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THE EFFECTIVENESS OF NICOTINE REPLACEMENT THERAPY DURING PREGNANCY; INVESTIGATING THE ROLE OF NICOTINE SUBSTITUTION, NICOTINE METABOLISM AND PREGNANT SMOKERS' EXPERIENCES

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Abstract

Smoking in pregnancy remains a significant public health issue. Nicotine replacement therapy (NRT) is effective in non-pregnant populations at assisting smoking cessation, but there is no evidence that NRT, used at standard dose, can help pregnant smokers to stop. It is established that nicotine metabolism increases during pregnancy, and so the nicotine dose delivered by NRT could be insufficient for ameliorating withdrawal symptoms throughout pregnancy. However, little is known about the level of nicotine substitution provided by NRT or the pattern of nicotine metabolism during pregnancy. Aside from increased nicotine metabolism, low adherence to NRT and the resulting insufficient substitution may explain why NRT does not appear to be effective during pregnancy. However, little is known about the experiences of women who do not adhere to the correct dosage of NRT during pregnancy. There may also be other factors involved in individuals' low adherence which have yet to be identified.

The overall aim of this thesis was to increase understanding as to why NRT does not appear to be effective during pregnancy. The thesis consists of three studies, each with a specific aim;

Study One: to compare cotinine levels in pregnant women whilst smoking and when using NRT

Study Two: to describe the longitudinal pattern of pregnant women's nicotine metabolism, as measured by the nicotine metabolite ratio (NMR)

Study Three: to understand pregnant smokers' reasons for non-adherence to NRT during a quit attempt

The first study involved analysing data on 33 pregnant participants from the NRT arm of a randomised control trial who had stopped smoking and were still using 15mg/16hr nicotine patches 1 month after quitting. Salivary cotinine levels when smoking at baseline were compared with levels on NRT at 1 month. Cotinine levels were lower than those achieved from smoking (median 98.5ng/ml while smoking and 62.8ng/ml while using NRT and remaining abstinent, p = 0.045).

The second study involved 101 pregnant smokers recruited from hospital antenatal clinics, who were asked to provide saliva samples to measure NMR at 8-14 weeks (n=98), 18-22 weeks (n=65), 32-36 weeks gestation (n=47), 4 weeks postpartum (n=44) and 12 weeks postpartum (n=47). NMR changed over time (p=0.0006). Compared with NMR at 12 weeks postpartum, NMR was significantly higher at 18-22 weeks (26% higher, 95% CI 12% to 38%) and 32-36 weeks (23% higher, 95% CI 9% to 35%). There was no significant difference between the 8-14 weeks gestation or 4 weeks postpartum NMR and 12 weeks postpartum.

The third study was a qualitative study that involved semi-structured telephone interviews with 14 pregnant smokers who had recently been prescribed NRT, but self-reported low NRT adherence or discontinuing treatment prematurely. Thematic analysis was used to analyse data. Most

women smoked regularly while using NRT and many used NRT to cut down their cigarette intake rather than to quit abruptly. Some women were concerned that using NRT instead of smoking could actually increase their nicotine intake and nicotine dependency or cause greater harm to the fetus, and consequently often underutilised NRT.

This thesis supports the hypothesis that NRT at standard doses may be ineffective in pregnancy due to increased metabolism. The effectiveness of NRT may be hindered by the way in which NRT is used in pregnancy. Women underutilise NRT, smoke simultaneously and may lack understanding about the safety of NRT in relation to continued smoking. The findings from this thesis will aid the development of future research to improve NRT adherence during pregnancy and highlights the need to investigate the effectiveness of NRT at higher doses.

Publications and conference papers

Publications from thesis work

K Bowker, S Lewis, T Coleman, L Vaz, S Cooper. Comparison of cotinine levels in pregnant women whilst smoking and when using Nicotine Replacement Therapy. Nicotine and Tobacco Research. 2014, 16 (6) doi: 10.1093/ntr/ntu029

K Bowker, S Lewis, T Coleman, S Cooper. Changes in the rate of nicotine metabolism across pregnancy: a longitudinal study. Addiction. 2015, 110 (9), doi: 10.1111/add.13029

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Conference poster presentations from thesis work

K Bowker S Lewis, T Coleman, S Cooper. Changes in the rate of nicotine metabolism across pregnancy: a longitudinal study. Society for Research on Nicotine and Tobacco, Philadelphia, 25th -28th February 2015

K Bowker, S Lewis, T Coleman, L Vaz, S Cooper. Comparison of cotinine levels in pregnant women whilst smoking and when using Nicotine Replacement Therapy. UK Society for Behavioural Medicine 9th annual scientific meeting, Oxford, 9th -10th December 2013

