

Graphical results of the interaction test produced for cessation at one month are depicted in **Figure 17**. **Figure 17** illustrates that the probability of cessation in women receiving NRT was non-significantly higher than those who received placebo. Increasing NMR barely changed the probability of cessation in NRT users.

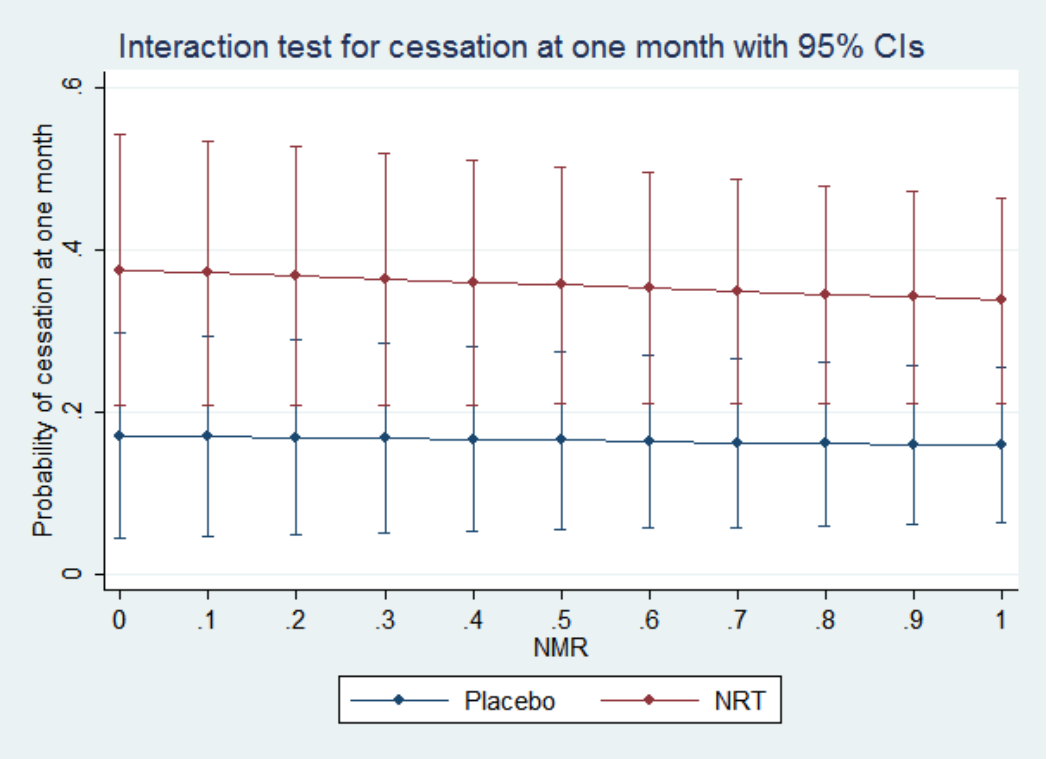


Figure 17 Interaction between NMR and treatment assignment for cessation at one month.

8.6. Discussion

8.6.1. Main findings

The distribution of NMR in pregnant women between 12 and 24 weeks gestation was found to be approximately normally distributed, with a mean of 0.44 (SD: 0.16). Women who metabolised nicotine faster were less likely to quit smoking and were found to have smoked more cigarettes prior to pregnancy. There was no evidence that NMR modified the association between assignment to NRT and increased odds of cessation earlier in pregnancy.

8.6.2. Strengths and limitations

There were three distinct advantages associated with the use of data from the SNAP trial in this investigation. Firstly, that the NMR was calculated using metabolites derived from blood samples, which measured the more stable 3HC and cotinine metabolites,[52,172] as opposed to a nicotine ratio based on nicotine and cotinine used in hair cotinine analysis, which is better suited to analysing

long term changes in nicotine metabolism.[174,175] Secondly, NMR from blood samples were collected within a restricted period of time at baseline, which limited the possibility that changes in the NMR were due to variation in gestational age.[124,174] NMR was collected for women between 12 and 24 weeks gestation, but the majority of women included were at approximately 16 weeks gestation. Thirdly, robust methods were used to collect data within the SNAP trial, which included high rates of outcome ascertainment and validation of smoking status.[224]

A limitation of this study was that women who gave samples for analysis were less likely to have partners who smoke and were more advantaged than women who did not give samples (**Table 20**). However, as these characteristics were unrelated to rate of metabolism (NMR), it is unlikely that there would be a different association between NMR and cessation in the women who did not give samples. It has been shown that women's rates of nicotine metabolism increase as pregnancy progresses.[124] However, while the interaction result might change with a significantly faster NMR, it is unlikely that gestational increases will alter the observed association between faster nicotine metabolism in pregnancy and lower rates of cessation. Likewise, this would not affect NMR's associations with baseline maternal factors or the direction of association with odds of cessation. As a result, the main findings presented are likely to be generalisable throughout gestation.

In this trial, to avoid possible toxicity to the foetus, women were asked not to continue using the patch if their cessation attempt failed. As the majority of quit attempts failed in the first month, only 7% were still using the patch by and after one month.[116] However, this may not have harmed the ability to detect an interaction between speed of metabolism and NRT as the outcome of this was

successful cessation. A possible limitation of the interaction test is that there may not have been enough power to detect a weak interaction.

8.6.3. Findings in the context of previous work

There have been few other studies investigating nicotine metabolism using NMR in pregnant smokers. Those that have done so examined the relationship between nicotine metabolism and progress of gestation. They used segmental hair analysis and found that nicotine and cotinine metabolism increases in the second and third trimesters.[174,175] No studies have investigated the relationship between nicotine metabolism and cessation in pregnancy; this study extends previous findings.

The results obtained from the analysis of NMR showed that it was similarly distributed in pregnant women between the gestations of 12-24 weeks compared to the non-pregnant population.[172] The mean NMR of the normal metaboliser genotypes in two separate studies was found to be 0.42[161] and 0.43 respectively[246] and the overall mean of all participants in studies of non-pregnant women ranged between 0.38 and 0.44.[169,170,172] The NMR was found to be weakly associated with the number of cigarettes in non-pregnant smokers[166] and, similarly, there was a strong relationship with the number of cigarettes smoked prior to pregnancy in this study as well. Also, there was also no association with HSI, a questionnaire measure of dependence, as studies in non-pregnancy have also found.[166]

The odds ratio for association between NMR and cessation remained significant, and only changed minimally from the unadjusted estimates when adjusted for HSI. In contrast, the statistical significance of the association between NMR and validated cessation became non-significant when the number of cigarettes smoked per day was adjusted for. This was anticipated as the number of

cigarettes smoked per day is likely to be a mediator of the relationship between the rate of nicotine metabolism and smoking cessation; more rapid metabolism is likely to lead to greater cigarette consumption and hence to decreased odds of cessation. Adjusting for this factor, therefore, is likely to result in over-adjustment bias and findings adjusted for the number of cigarette smoked are presented for illustration only.

A systematic review of the NMR in the non-pregnant population found a similar relationship between nicotine metabolism and cessation. There was conflicting evidence for the impact of fast or slow nicotine metabolism on cessation being modified by use of NRT in non-pregnant smokers who were trying to stop smoking.[166] One study that demonstrated such moderation reported better cessation outcomes in slower metabolisers who used 21mg/24hr transdermal patches.[169] However, this significant interaction was only demonstrated with a higher therapeutic dose of nicotine than that received by pregnant smokers who were included in the present analyses. Another study that investigated cotinine levels during and after pregnancy found that the ratio of cotinine per cigarette smoked postnatally was significantly higher than that during pregnancy.[247] This suggests that the amount of nicotine obtained by non-pregnant individuals is higher than that of pregnant smokers per cigarette, perhaps due to slower metabolism; a similar number of cigarettes, or NRT's, may not provide equivalent levels of nicotine in both these states. NMR may increase later in pregnancy and change the relationship between NMR and cessation, as well as that of the interaction investigated in this study. The exact mechanism by which increases in nicotine metabolism later in pregnancy occur is not currently known and changes to the NMR later in pregnancy should be investigated. As NRT is of proven efficacy outside of pregnancy[102] and, in this sample, was shown to be effective for cessation at one month after randomisation,[116] it was hypothesised that, at one month, the rate of nicotine metabolism would modify the

relationship between NRT and cessation. We found no evidence of such an interaction between standard dose NRT, NMR and cessation at one month post-quit, and so, there is no indication that faster metabolism reduces the effectiveness of NRT in earlier pregnancy. By providing pregnant women that have slow nicotine metabolism with the same strength nicotine patches as those women that had faster nicotine metabolism, women who metabolised nicotine more slowly effectively received a higher dose. However, these analyses found no significant improvement for the effectiveness of NRT in women who metabolised nicotine more slowly either. These results support those found by Berlin and colleagues[220] and provide no evidence to suggest that the effectiveness of nicotine replacement therapy varies with women's rates of nicotine metabolism. More research using higher therapeutic doses of nicotine are required to investigate this hypothesis further.

8.6.4. Conclusions

In a smoking cessation trial investigating the effectiveness of nicotine patches in pregnancy, women with faster nicotine metabolism were less likely to achieve cessation even whilst using 15mg/16hr NRT patches. There was no evidence to suggest the rate of nicotine metabolism modifies the efficacy of NRT used in pregnancy. It is possible that nicotine metabolism increases as pregnancy progresses and beyond the gestations studied here, even faster rates of nicotine metabolism occur. Consequently, further research aimed at developing an understanding of the influence of nicotine metabolism on women's smoking throughout pregnancy, is required. It is possible that increased nicotine metabolism renders trialled doses of NRT ineffective and research or trials using higher doses of NRT, may be necessary to blunt the effect of accelerated nicotine metabolism later in pregnancy and to fully test whether or not NRT is efficacious in pregnancy.

Chapter 9

Conclusions and recommendations

9.1 Introduction

The overall aim of the thesis was to explain the findings of the original SNAP trial report, to investigate the use and safety of NRT and to raise hypotheses about ways in which NRT might be used more effectively for smoking cessation in pregnancy. This chapter summarises results relevant to each of the objectives put forth in **Chapter 2**. This chapter also discusses how these results have been disseminated, potential impact upon policy and practice, and suggests further avenues of research relating to the use of NRT for smoking cessation in pregnancy that may be undertaken. A full list of courses attended, training, and publications produced from and during the writing of this thesis are detailed in **Appendix B**. Re-prints of published papers from this thesis are included in **Appendix C**.

9.2 Summary of thesis findings

9.2.1 Objective I: To investigate the associations between baseline maternal characteristics and validated cessation at one month after randomisation and at delivery

Using a cohort of participants from the SNAP trial, independent associations between baseline maternal characteristics and smoking cessation at both one month post-quit date and at delivery were investigated. Women that were more nicotine dependent and more socially disadvantaged, i.e. women who smoked more, had reduced time until their first cigarette was smoked and left education earlier were less likely to achieve smoking cessation. The findings of this study were shared through publication in *Nicotine & Tobacco Research,*[248] and presented at a the 2012 Society for Research on Nicotine and Tobacco (SRNT) in Helsinki, and at a "Tackling smoking in the 21st Century" conference in York.

Prior to this study, the majority of investigations into predictors of cessation had been restricted to the non-pregnant population[95] and those in pregnancy used

survey [99] or self-reported cessation data.[97] There was little evidence on which factors predict cessation when pregnant women are using NRT for cessation and this study investigated this question using prospective trial data with biochemically verified measures of cessation. We now know that women with lower levels of education (i.e. more socially disadvantaged) and had higher pre-treatment cotinine concentrations (i.e. more nicotine dependent) have reduced odds of achieving cessation. The results suggest these women may require different kinds of smoking cessation support than others.

9.2.2 Objective II: To investigate whether or not the relationship between social disadvantage and smoking cessation in pregnancy is mediated by more proximal influences on behaviour

In a cohort of pregnant women from the SNAP trial, this study investigated whether or not nicotine dependence or smoking by pregnant women's partners were factors that mediated the association between social disadvantage and smoking cessation at one month post-quit date. A significant proportion of the association between social disadvantage and maternal cessation was explained by individual's nicotine dependence. However, smoking by the partners of pregnant women did not mediate this association. Findings from this study were presented at a UK Society for Behavioural Medicine (UKSBM) conference, and a paper is due to be submitted shortly.

Previously, no studies had attempted to explain the nature of the relationship between social disadvantage and smoking cessation in pregnancy. Studies outside of pregnancy have shown that social gradient is associated with smoking cessation[149] and that the association between SES and cessation is mediated by social support, neighbourhood disadvantage, negative affect/stress and agency (self-efficacy).[207] This study suggests that by targeting nicotine dependence the

hindrance to achieving smoking cessation which is attributable to social disadvantage could be reduced.

9.2.3 Objective III: To investigate any associations between adherence with NRT and infants' birth outcomes

This study aimed to investigate whether greater reported use of NRT transdermal patches by pregnant smokers was associated with better birth outcomes, specifically birth weight and being born small for gestational age (SGA). An exploratory analysis was also performed, investigating associations between use of NRT and any other birth outcomes measured in the SNAP trial. Within women who delivered singleton births, there was no association between use of NRT reported at one month into the SNAP trial and either birth weight or SGA. Similarly, NRT use reported at delivery was not associated with birth weight or SGA. Greater use of NRT was not associated with any other exploratory birth outcomes investigated in the SNAP trial.

Currently, the only evidence on the effect of greater NRT use on birth outcomes has been from cohort studies which only measured use or non-use of NRT rather than the amount used. Furthermore, it is difficult to clearly characterise smoking status from these studies. These studies have found no association between NRT use and increased birth weight compared to birth weight from mothers that continued smoking, irrespective of the type of NRT used. This literature supports the findings in this study chapter. This study is the first prospective investigation, and the first that used reported use of NRT as a unit of measurement to investigate whether greater use of NRT was associated with either birth weight or SGA. It therefore provides the most robust evidence that there is no association between NRT use and birth weight or being born SGA.

9.2.4 Objective IV: To investigate relationships between adherence with NRT and validated smoking cessation at one month after randomisation and delivery, including an assessment of whether or not 'reverse causality' might explain these

This study investigated the nature of the relationship between adherence to NRT and smoking cessation. Firstly, this was done through investigating the characteristics of women that have higher adherence, compared with lower adherence. Secondly, by investigating the relationship between adherence and achieving cessation, and whether this association was a function of NRT use or not. Other reasons for the association between adherence and cessation were also investigated including confounding factors, reverse causation and whether adherence was driven by individual characteristics rather than by the nicotine gained from patches. Multivariable analyses found that only the inverse association between baseline cotinine concentration and adherence, and the positive association between assignment to receive NRT and adherence, remained significant. Furthermore, treatment assignment to NRT was associated with cessation, and HSI was inversely associated with cessation. After adjusting for confounders, shorter and longer term adherence to NRT were both associated with increased odds of cessation. However, women who were failing to stop smoking, and therefore not using NRT, could not be discounted as a cause of these associations. Finally, within a restricted sample of women who were abstinent for one month, there was no significant difference in the odds cessation between highly adherent NRT or placebo patch users, indicating that adherence to patches may be more attributable to individual characteristics rather than the nicotine provided by standard dose NRT patches.

Prior to this analysis, no studies had investigated the degree to which the effectiveness of NRT in pregnancy might be affected by adherence. In the general population, adherence with NRT has been found to promote smoking

cessation, including after having controlled for reverse causation.[139-142] This thesis provides the first data to demonstrate that adherence to NRT in pregnancy is associated with increased odds of cessation, though the possibility of reverse causation could not be ruled out and adherence to NRT resulted in no greater odds of cessation than adherence to placebo. The observed adherence-cessation association therefore, might be due to women that failed to stop smoking, not using NRT, or the characteristics of women who adhere; women that follow instructions to use NRT might also be more likely to follow instructions to stop smoking.

9.2.5 Objective V: To describe rates of nicotine metabolism amongst participants and to investigate associations between nicotine metabolism, maternal characteristics and smoking cessation outcomes

In a cohort of women who provided blood samples for plasma estimation of cotinine levels, this study described the Nicotine Metabolite Ratio (NMR), a measure of nicotine metabolism, and its relationship with smoking cessation in pregnancy. The range and distribution of the NMR was similar in pregnant women to that of non-pregnant individuals. Women with faster nicotine metabolism smoked more cigarettes and women with faster nicotine metabolism were less likely to achieve cessation at either one month post-quit date or at delivery. However, there was no evidence that NRT was more effective in women who metabolised nicotine more slowly. The findings of this study were disseminated through publication in *Nicotine & Tobacco Research,*[249] and presented at both a regional Society for Academic Primary Care (SAPC) meeting in Lincoln, and national SAPC meeting in Edinburgh.

Previously, NMR's association with cessation had not been investigated in pregnancy. Studies of NMR in pregnancy have used segmental hair analysis to

analyse if NMR changes with gestation in the second and third trimesters,[174,175] or have been small.[172] The distribution of NMR at 12-24 weeks gestation was similar to that in non-pregnancy. We now know that the NMR is inversely associated with cessation in pregnancy. This was also the first study to investigate whether slower nicotine metabolisers have greater odds of cessation than faster metabolisers when using NRT; it found they did not, but associations may not have been detected due to a lack of power.

9.3 Personal development and development of research skills

Whilst conducting this thesis I was able to enhance my research skills and learn a variety of new methods and techniques. Skills obtained on the Nottingham Systematic Review Course were used to enhance the efficiency and effectiveness of searching for relevant journal articles, materials and references used in this thesis. All analyses used either logistic or linear regression methods within Stata, but the mediation analysis involved learning factor analysis, how to implement direct acyclic graph techniques for confounder selection and structural equation modelling techniques using Mplus. Furthermore, through giving poster and oral presentations of my research at both national and international conferences, as well as writing papers as a first author from these (**Appendix B** and **Appendix C**), I have been able to enhance my capacity as an independent researcher.

In addition, I have worked concurrently as a research associate during the fourth year of my PhD, for a National Institute for Health Research (NIHR)-Programme Grants for Applied Research (PGfAR) funded programme investigating smoking cessation in pregnancy. In doing so, I also developed new skills in Markov Chain Monte Carlo modelling methods using MLwiN and an understanding of routinely collected primary care research data through using Clinical Practice Research Datalink (CPRD) data. I was able to apply skills gained on the Data Organisation

and Management in Epidemiology (DOME) masters module to manage these large datasets.

9.4 Policy and practice implications

Current guidelines and recommendations for quitting smoking in pregnancy and following childbirth set forth by NICE's Public Health Interventions Advisory Committee (PHIAC) were last reviewed in 2010,[109] and were due to be reviewed again in July 2015, but the recommendations were judged as being up to date. Research from this thesis adds to the current knowledge base on smoking in pregnancy and will inform the next iteration of the guidelines.

Current guidelines suggest that there is limited evidence on whether helping partners or 'significant others' to quit, and having them encourage pregnant women to quit is effective.[109] Findings from **Chapter 4** and **Chapter 5** provide evidence to suggest that partners' smoking is not associated with cessation, and targeting the partner may have a minimal effect of reducing the negative effect that social disadvantage has on whether pregnant women achieve cessation. NICE guidelines also recommend that NRT only be prescribed when women have stopped smoking, once the dangers have been discussed and if they express a desire to use NRT.[109] However, in **Chapter 6**, there was no evidence that greater use of NRT was associated with either better or worse birth outcomes in a population of pregnant smokers attempting to quit. This may help to allay some concerns there may be about prescribing NRT in pregnancy.

There is still insufficient evidence to make practice or policy implications with regards to encouraging adherence (**Chapter 7**) or targeting women with slower or faster nicotine metabolism with NRT (**Chapter 8**). Further research is needed and avenues of further research are detailed in the following section.

9.5 Avenues for further research

There is currently little evidence on the use of NRT for smoking cessation in pregnancy. The research summarised in **Section 9.2** adds to the current knowledge on this subject. However, there are further areas for research and questions that have arisen from this thesis are detailed below.

Chapter 4 investigated factors associated with smoking cessation in pregnancy, and as the first study using multivariable methods to do so, the findings are important. However, a limitation of this study was that there were a restricted number of variables that were collected, that could be investigated. Few behavioural or socioeconomic factors, which have been shown to be associated with cessation in non-pregnancy, were investigated in this study and these should be investigated as potential factors associated with cessation in future studies. Furthermore, whilst it is now known that heavier smoking, more socially disadvantaged pregnant smokers are less likely to quit, the nature of support that these women might find most helpful remains unknown. Using data from the SNAP trial it was impossible to investigate this question further, but future research on smoking in pregnancy should address this.

In **Chapter 5**, nicotine dependence was found to mediate the negative effect of social disadvantage on smoking cessation. Nicotine dependence explained approximately a fifth of this association, and since partners' smoking status explained very little of this association (~2%), there is still scope for mediation of this association by other unmeasured factors. Research outside of pregnancy has identified agency (self-efficacy) as a potential mediator of this association,[207] and the number of other household members who are smokers could also act as a mediator of this association, but these factors were unmeasured in SNAP. Identifying other potential mediators of this association could suggest additional avenues for providing support or implementing cessation interventions which

might ameliorate the negative effect on quitting that social disadvantage may have, in addition to that of addressing nicotine dependence.

Chapter 6 addressed NRT use and birth outcomes, and found that NRT use was not associated with either birth weight or SGA. These elements are important indicators of development, but further research should investigate the use of NRT with the more rare exploratory outcomes also detailed in this study. The study detailed in **Chapter 6** may have lacked the power to detect these associations, if they existed; to investigate the association with NRT use and birth outcomes further, larger sample sizes are needed. Though, where large numbers of pregnant women are studied, for example in epidemiological cohort studies, the NRT use data is poor. One possible way to investigate the exploratory outcomes further is by combining evidence from currently published NRT trials to perform an individual patient data (IPD) meta-analysis for birth outcomes, using trials that had data on the reported number of NRT used and birth outcome data.

In **Chapter 7**, the association between adherence to NRT and cessation was investigated. Further research concerning adherence to NRT in pregnancy would be aided by more accurate measures of adherence and recording of concurrent smoking, possibly using daily diaries. However, being able to collect this type of data is not practical for a large number of people as it involves a concerted effort on smokers' part, and is also subject to reporting bias. One aspect that could be improved by having more data is the further investigation of reverse causation. A study similar to that described to address the limitations of **Chapter 6**, an IPD meta-analysis of studies already completed may provide a larger sample of completely abstinent women in which to investigate reverse causation and whether it was women with adherent characteristics or nicotine in NRT that causes greater adherence.

Chapter 8 discussed the first large study to investigate the metabolism of nicotine in pregnancy using the nicotine metabolite ratio (NMR). However limitations such as small sample size may have hindered the chance of detecting a weak interaction between NMR and active/placebo NRT. Furthermore, investigating within a restricted gestational range of pregnant women, due to blood samples for NMR being taken at between 12-24 weeks, may mean that an interaction may exist in later pregnancy since the rate of metabolism is hypothesised to increase with gestation. Future studies investigating the influence of nicotine metabolism in pregnancy should also investigate its influence on the effectiveness of NRT in the first and third trimesters. An alternative hypothesis is that the doses of nicotine in the SNAP trial were not sufficient to produce an interaction with NMR. One study in non-pregnant individuals did demonstrate that the effectiveness of NRT varied by the individuals' speed of nicotine metabolism, however this study used higher doses of NRT than those given in the SNAP trial.[169] The doses of nicotine provided in the SNAP trial might not have been sufficient to demonstrate such moderation by NMR in this study. Given this finding, along with the finding that greater NRT use was not associated with better or worse birth outcomes in this sample, it is conceivable that further research might be conducted to investigate moderation by NMR using higher therapeutic doses of nicotine which may be required to demonstrate this.

9.6 Overall conclusions

This thesis has provided evidence to illustrate some ways in which the use of NRT in pregnancy might be changed, such that it has greater potential to be found effective, as it is in non-pregnant smokers. Women who are more socially disadvantaged, having lower levels of education and smoking more cigarettes, are less likely to achieve smoking cessation. Treatment of nicotine dependence

could provide a means of reducing the impact of social disadvantage on smoking cessation in pregnancy which, at least in theory, should be effective. Infants born to women who use NRT have no worse birth outcomes (with respect to birth weight and SGA) than those given placebo patches, providing no evidence to suggest the use of NRT causes harm in pregnancy. There is also burgeoning evidence that greater adherence to NRT is associated with increased odds of cessation in pregnancy, though it was not possible to rule out reverse causation as a cause of this association and it may be due to the characteristics of women who are more likely to be adherent instead of any effect from NRT. There is also evidence to suggest that women that metabolise nicotine more quickly have reduced odds of cessation, but with no evidence to suggest that NRT is more effective in slower metabolisers, it may be that higher doses are required to observe the effect produced in the non-pregnant population. Overall this thesis should provide a spur to encourage further investigation of adherence to NRT and the metabolism of nicotine and its effects on smoking cessation in pregnancy.

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Appendix A

SNAP trial data

Table 22 Variables collected at baseline in the SNAP trial and description

Baseline variable	Coding
Date of birth	days / month / year
Initials (UID)	first and last initials
Ethnicity	white British / other
Age full time education completed	years
Number of cigarettes prior to pregnancy	n
Number of cigarettes currently smoked	n
Time until first cigarette smoked	<15mins / 15-60mins / >60mins
Time since last cigarette	
Partners smoking status	yes / no / NA
Number of previous pregnancies <24 weeks	0-9
CO reading	CO parts per million
CO date	days / month / year
Quit date	days / month / year
Maternal height	cm
Maternal weight	kg
Number of previous preterm pregnancies	0-4
Length of first behavioural support session	16-30mins / 31-45mins / >60mins
Previous use of NRT	yes / no
Date that NRT was last used	days / month / year
Primary care trust/Hospital	Trusts 1-7
Smoking cessation support centre	Hospital attached centres 1-7
Gestational age at baseline	Weeks
Treatment assignment	Nicotine replacement therapy / Placebo patches
Randomisation date	days / month / year
Free cotinine	ng/ml
Free trans-3'-hydroxycotinine	ng/ml
Total cotinine	ng/ml
Total trans-3'-hydroxycotinine	ng/ml
3HC:Cot ratio (measured from free cotinine)	ratio
3HC:Cot ratio (measured from total cotinine)	ratio

Table 23 Variables collected at one month follow-up in the SNAP trial and description

One Month follow-up variable	Coding
Attended one month follow-up	yes / no
Date of follow-up	days / month / year
CO reading	CO parts per million
Smoked cigarettes in the past 24hrs	yes / no
Smoked since quit date	yes / no
How often smoked since quit	5 times or less in total / at least weekly but less than daily / most days
Number of days NRT used on	0-56
Number of days non-trial NRT used	n
NRT use validated	yes / no
Number of support sessions received	telephone support / text support / face-to-face support
NRT use in the past 24hrs	yes / no
Saliva cotinine	ng/ml
Maternal weight	kg

Table 24 Variables collected at delivery in the SNAP trial and description

Delivery variable	Coding
Date of follow-up	days / month / year
CO reading	CO parts per million
CO date	days / month / year
Saliva cotinine measurement	ng/ml
Saliva cotinine date	days / month / year
Smoked cigarettes in the past 24hrs	yes / no
Number of cigarettes smoked since quit date	n
How often smoked since quit	5 times or less in total / at least weekly but less than daily / most days
Number of days non-trial NRT used	n
Number of support sessions received	telephone support / text support / face-to-face support
Hypertension	mmHg
Antenatal admission	yes / no
Miscarriage <24 weeks	n
Labour mode	induced / no labour / not applicable / spontaneous
Birth status	live birth / still birth
Gestational age at birth	weeks
Delivery mode	assisted vaginal / caesarean / spontaneous vaginal
Gender	male / female
APGAR	0-10
Umbilical cord pH <7	yes / no / unknown
Birthweight	g
Neonatal admission	yes / no
Intraventricular haemorrhage	iv haemorrhage / vetriculomegaly / none
Ventilated for >24hrs	yes / no / NA
Necrotic enterocolitis	yes / no
Neonatal convulsions	yes / no
Congenital abnormalities	yes / no
Completed primary outcome	yes / no
Infant date of birth	days / month / year
Withdrawal reason	Free text

Appendix B

Professional development

Masters modules [Credits]

- Tobacco Control Interventions. [7.5]
- Epidemiology of tobacco use and the tobacco industry. [7.5]
- Data Organisation and Management in Epidemiology – A practical course in Stata. [10]

Graduate school courses [Credits]

- Using posters to communicate research. [1]
- Further LaTeX for researchers – developing your skills in LaTeX. [1]
- Getting going on your thesis. [2]
- Presentation skills for researchers. [2]
- The viva: the students side of the table.
- Professional networking for researchers.

External courses

- The Cochrane Schizophrenia group – The Nottingham Systematic review course 2012 – Nottingham 3rd/6th July 2012.

Conferences and Seminars [Credits]

- UK Centre for Tobacco Control Studies Post Graduate Student Conference – Bath – 21st/22nd November 2011. Presented: An introduction to medicinal nicotine in pregnancy.
- Fifty years since Smoking and Health progress, lessons and priorities for a smoke-free UK – London – 6th March 2012. (Attendance only)
- XIV Annual Meeting of the SRNT Europe – Helsinki, Finland – 29th August/2nd September 2012. Presented: Factors associated with smoking cessation in early and late pregnancy in a trial of standard dose nicotine replacement therapy.
- Tackling Smoking in 21st Century Britain Conference – York, UK – 7th/9th November 2012. Presented: Factors associated with smoking cessation in early and late pregnancy in a trial of standard dose nicotine replacement therapy.
- School of Community Health Sciences postgraduate research Conference – Nottingham, UK – 7th December 2012. Presented: Factors associated with smoking cessation during pregnancy (poster). [4]
- UK Society for Behavioural Medicine – Oxford, UK – 9th-10th December 2013. Presented: The relationship between social disadvantage and smoking behaviour in pregnancy: mediation via nicotine dependence and partners smoking (poster).
- Society for Academic Primary Care Trent Regional Meeting – Lincoln, UK – 25th March 2014. Presented: The 3HC: Cot ratio (Nicotine Metabolite Ratio) as a proxy for nicotine metabolism in pregnancy.
- Society for Academic Primary Care National Conference – Edinburgh, UK – 9th July/11th July 2014. Presented: The 3HC: Cot ratio (Nicotine Metabolite Ratio) as a proxy for nicotine metabolism in pregnancy.

PhD related publications

- Vaz, LR; Leonardi-Bee, J; Aveyard, P; Cooper, S; Grainge, M; Coleman, T; on behalf of the SNAP trial team. Factors Associated With Smoking Cessation in Early and Late Pregnancy in the Smoking, Nicotine, and Pregnancy Trial: A Trial of Nicotine Replacement Therapy. *Nicotine & Tobacco Research* 2013. DOI: 10.1093/ntr/ntt156.
- Bowker, KA; Lewis, S; Coleman, T; Vaz, LR; Cooper, S. Comparison of Cotinine Levels in Pregnant Women Whilst Smoking and When Using Nicotine Replacement Therapy. *Nicotine & Tobacco Research.* 2014. DOI:

10.1093/ntr/ntu029. *[Contribution of metabolism data and contribution to interpretation]*

- Vaz, LR; Coleman, T; Aveyard, P; Cooper, S; Leonardi-Bee, J; on behalf of the SNAP trial team. The nicotine metabolite ratio in pregnancy measured by trans-3'-Hydroxycotinine to Cotinine ratio: characteristics and relationship with smoking cessation. *Nicotine & Tobacco Research.* 2015. DOI: 10.1093/ntr/ntu342.

Research associate related publications and contributions

- The SNAP trial: a randomised placebo-controlled trial of nicotine replacement therapy in pregnancy; effectiveness and safety until 2 years after delivery, with economic evaluation. (Part of the SNAP trial team, secondary adherence analysis).
- Vaz, LR; Coleman, T; Fahy, SJ; Cooper, S; Bauld, L; Szatkowski, L; Leonardi-Bee, J. Factors associated with the Effectiveness and Reach of NHS Stop Smoking Services for pregnant women in England. *(Submission to Nicotine & Tobacco Research expected within 2 weeks).*

Memberships

- UK Society for Behavioural Medicine (UKSBM).
- Society for Academic Primary Care (SAPC).

Appendix C

Publications from the thesis

ORIGINAL INVESTIGATION

Factors Associated With Smoking Cessation in Early and Late Pregnancy in the Smoking, Nicotine, and Pregnancy Trial: A Trial of Nicotine Replacement Therapy

Luis R. Vaz MSc[1], **Jo Leonardi-Bee** PhD[2], **Paul Aveyard** PhD[3], **Sue Cooper** PhD[1], **Matthew Grainge** PhD[2], **Tim Coleman** MD[1]; **on behalf of the SNAP trial team**

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ABSTRACT

Introduction: Previous studies have found partners' smoking status, multiparity, and nicotine dependence to be associated with smoking cessation in pregnancy. However, no studies have investigated influences on cessation among women using nicotine replacement therapy (NRT). We analyzed data from a trial of NRT in pregnancy to determine factors associated with shorter and longer term cessation.

Methods: Data were collected at baseline, 1 month, and delivery from 1,050 pregnant women. Two multivariable logistic models for validated cessation at 1 month and delivery were created using a systematic strategy for selection of included factors.

Results: All findings are from multivariable analyses. At 1 month, odds of cessation were greater among those who completed full time education at >16 years of age (odds ratio [*OR*]: 1.82, 95% confidence interval [95% *CI*]: 1.24–2.67, $p = .002$) but they were lower in women with higher baseline cotinine levels (*OR*: 0.93, 95% *CI*: 0.90–0.95, $p < .001$). At delivery, the odds of cessation were greater among those who completed full time education at >16 years of age (*OR*: 1.89, 95% *CI*: 1.16–3.07, $p = 0.010$) but were inversely associated with higher baseline cotinine levels (*OR*: 0.96, 95% *CI*: 0.92–0.99, $p = .010$).

Conclusions: Women who are better educated and have lower pretreatment cotinine concentrations had higher odds of stopping smoking and factors associated with shorter and longer term cessation were similar.

INTRODUCTION

Smoking in pregnancy is a significant public health problem. In the United Kingdom, a country with strong tobacco control culture, a survey in 2011 found that 26% of pregnant women smoked at some point before or during pregnancy and 12% smoked constantly throughout gestation (Eastwood, 2011). As smoking is a completely preventable cause of poor health outcomes for mothers and their babies, stopping smoking before or during pregnancy is vital. Unfortunately, though, there are few evidence-based cessation interventions that are proven to work for cessation in pregnancy.

A systematic review investigating the predictors of quit attempts made by nonpregnant smokers, found that a lower number of previous quit attempts and higher levels of nicotine dependence were both inversely associated with cessation (Vangeli, Stapleton, Smit, Borland, & West, 2011). Factors that have been associated with increased number of quit attempts in pregnancy also include age and smoking duration (Yu, Park, & Schwalberg, 2002). However, a recent systematic review found that having a partner who smoked, multiparity and increasing nicotine dependence had, in many studies, been found inversely associated with likelihood of achieving cessation (Schneider, Huy, Schutz, & Diehl, 2010). Additionally, socioeconomic factors such as increased income and educational levels of the mother and partner have also been shown to be associated with cessation in pregnancy (Ebert & Fahy, 2007; Mohsin & Bauman, 2005; Schneider et al., 2010), but these associations may be due to decline in smoking rates, which has been found to be lower in women from lower socioeconomic groups (US DHHS, 2004). Data from surveys conducted in the United Kingdom and Spain have also found that pregnant women with lower educational and socioeconomic levels have lower chances of cessation, whereas women who smoked fewer cigarettes, started smoking at an older age, had a partner who did not smoke or were primiparous were more likely to quit (Torrent et al., 2004).

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There is less evidence, though, about which factors might influence women's success when using nicotine replacement therapy (NRT) in cessation attempts made during pregnancy. Although nicotine dependence appears central to maintaining smoking behavior in pregnancy, attempts to promote cessation in pregnancy by addressing this with NRT have thus far been unsuccessful (Coleman, Chamberlain, Davey, Cooper, & Leonardi-Bee, 2012a). Further investigation of factors associated with cessation in pregnancy is warranted and analyses using data from studies in which an attempt has been made to treat nicotine dependence would be particularly informative. Recently, the Smoking, Nicotine, and Pregnancy (SNAP) trial, a large trial investigating the use of NRT for smoking cessation in pregnancy was conducted (Coleman et al., 2012b), and using the cohort of participants from this trial, we investigate independent associations between participants' baseline characteristics and cessation at both early and late follow-up points to help ascertain whether or not any might be potential determinants of successful cessation.

METHODS

Data Source

Data for explanatory variables in these analyses were collected at baseline and outcome variable data were collected at two subsequent follow-up points within the SNAP trial (Coleman et al., 2012b). Trial participants were aged 16–45 years; of 12–24 weeks gestation; smoked ≥10 cigarettes prior to pregnancy and smoked ≥5 cigarettes currently; and had exhaled carbon monoxide (CO) readings of >8 parts per million (ppm).

Treatment Protocol

Between May 2007 and February 2010, 1,050 participants were recruited to the trial from seven English hospital antenatal clinics. Research midwives collected baseline data, prescribed trial patches and provided face-to-face behavioral support at enrollment, and collected follow-up data at contacts; 1 month and delivery. Women received a behavioral support session lasting up to 1 hr at enrollment. A quit date was also set within 2 weeks of enrollment and the follow-up points were measured from this. Women were offered additional behavioral support from the local National Health Service (NHS) stop smoking services throughout the trial to all participants according to the national standards, and research midwives provided telephone support when women were contacted on their quit date, 3 days after this and at 1 month. Participants were randomized to receive either NRT (15 mg/16 hr) or identical placebo patches. The first 4 weeks supply of patches was issued on the quit date, with a second batch of 4 weeks of patches given to those women reported not smoking and who had CO validation at the 1-month follow-up. Full methods (Coleman et al., 2012b) including the initial (Coleman et al., 2007) and final (Coleman et al., 2009) protocols for this study are published elsewhere.

Baseline Data: Explanatory Variables

Prior to randomization, the following data were collected from participants: date of birth, ethnicity, age on completion of full time education, partner's smoking status, parity, gestational age, body mass index, and previous use of NRT during their current pregnancy. Saliva and blood samples were taken for cotinine estimation, along with exhaled CO readings to estimate smoke and nicotine intake, respectively. Trial recruitment site and participants' treatment assignment (i.e., NRT or placebo) were also available from the trial database.

Outcome Variables

For analyses in this paper, we used validated cessation at 1-month postquit date and at delivery as outcome variables. At 1 month, cessation was defined as continuous abstinence from quit date to 1 month, validated by an exhaled CO reading of ≤8 ppm; and, at delivery, cessation was defined as continuous abstinence from a quit date until delivery, validated by an exhaled CO reading of ≤8 ppm and/or a saliva cotinine level of <10 ng/ml. Participants who were lost to follow-up were coded as continuing smokers.

Analysis Strategy

This analysis investigated associations between baseline characteristics of participants and cessation at 1 month after initiating treatment (i.e., from quit date) and at delivery. Two multivariable logistic models were built. Initially, for both models, variables were identified which had significant univariate associations ($p \leq .05$) with validated cessation at each timepoint. Secondly, these variables were all entered into a multivariable model using stepwise backwards elimination to remove variables found to have nonsignificant associations with outcome ($p > .05$). Finally, variables which showed no association at the univariate level were entered into the models individually, to determine if they were subsequently associated with validated cessation. Treatment assignment was included as an *a-priori* confounder.

To maximize the number of participants included in the analysis, where possible, a missing category was created for categorical variables with missing data and imputation was planned for continuous variables (baseline cotinine) where >10% of cases had missing data. Where all missing values for an exposure occurred among people in the same outcome category (i.e., continuing smokers) or a small percentage of data was missing for a continuous variable, a univariate sensitivity analysis comparing all participants against those with complete data was conducted, to verify that they did not differ in their baseline characteristics.

All analyses were conducted using Stata 11.2 (College Station, TX).

RESULTS

In the 1 month and delivery multivariate analyses, missing data for the categorical variable "age full time education finished" could not be included as all those with missing data were smokers at follow-up and inclusion as an extra category would perfectly predict the outcome. Furthermore, imputation for the continuous variable "baseline cotinine level" was not carried out due to data being missing for only 80 participants (7.6%). As a result, analysis was undertaken on 957/1,050 participants (91.1%), for whom complete exposure data were available. Including the 93 participants for whom some baseline data were missing in the final multivariable model, did not alter the results. An analysis based on achieving validated cessation was conducted and characteristics of the women included are detailed in Table 1. At 1 month 167 (17.5%), and at delivery 84 (8.8%) of the participants achieved validated cessation.

Table 1. Participant Characteristics for Cessation at 1 Month and Delivery

	One month		Delivery	
Variable	Smoking (n = 790)	Abstinent (n = 167)	Smoking (n = 873)	Abstinent (n = 84)
Age (median [IQR]) (years)	25 (21–30)	25 (21–31)	25 (21–30)	25 (21–31)
Primiparous	36.8%	37.1%	36.3%	42.9%
Age full time education finished (years)				
≤16	80.5%	68.9%	79.6%	66.7%
>16	19.5%	31.1%	20.4%	33.3%
Ethnicity				
British: White	97.3%	96.4%	97.4%	95.2%
Other	2.7%	3.6%	2.6%	4.8%
Gestational age (median [IQR]) (weeks)	15 (13–18)	15 (13–18)	15 (13–18)	15 (13–20)
Baseline cotinine (median [IQR]) (ng × 10^{-3}/ml)	12.7 (8.7–18.2)	9.1 (5.8–13.8)	12.4 (8.1–17.8)	10.6 (6.0–15.3)

Note. IQR = interquartile range.

Factors Associated With Smoking Cessation at 1 Month

At 1 month, in the univariate analysis, women who finished full time education when they were >16 years had greater odds of cessation; women who had a higher baseline cotinine levels, and those who were recruited from trial site 4, were found to have lower odds of cessation (Table 2). The final multivariable model shows which baseline variables were independently associated with validated cessation at 1 month. Women who were aged >16 years when they finished full time education (odds ratio [*OR*]: 1.82, 95% confidence interval [95% *CI*]: 1.24–2.67, p = .002) had significantly increased odds of achieving validated cessation. Participants who had a higher baseline cotinine (*OR*: 0.94, 95% *CI*: 0.91–0.96, p < .001 for a 10 ng/ml increase) had significantly lower odds of cessation at 1 month after quit date. The effect of trial recruitment site 4 did not remain significant when added to the multivariable model (*OR*: 0.69, 95% *CI*: 0.36–1.34, p = .277).

Factors Associated With Smoking Cessation at Delivery

Table 3 shows the univariable and multivariable associations with validated cessation at delivery; the univariable results found women who finished full time education at >16 years and had lower baseline cotinine levels had increased odds of cessation and those with higher baseline cotinine levels had lower odds of cessation. In the final multivariable model, women who continued school beyond the compulsory minimum age (16 years) were more likely to stop smoking (*OR*: 1.89, 95% *CI*: 1.16–3.07, p = .010) and women with a higher baseline cotinine level were less likely to achieve cessation (*OR*: 0.96, 95% *CI*: 0.92–0.99, p < .010).

DISCUSSION

Main Findings

We found that, among participants in a trial of transdermal nicotine patches in pregnancy, smoking cessation of 1-month duration and also until delivery were positively associated with finishing full time education beyond the compulsory age of 16 years and negatively associated with baseline cotinine levels. Leaving school at 16 years is a marker of social disadvantage and also an indicator of lower socioeconomic status, which is associated with decreased probability of quitting.

Strengths and Limitations

The main limitation of this study was that a relatively restricted variety of variables were collected in the trial; in particular, there were few behavioral or socioeconomic measures, which, in some studies have been shown to influence cessation (Schneider et al., 2010). It also remains possible that differences in cessation rates observed in early and late pregnancy might be explained by unmeasured factors. Furthermore, as a number of significance tests were performed, some of the observed associations may have occurred by chance (i.e., Type I errors). However, as this is the first analysis employing multivariable methods to determine factors which were independently associated with smoking cessation in a trial of NRT patches, findings remain interesting. Additionally, our study sample was large and was mostly complete, permitting inclusion of 91% of trial participants in analyses. This will have increased the likelihood that weak associations between baseline factors and validated cessation in the trial database could be discovered. A final advantage of investigating predictors in a trial of NRT is that biochemically validated cessation was used; accuracy of self-reported cessation in pregnancy is typically low due to the perceived social acceptability of smoking during pregnancy.

Findings in the Context of Previous Work

Two previous systematic reviews, in nonpregnant (Vangeli et al., 2011) and pregnant smokers (Schneider et al., 2010), respectively, have investigated predictors of smoking cessation. Both found that nicotine dependence is an important predictor of quit attempt success, with higher levels reducing the likelihood of a successful quit attempt. Our analyses provide complementary data showing, in the context of an NRT trial, that lower cotinine concentration increased the odds of

Table 2. Univariate and Multivariate Associations With Validated Cessation at 1 Month

Variable	*N*	Smoking at 1 month (%)	Cessation at 1 month (%)	Crude *OR*	95% *CI*		*p* value	Adjusted *OR*	95% *CI*		LRT *p* value
					Lower	Upper			Lower	Upper	
Age (years)†											
<20	147	129 (87.8)	18 (12.2)	0.64	0.36	1.12	0.250*	–	–	–	–
20–24	322	264 (82.0)	58 (18.0)	1.00	–	–		–	–	–	
25–29	228	183 (80.3)	45 (19.7)	1.12	0.73	1.73		–	–	–	
30–34	158	134 (84.8)	24 (15.2)	0.82	0.49	1.37		–	–	–	
35–39	78	63 (80.8)	15 (19.2)	1.08	0.58	2.04		–	–	–	
≥40	24	17 (70.8)	7 (29.2)	1.87	0.74	4.73		–	–	–	
Ethnicity											
Other	27	21 (77.8)	6 (22.2)	1.00	–	–	0.521	–	–	–	–
British: White	930	769 (82.7)	161 (17.3)	0.73	0.29	1.84		–	–	–	
Age full time education finished											
≤16	751	636 (84.7)	115 (15.3)	1.00	–	–	0.001	1.00	–	–	0.002
>16	206	154 (74.8)	52 (25.2)	1.87	1.29	2.71		1.82	1.24	2.67	
Partner smokes											
No	225	186 (82.7)	39 (17.3)	1.00	–	–	0.412	–	–	–	–
Yes	654	544 (83.2)	110 (16.8)	0.96	0.65	1.44		–	–	–	
Not applicable	78	60 (76.9)	18 (23.1)	1.43	0.76	2.69		–	–	–	
Parity											
≤1	649	535 (82.4)	114 (17.6)	1.00	–	–	0.919*	–	–	–	
2 or 3	256	211 (82.4)	45 (17.6)	1.00	0.68	1.46		–	–	–	
≥4	52	44 (84.6)	8 (15.4)	0.85	0.39	1.86		–	–	-	
Baseline cotinine (ng × 10^{-1}/ml)											
12.1 (7.8–17.6), median (IQR)	957	790 (82.6)	167 (17.4)	0.93	0.90	0.95	<0.001	0.93	0.90	0.95	<0.001
BMI											
<18.5	28	27 (96.4)	1 (3.6)	0.18	0.02	1.34	0.987*	–	–	–	–
18.5–24.9	362	300 (82.9)	62 (17.1)	1.00	–	–		–	–	–	
25–29.9	267	213 (79.8)	54 (20.2)	1.23	0.82	1.84		–	–	–	
>30	254	212 (83.5)	42 (16.5)	0.96	0.62	1.47		–	–	–	
Missing	46	38 (82.6)	8 (17.4)	1.02	0.45	2.29		–	–	–	
Length of first behavioral support session											
16–30	143	123 (86.0)	20 (14.0)	1.00	–	–	0.478	–	–	–	–
31–45	791	648 (81.9)	143 (18.1)	1.36	0.82	2.25		–	–	–	
>60	23	19 (82.6)	4 (17.4)	1.29	0.40	4.20		–	–	–	
Previous preterm births											
0	872	718 (82.3)	154 (17.7)	1.00	–	–	0.577	–	–	–	–
≥1	85	72 (84.7)	13 (15.3)	0.84	0.45	1.56		–	–	–	

(Continued)

Table 2. Continued

Variable	*N*	Smoking at 1 month (%)	Cessation at 1 month (%)	Crude *OR*	95% *CI*		*p* value	Adjusted *OR*	95% *CI*		LRT *p* value
					Lower	Upper			Lower	Upper	
Use of NRT since pregnancy began											
No	914	756 (82.7)	158 (17.3)	1.00	–	–	0.548	–	–	–	–
Yes	43	34 (79.1)	9 (20.9)	1.27	0.60	2.69		–	–	–	
Trial recruitment site											
Site 1	102	81 (79.4)	21 (20.6)	1.00	–	–	0.032	–	–	–	–
Site 2	113	97 (85.8)	16 (14.2)	0.64	0.31	1.30		–	–	–	
Site 3	188	151 (80.3)	37 (19.7)	0.95	0.52	1.72		–	–	–	
Site 4	238	212 (89.1)	26 (10.9)	0.47	0.25	0.89		–	–	–	
Site 5	165	127 (77.0)	38 (23.0)	1.15	0.63	2.11		–	–	–	
Site 6	77	63 (81.8)	14 (18.2)	0.86	0.40	1.82		–	–	–	
Site 7	74	59 (79.7)	15 (20.3)	0.98	0.47	2.06		–	–	–	
Gestational age (weeks)											
12–19	766	637 (83.2)	129 (16.8)	1.00	–	–	0.326	–	–	–	–
20–24	191	153 (80.1)	38 (19.9)	1.23	0.82	1.83		–	–	–	

Note. BMI = body mass index; *CI* = confidence interval; IQR = interquartile range; LRT = likelihood ratio test; NRT = nicotine replacement therapy; *OR* = odds ratio.
*p value: test for trend.
†Age included as continuous variable in multivariate model to derive p value, equivalent to test for trend adjusting for covariates.

Table 3. Univariate and Multivariate Associations With Validated Cessation at Delivery

Variable	*N*	Smoking at delivery (%)	Cessation at delivery (%)	Crude *OR*	95% *CI*		*p* value	Adjusted *OR*	95% *CI*		LRT *p* value
					Lower	Upper			Lower	Upper	
Age											
<20	147	135 (91.8)	12 (8.2)	0.93	0.46	1.89	0.403*	–	–	–	–
20–24	322	294 (91.3)	28 (8.7)	1.00	–	–		–	–	–	
25–29	228	206 (90.4)	22 (9.7)	1.12	0.62	2.02		–	–	–	
30–34	158	145 (91.8)	13 (8.2)	0.94	0.47	1.87		–	–	–	
35–39	78	74 (94.9)	4 (5.1)	0.57	0.19	1.67		–	–	–	
≥40	24	19 (79.2)	5 (20.8)	2.76	0.96	7.96		–	–	–	
Ethnicity											
Other	27	23 (85.2)	4 (14.8)	1.00	–	–	0.300	–	–	–	–
British - White	930	850 (91.4)	80 (8.6)	0.54	0.18	1.60		–	–	–	
Age full time education finished											
≤16	751	695 (92.5)	56 (7.5)	1.00	–	–	0.009	1.00	–	–	0.010
>16	206	178 (86.4)	28 (13.6)	1.95	1.20	3.16		1.89	1.16	3.07	
Partner smokes											
No	78	199 (88.4)	26 (11.6)	1.00	–	–	0.193	–	–	–	–
Yes	225	604 (92.4)	50 (7.6)	0.63	0.38	1.04		–	–	–	
Not applicable	654	70 (89.7)	8 (10.3)	0.87	0.38	2.02		–	–	–	
Parity											
≤1	649	588 (90.6)	61 (9.4)	1.00	–	–	0.304*	–	–	–	–
2 or 3	256	235 (91.8)	21 (8.2)	0.86	0.51	1.45		–	–	–	
≥4	52	50 (96.2)	2 (3.8)	0.39	0.09	1.62		–	–	–	
Baseline cotinine (ng × 10^{-1}/ml)											
12.1 (7.8–17.6), median (IQR)	957	873 (91.2)	84 (8.8)	0.95	0.92	0.98	0.002	0.96	0.92	0.99	0.010
Length of first behavioral support session											
16–30	143	135 (94.4)	8 (5.6)	1.00	–	–	0.258	–	–	–	–
31–45	791	718 (90.8)	73 (9.2)	1.72	0.81	3.64		–	–	–	
>60	23	20 (87.0)	3 (13.0)	2.53	0.62	10.34		–	–	–	
BMI											
<18.5	28	27 (96.4)	1 (3.6)	0.44	0.06	3.37	0.273*	–	–	–	–
18.5–24.9	362	334 (92.3)	28 (7.7)	1.00	–	–		–	–	–	
25–29.9	267	243 (91.0)	24 (9.0)	1.18	0.67	2.08		–	–	–	
>30	254	225 (88.6)	29 (11.4)	1.54	0.89	2.65		–	–	–	
Missing	46	44 (95.7)	2 (4.3)	0.54	0.12	2.35		–	–	–	
Previous preterm births											
0	872	795 (91.2)	77 (8.8)	1.00	–	–	0.852	–	–	–	–
≥1	85	78 (91.8)	7 (8.2)	0.93	0.41	2.08		–	–	–	

(Continued)

Table 3. Continued

Variable	*N*	Smoking at delivery (%)	Cessation at delivery (%)	Crude *OR*	95% *CI*		*p* value	Adjusted *OR*	95% *CI*		LRT *p* value
					Lower	Upper			Lower	Upper	
Use of NRT since pregnancy began											
No	914	837 (91.6)	77 (8.4)	1.00	–	–	0.106	–	–	–	–
Yes	43	36 (83.7)	7 (16.3)	2.11	0.91	4.91		–	–	–	
Trial recruitment site											
Site 1	102	90 (88.2)	12 (11.8)	1.00	–	–	0.397	–	–	–	–
Site 2	113	106 (93.8)	7 (6.2)	0.50	0.19	1.31		–	–	–	
Site 3	188	172 (91.5)	16 (8.5)	0.70	0.32	1.54		–	–	–	
Site 4	238	221 (92.9)	17 (7.1)	0.58	0.26	1.26		–	–	–	
Site 5	165	152 (92.1)	13 (7.9)	0.64	0.28	1.47		–	–	–	
Site 6	77	69 (89.6)	8 (10.4)	0.87	0.34	2.24		–	–	–	
Site 7	74	63 (85.1)	11 (14.9)	1.31	0.54	3.15		–	–	–	
Gestational age (weeks)											
12–19	766	704 (91.9)	62 (8.1)	1.00	–	–	0.147	–	–	–	–
20–24	191	169 (88.5)	22 (11.5)	1.48	0.88	2.47		–	–	–	

Note. BMI = body mass index; *CI* = confidence interval; IQR = interquartile range; LRT = likelihood ratio test; NRT = nicotine replacement therapy; *OR* = odds ratio.
*p value: test for trend.

cessation among trial participants in both early and late pregnancy. Cotinine levels are a marker of tobacco smoke exposure rather than being a measure of nicotine dependence. However, within these trial participants both plasma and saliva measures of cotinine have been found to be highly correlated with a validated measure of nicotine dependence in pregnancy (Kwok, Taggar, Cooper, Lewis, & Coleman, 2013), so it is likely that, lower levels of nicotine dependence, would also increase the likelihood of women in this study quitting too.

The previous reviews also investigated associations between socioeconomic factors and smoking cessation and found strong evidence for a positive association between higher socioeconomic status or, higher income levels and cessation in pregnancy (Schneider et al., 2010); but little evidence was found for an association between income, level of education or employment status, and cessation in nonpregnant smokers (Vangeli et al., 2011). The present study concurs with the previous findings that higher levels of social disadvantage are associated with worse outcomes in pregnant women who attempted cessation as part of an NRT trial.

In the previous review that investigated pregnant smokers' quit attempts, all included studies used self-reported cessation measures (Schneider et al., 2010), which may be prone to underreporting of smoking behavior due to social desirability bias and, so, the validity of findings from empirical studies included in this review could be questioned. As nonsmokers are unlikely to report themselves as smokers, misclassification of women currently smoking as nonsmokers could bias the strength of associations between factors relating to smoking behavior and addiction and those measuring social disadvantage and cessation toward the null. In our dataset, misclassification of smoking status due to such biases in self-reported data cannot have occurred; consequently, our demonstration of the importance of nicotine dependence and social disadvantage to smoking cessation is likely to be valid.

Only one previous report has used validated cessation data to investigate factors associated with successful cessation in pregnancy (Fish et al., 2009). This analysis used data from the U.S. "Baby Steps" trial (Pollak et al., 2007) and found that women who were primiparous and who used more NRT were more likely to report cessation at 38 weeks gestation (Fish et al., 2009); however, the study sample was small (104 women) and only univariable associations were reported, so these findings are difficult to interpret. Our much larger study has greater power and used a multivariable analysis, which investigated the independent associations with cessation.

The analyses presented in this paper suggest that, in pregnant women who use NRT to attempt cessation, higher levels of social disadvantage and higher pretreatment cotinine levels are associated with worse cessation outcomes. It is possible that both associations are causal, though a mechanism for cotinine concentration affecting cessation through its contribution to nicotine dependence is more immediately obvious. Outside of pregnancy, nicotine dependence is also more easily remedied, for example, by NRT, which has been shown to reduce the strength of craving and be effective (Stead, Perera, Mant, & Lancaster, 2008). These findings have research implications; further work investigating how socioeconomic status may influence success in quit attempts could uncover factors that are amenable to intervention. Similarly, further research into the treatment of nicotine dependence may be indicated; there is currently no evidence that NRT is effective in pregnancy (Coleman et al., 2012a), but future studies using higher doses of nicotine than those which have been trialed could be undertaken. Nicotine metabolism is faster in pregnancy (Dempsey, Jacob, & Benowitz, 2002) and the standard doses of NRT that have been investigated may be too low to be effective. The finding that increasing cotinine concentration, which is strongly correlated with nicotine dependence (Kwok et al., 2013), is strongly associated with cessation failure should provide a spur to investigate this possibility.

Conclusions and Recommendations

Among pregnant participants in a trial of NRT for smoking cessation, women who were not educated beyond the compulsory age for finishing school and those who had higher pretreatment cotinine concentrations were less likely to stop smoking throughout pregnancy. Women with these characteristics may require a different kind of smoking cessation support in pregnancy than others; however, it is not possible, from our data, to determine the nature of support that these women would find most helpful.

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DECLARATION OF INTERESTS

Tim Coleman, Jo Leonardi-Bee, and Paul Aveyard are members of the UKCTCS, a UKCRC Public Health Research: Centre of Excellence. Tim Coleman, Sue Cooper, and Paul Aveyard are members of the NIHR School for Primary Care Research. There are no potential conflicts of interest relevant to this study.

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Original investigation

Original investigation

The Nicotine Metabolite Ratio in Pregnancy Measured by *trans*-3′-Hydroxycotinine to Cotinine Ratio: Characteristics and Relationship With Smoking Cessation

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Abstract

Introduction: Nicotine replacement therapy (NRT) helps nonpregnant smokers quit, but there is no evidence that standard dose NRT is effective in pregnancy. As nicotine metabolism increases in pregnancy, this could reduce NRT efficacy. Using the ratio of *trans*-3′-hydroxycotinine to cotinine, the nicotine metabolite ratio (NMR), we investigated relationships between the rate of nicotine metabolism, maternal characteristics and smoking cessation in pregnant women recruited to a randomized controlled trial of NRT.

Methods: Data from 1,050 pregnant smokers in the Smoking, Nicotine and Pregnancy trial who were of 12–24 weeks gestation had exhaled carbon monoxide readings of ≥8 ppm at recruitment and who were randomized to NRT or placebo patches were used. Linear and logistic regression investigated associations between maternal characteristics and NMR and also between NMR and subsequent validated cessation from smoking.

Results: Six hundred and sixty-two women (63%) provided blood samples for NMR estimation. Higher NMR was associated with increased cigarette consumption prior to pregnancy. At 1 month (odds ratio [*OR*] = 0.87; 95% CI = 0.76–0.99; $p = .043$) and delivery (*OR* = 0.79; 95% CI = 0.66–0.95; $p = .010$), there was a significant negative association between a 0.1 unit increase in NMR and odds of achieving cessation after adjusting for possible confounders. There was no evidence for an interaction between a 0.1 unit increase in NMR and treatment assignment on the odds of cessation at 1 month post-quit date ($p = .556$).

Conclusion: Pregnant women who metabolize nicotine more rapidly are less likely to achieve cessation when they try to quit smoking. There is no evidence that NRT is more effective in women who metabolize nicotine more slowly.

Introduction

Smoking is the leading preventable cause of morbidity in pregnant women and causes harm to the developing child.[1] It has also been associated with adverse birth outcomes including low birth weight, being born prematurely or being born small for gestational age.[2] Smoking whilst pregnant is harmful and should ideally be stopped before or during pregnancy.[3]

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In this article, we examine the importance of the rate of nicotine metabolism in pregnancy. Nicotine is rapidly metabolized by the cytochrome P450 enzyme CYP2A6[4,5] to cotinine, which is not psychoactive. The same enzyme metabolizes cotinine to *trans*-3′-hydroxycotinine (3HC), which is then metabolized by other enzymes.[6] Most of the enzyme sites are occupied and metabolism occurs at its maximal rate and does not change in proportion to drug concentration (zero order kinetics). As a result, a fixed amount of nicotine is metabolized over time.[6,7] Additionally, binding to plasma proteins is low, at <5%[8] and the nicotine metabolite ratio (NMR) is likely to be unaffected by free nicotine binding to these proteins in the blood. Changes in metabolic enzymes may occur as gestation progresses, but there is no evidence of induced expression of more of the major metabolizing enzyme (CYP2A6) in extrahepatic tissues by xenobiotics in humans.[9] As nicotine is metabolized very quickly, the more direct measure of nicotine/cotinine ratio depends upon recent smoking. The 3HC/cotinine ratio (NMR) is much more stable because the half-life of both compounds is much longer than nicotine's,[8,10] and therefore little affected by recent smoking. It provides an indirect measure of the speed of metabolism of nicotine because it reflects the efficiency of the system responsible for 70%–80% of nicotine metabolism.[8,11] In this article, we provide descriptive data for this ratio in a population of pregnant smokers.

Tobacco smokers titrate the amount they smoke to the level of nicotine required from smoking[12]; as such, smoking behavior is driven by nicotine dependence and continuing to smoke is, in part, attributable to the addictive properties of nicotine contained in cigarettes. If people do smoke to regulate nicotine intake, those who metabolize nicotine more quickly would be expected to smoke more cigarettes per day on average, and there is limited evidence of this in nonpregnant smokers.[13,14] Faster metabolizers remove nicotine more quickly and therefore will experience nicotine withdrawal more rapidly after smoking than slower metabolizers. They might, therefore, be expected to smoke more often and, perhaps also to draw more deeply on their cigarettes to restore their nicotine concentration and ameliorate their withdrawal; this could lead to them to become more nicotine dependent than slow metabolizers. Here we examine whether faster nicotine metabolism is associated with a reduced chance of cessation in pregnant women.

During the second trimester of pregnancy CYP2A6 increases its activity and, thus more rapidly metabolizes the conversion of nicotine to cotinine and then to 3HC.[15] We report on data from a trial of nicotine replacement therapy (NRT) to assist cessation in pregnancy. Although there was evidence that NRT improved the rate of cessation four weeks into pregnancy,[16] there was no evidence that NRT was effective by late pregnancy, a finding consistent with a prior meta-analysis[17] and a recent randomized control trial (RCT) of NRT in pregnancy.[18] Almost all women recruited in the Smoking, Nicotine and Pregnancy (SNAP) trial tried to stop smoking in the second trimester of pregnancy when the nicotine in the patch may have been more rapidly metabolized than would have been the case prior to pregnancy.[15,19–21] This could have undermined its effectiveness. We therefore examine whether the rate at which women metabolize nicotine modified the effectiveness of NRT in pregnancy. Such a finding would suggest that higher dose NRT may be needed to support cessation in pregnant women.

In this study, we used data from a large RCT of NRT in pregnancy to describe the characteristics of NMR in pregnant smokers, investigate whether or not the rate of nicotine metabolism was associated with cessation and, if increased metabolism was associated with decreased effectiveness of NRT in pregnancy.

Methods

Data Source

Data for this study were derived from the SNAP trial, The SNAP trial was a randomized controlled trial of nicotine replacement therapy for smoking cessation in pregnant women motivated to quit.[16] One thousand and fifty participants, who were aged 16–45 years, 12–24 weeks gestation at recruitment, smoked ≥10 cigarettes prior to pregnancy and smoked ≥5 cigarettes currently in pregnancy, and had exhaled carbon monoxide (CO) readings of ≥8 ppm, were randomized to receive up to 8 weeks supply of either 15 mg/16 hr nicotine patch or matched placebo. Participants set a quit date and were followed up 1 month after this, and at delivery or shortly after.

Baseline Data

Prior to randomization, data from the participants were collected which included: date of birth, ethnicity, age on completion of full time education, heaviness of smoking index (HSI),[22] partners' smoking status, parity, gestational age and previous use of NRT during their current pregnancy. Blood samples were taken for cotinine estimation, along with exhaled CO readings to estimate nicotine and tobacco smoke intake respectively. Trial recruitment site and participants' treatment assignment (i.e., NRT or placebo) were also available from the trial database.

Derivation of NMR

Analysis of all baseline blood samples was carried out by ABS Laboratories Ltd., at the end of the study. Blood samples were frozen at −20 °C on the day of collection in BD Vacutainer Gold top tubes. Samples were later transported from sites in insulated containers and further storage of the haemolyzed samples was carried out at −80 °C until they underwent cotinine analysis. The stability of the cotinine levels was assured by the laboratory, and they assayed approximately 10% of samples twice to demonstrate robustness of the method. Cotinine and 3HC measurements were derived from samples using Liquid Chromatography tandem mass spectrometry assay, which is a widely used a reliable method.[23] Blood samples underwent following plasma assays: free cotinine (ng/ml), free 3HC (ng/ml), total cotinine (ng/ml) and total 3HC (ng/ml). From this, free and total 3HC: Cot ratios were calculated.[24] Pregnant women who smoke more cigarettes have higher concentrations of "free" and "total" cotinine. The affinity of nicotine to nicotinic acetylcholine receptors is higher in smokers than nonsmokers,[8] but since all those included in the SNAP trial were verified as smokers before inclusion, nicotine receptor affinity was not assumed to differ greatly between individuals and affect the NMR. A higher NMR (3HC divided by total cotinine) indicates faster nicotine metabolism, with more of the parent metabolite, cotinine, having being metabolized into the downstream metabolite, 3HC. The present analysis used a NMR derived from total cotinine and total 3HC as the analysis by ABS Laboratories indicated no significant differences between the "free" and "total" values when glucuronides were hydrolyzed, that is, free and total cotinine values were the same.[25] When investigated, we found the values to be highly correlated ($R^2 = 0.865$).

Outcome Data: Validated Cessation

Cessation was defined as continuous abstinence from quit date to the respective follow-up point. At 1 month, a CO reading of <8ppm was used for validation. At delivery, either CO validation as above, validation using saliva cotinine (<10 ng/ml), or both of these methods

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were required. Women who had a missing value for whether they achieved cessation, that is, were lost to follow-up, were categorized as continuing smokers, which is consistent with the Russell standard.[26]

Analysis Strategy

Characteristics of the Study Sample

Participants were included in the analysis if we were able to derive values for free and total cotinine measurements from the blood sample provided at baseline. The baseline characteristics of participants included in analyses were compared with those excluded from the analyses.

Associations Between Baseline Variables and the NMR

Univariate linear or logistic regression was used to describe any potential associations between selected baseline characteristics (i.e., baseline cotinine level, HSI, gestation, maternal age and ethnicity) and NMR. As NMR was approximately normally distributed it was used as a continuous variable in analyses.

Associations Between the NMR and Cessation

Logistic regression was used to investigate the associations between NMR and achieving cessation at 1 month post-quit date and at delivery, adjusted for treatment assignment. Treatment assignment was adjusted for because the main results from the SNAP trial found NRT significantly increased the odds of cessation at 1 month post-quit date[16] and the data used in the present study is from a RCT. Since in nonpregnancy, there are some studies that show weak associations between NMR and the number of cigarettes smoked, and some that show none,[14] when examining the association between NMR and cessation, we adjusted for markers of dependence (HSI and the number of cigarettes). This is done to permit comparison with and discussion of other studies.

Interaction Between Treatment Assignment and NMR With Respect to Cessation

We were interested to know whether the relation between NMR and cessation was the same in both NRT and placebo trial groups and whether any impact of NMR on cessation might be modified by treatment with NRT. NRT may not be as effective in pregnancy because of an increase in rate of nicotine metabolism.[13] We next tested for any interaction between nicotine metabolism (NMR) and treatment allocation (i.e., placebo or NRT patches) on the odds of cessation at 1 month. To maintain power for the interaction test, NMR was included as a continuous variable.

All analyses were conducted using Stata 11.2. Significant associations in these tests were defined by $p \leq .05$ significance level.

Results

Characteristics of Study Sample

Of the 1,050 participants enrolled to the SNAP trial,[16] baseline blood samples for measuring NMR were taken from 673 (64.1%); 9 individuals were excluded because blood samples were not received at the laboratory. Two participants were excluded because they reported using NRT in the 7 days prior to blood samples being given and this could have affected cotinine levels. All analyses were undertaken in the remaining 662 (63.0%) participants. This sample of 662 women consisted of those who provided blood samples and received either placebo or NRT patches.

Baseline characteristics of participants included and excluded from the analysis are detailed in Table 1. Women who provided samples for cotinine analysis and those who did not were similar in all baseline characteristics compared, apart from being less likely to have a partner that smoked and being less likely to be socially disadvantaged. The distribution of the NMR ranged from 0 to 0.9 and was almost normally distributed, being only weakly positively skewed; mean: 0.44 (*SD* = 0.16).

Table 1. Baseline Characteristics of Women Included and Excluded in the Study Sample

Characteristics	Included (662)	Excluded (388)	*p* value
Maternal age (years) (mean [*SD*])	26.7 (6.2)	25.6 (6.0)	.999
Ethnicity			
White British	644 (97.3%)	374 (96.4%)	.418
Asian/other background	18 (2.7%)	14 (3.6%)	
Age full time education finished (years) (mean [*SD*])	16.3 (1.46)	16.2 (1.69)	.913
Heaviness of smoking index			
0–3	439 (66.3%)	245 (63.1%)	.298
4–6	223 (33.7%)	143 (36.9%)	
Gestational age at baseline (mean [*SD*])	16.3 (3.5)	16.1 (3.5)	.829
Partners smoking status			
No partner	49 (7.4%)	38 (9.8%)	.006
Nonsmoking	176 (26.6%)	71 (18.3%)	
Smoking	437 (66.0%)	279 (71.9%)	
Parity			
0–1	455 (68.7%)	264 (68.0%)	.962
2–4	170 (25.7%)	101 (26.0%)	
>4	37 (5.6%)	23 (5.9%)	
Baseline cotinine level (ng/ml) (median [*IQR*])	136.2 (80.7)	133.9 (79.4)	.660
Treatment assignment			
NRT	341 (51.5%)	200 (51.5%)	.339
Placebo	321 (48.5%)	188 (48.5%)	
Index of multiple deprivation (mean [*SD*])	31.6 (17.1)	34.5 (16.1)	.004

Note. IQR = interquartile range; NRT = nicotine replacement therapy.

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Associations Between Baseline Variables and the NMR

The results from crude linear and logistic regressions between selected baseline variables and NMR for women were as follows. The number of cigarettes smoked prior to pregnancy was the only factor to be significantly associated with the NMR ($p = .006$); higher NMR was found in women for each additional cigarette that was smoked prior to pregnancy. There were nonsignificant associations between maternal age ($p = .190$), ethnicity ($p = .369$), HSI ($p = .560$), time to first cigarette ($p = .480$), gestational age at baseline ($p = .143$) and NMR.

Association Between NMR and Cessation

The univariable associations between NMR and validated cessation at both 1 month and delivery were strong. At 1 month, there was a 14% decrease in the odds of achieving validated cessation for every 0.1 unit increase in NMR (*OR* = 0.86; 95% CI = 0.76–0.98; $p = .026$). When adjusted for treatment assignment and HSI, the association changed minimally (*OR* = 0.87; 95% CI = 0.76–0.99; $p = .043$). The strength of association was weakened further when adjusting for the number of cigarettes smoked per day, which might be expected as it is likely to act as a mediator of the NMR: cessation relationship (*OR* = 0.88; 95% CI = 0.77–1.00; $p = .057$). At delivery, there was a 22% decrease in the odds of achieving validated cessation for every 0.1 unit increase in NMR (*OR* = 0.78; 95% CI = 0.65–0.93; $p = .005$) and after adjustment for treatment assignment and HSI there remained a strong and significant negative association (*OR* = 0.79; 95% CI = 0.66–0.94; $p = .008$). Adjusting for the number of cigarettes at delivery did not significantly change the odds of cessation either (*OR* = 0.79; 95% CI = 0.66–0.95; $p = .010$).

Interaction Between Treatment Assignment and NMR With Respect to Cessation

An interaction was fitted between NMR and treatment assignment on the odds of cessation at 1 month post-quit date, adjusted for HSI. There was no evidence of interaction at 1 month (likelihood ratio test, $p = .556$). This interaction test finding provides no evidence for an interaction between NMR, use of NRT and cessation (Supplementary Figure 1); the chance of successful cessation with NRT does not vary with NMR. If NMR does undermine the odds of cessation in pregnant women between 16 and 28 weeks' gestation, then the effect is very slight. An interaction test was not carried out for longer term cessation at delivery, as this was likely to lack power due to the small number of women who maintained cessation until delivery.

Discussion

Main Findings

In a cohort of pregnant women between 12 and 24 weeks gestation, NMR was found to be almost normally distributed, with a mean of 0.44 (*SD* = 0.16). Women with higher NMR smoked more cigarettes prior to pregnancy and had significantly decreased odds of achieving cessation. There was no evidence that NMR modified the association between assignment to NRT and increased odds of cessation earlier in pregnancy.

Strengths and Limitations

There were several advantages associated with using data from the SNAP trial for addressing the aims of this study. A first strength is that NMR was calculated using metabolites measured from plasma and this method uses a more stable measure of the NMR derived from 3HC and cotinine,[8,21] rather than nicotine and cotinine used in hair sample assays better suited to long term changes in nicotine metabolism.[19,20] Another is that, restricted time between when plasma samples for NMR were collected in all participants (most women were approximately 16 weeks pregnant), meant that the associations between NMR and cessation, as well as those between NMR and baseline maternal characteristics were not biased by variation in NMR due to changes in gestational age. In addition, robust methods were used to collect data within the SNAP trial, which included high rates of outcome ascertainment and validation of smoking status.

One limitation of this study was that women who gave samples for analysis were less likely to have partners who smoke and were more advantaged than women who did not give samples (Table 1). However, as these characteristics were unrelated to rate of metabolism (NMR), there is little reason to imagine that there would be a different association between NMR and cessation in the women who did not give samples. A possible disadvantage is that NMR was measured in a restricted gestational range, and the result of the interaction test may vary in later pregnancy. It has been shown that women's rates of nicotine metabolism will become faster as pregnancy progresses.[13] However, while the interaction result might change with a drastically faster NMR, it is unlikely that gestational increases will alter the observed association between faster nicotine metabolism in pregnancy and lower rates of cessation. Likewise, this would not affect NMR's associations with baseline maternal factors or the direction of association with cessation. As a result, the main findings presented are likely to be generalizable throughout gestation.

In this trial, women were asked not to continue using the patch if their cessation attempt failed to avoid possible toxicity to the fetus. As the large majority of quit attempts failed in the first month, only 7% were still using the patch by and after 1 month.[16] However, this may not have harmed our ability to detect an interaction between speed of metabolism and NRT as the outcome of this was successful cessation. A possible limitation of the interaction test is that there may not have been enough power to detect a weak interaction.

Findings in the Context of Previous Work

We found that the NMR was similarly distributed in pregnant women between the gestation of 12–24 weeks to that of the nonpregnant population.[21] The mean NMR of the normal metabolizer genotypes in two separate studies was found to be 0.42[27] and 0.43 respectively[28] and the overall mean of all participants in studies of nonpregnant women ranged between 0.38 and 0.44.[21,29,30] These are similar to our findings in pregnant women in the second trimester. The NMR has been found to be weakly associated with the number of cigarettes in nonpregnant smokers[14] and, similarly, there was a strong relationship with the number of cigarettes smoked prior to pregnancy in this study. Also, in the analysis of baseline characteristics associated with NMR, we found no association between HSI and NMR, which is similar to studies outside of pregnancy.[14] When adjusting for HSI the odds ratio for association between NMR and cessation remained significant, changing only minimally from the unadjusted estimates. In contrast, the statistical significance of the NMR: cessation association became nonsignificant when the number of cigarettes smoked daily (CPD) was adjusted for. However, this could be anticipated as CPD is likely to be a mediator of the relationship between the rate of nicotine metabolism and smoking

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cessation; more rapid metabolism is likely to lead to greater cigarette consumption and hence to decreased odds of cessation. Adjusting for this factor, therefore, is likely to result in over-adjustment bias and findings adjusted by CPD are presented for illustration only.

A systematic review of the NMR outside of pregnancy found a similar relationship between nicotine metabolism and cessation, but conflicting evidence for the impact of fast or slow nicotine metabolism on cessation being modified by use of NRT in nonpregnant smokers who were trying to stop smoking.[14] One study did demonstrate such moderation and this reported better cessation outcomes in slower metabolizers who used 21 mg/24 hr transdermal patches.[29] However, this was exhibited with a higher therapeutic dose of nicotine than that received by pregnant smokers who were included in analyses for this paper. As NRT is of proven efficacy outside of pregnancy[31] and, in this sample, was shown to be effective for cessation at 1 month after randomization,[16] we hypothesized that, at 1 month, the rate of nicotine metabolism would modify the relationship between NRT and cessation. We found no evidence of such an interaction between NRT, NMR and cessation at 1 month post-quit, and so, there is no indication that faster metabolism reduces the effectiveness of NRT in earlier pregnancy. By providing pregnant women that have slow nicotine metabolism, with the same strength nicotine patches as those women that had faster nicotine metabolism, we effectively gave higher dose treatment to women who metabolize nicotine more slowly. However, we found no significant improvement for the effectiveness of NRT in these women. These results support those found by Berlin and colleagues[18] and provide no evidence to suggest that the effectiveness of nicotine replacement therapy varies with women's rates of nicotine metabolism. More research using higher therapeutic doses of nicotine are required to investigate this hypothesis.

Conclusion

Pregnant women who metabolize nicotine more quickly were less likely to achieve cessation. We found no evidence to suggest that the rate of nicotine metabolism modifies the efficacy of NRT used in pregnancy.

Supplementary Material

Supplementary Figure 1 can be found online at http://www.ntr.oxfordjournals.org

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Declaration of Interests

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