Oral presentations and seminars from thesis work

K Bowker S Lewis, T Coleman, S Cooper. Changes in the rate of nicotine metabolism across pregnancy: a longitudinal study. UK Nicotine & Smoking Cessation Conference, Manchester, 11th-12th June 2015

K Bowker. Changes in the rate of nicotine metabolism across pregnancy: a longitudinal study. School of Epidemiology and Public Health, University of Nottingham, lunch time seminar session 16th March 2015

K Bowker. Changes in the rate of nicotine metabolism across pregnancy: a longitudinal study. University of Nottingham, School of Medicine presentation event for postgraduate research students, 6th January 2015

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Declaration

My supervisors Dr Sue Cooper, Professor Tim Coleman and Professor Sarah Lewis provided guidance for this thesis, advised on the design of the studies and contributed to the arising thesis drafts and publications. The role I played in the three studies conducted as part of this thesis is as follows:

Study One: Comparison of cotinine levels in pregnant women whilst smoking and when using nicotine replacement therapy

I designed this study with the assistance of my supervisors. This study involved secondary analysis of data from the Smoking Nicotine and Pregnancy (SNAP) trial that investigated whether NRT is effective during pregnancy, which was led by Professor Tim Coleman. All supervisors provided statistical advice for the analysis of this study and commented on both thesis and publication drafts.

Study Two: Changes in the rate of nicotine metabolism across pregnancy: a longitudinal study

I designed and sought the necessary ethical and research approvals for this study, with advice from my supervisors. Along with Denise Lochrie, a midwifery researcher from the Clinical Research Network we recruited all participants into this study. I coordinated and managed the follow up samples and survey collections. Nottingham Health Science Biobank stored the study samples. ABS laboratories analysed all the study samples. All supervisors provided statistical advice for the analysis of this study,

guidance on the study management and commented on both thesis and publication drafts.

Study Three: Understanding pregnant smokers' adherence to nicotine replacement therapy during a quit attempt: a qualitative study

I designed and sought the necessary ethical and research approvals for this study, with advice from my supervisors. Nottinghamshire New Leaf and New Leaf Nottingham CityCare stop smoking services assisted with recruitment for this study, by referring women who were interested in taking part in the study. I contacted all potential study participants and was responsible for obtaining informed consent. I conducted all the surveys and interviews. Clayton Research Support transcribed all the interviews for this study. I led the analysis and writing of the study findings with support from Dr Kasia Campbell from the University of Nottingham, Dr Felix Naughton from the University of Cambridge and my supervisors.

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Abbreviations

BMI	Body Mass Index
CBT	Cognitive Behavioural Therapy
CHN	City Hospital Nottingham
CI	Confidence Interval
CO	Carbon Monoxide
COT	Cotinine
ETS	Environmental Tobacco Smoke
FTND	Fagerstrom Test for Nicotine Dependency
GP	General Practitioner
HSI	Heaviness of Smoking Index
IFS	Infant Feeding Survey
IQR	Interquartile Range
MCS	Millennium Cohort Survey
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NMR	Nicotine Metabolite Ratio
NRT	Nicotine Replacement Therapy
ONS	Office for National Statistics
OR	Odds Ratio
OTC	Over The Counter
PIS	Participant Information Sheet
QMC	Queens Medical Centre
RCT	Randomised Control Trial
RR	Risk Ratio
SATOD	Smoking at the Time of Delivery
SNAP	Smoking Nicotine and Pregnancy Trial
SD	Standard Deviation
SSS	Stop Smoking Services
WHO	World Health Organisation
3HC	3 Hydroxycotinine Cotinine

Chapter One: Background

1.1 Harmful effects of smoking in pregnancy

Smoking in pregnancy is a major public health issue; tobacco exposure is one of the most dangerous risks to pregnancy [1,2]. This section will review the large body of research that has explored the risks between maternal smoking and adverse health outcomes during pregnancy and the subsequent health and development of the offspring.

1.1.1 Complications in pregnancy

1.1.1.1 Miscarriage

Smoking in pregnancy is associated with many adverse pregnancy outcomes, such as placenta abruption, placenta previa [3] and fetal growth restriction[4]. A systematic review and meta-analysis of the risk of tobacco exposure and miscarriage found women who reported smoking at least one cigarette per day were at an increased risk of miscarriage (RR, 1.23; 95% CI, 1.16 to 1.30, n=50 studies). The risk increased with the reported number of cigarettes smoked per day; for every additional cigarette smoked per day there was a 1% increase in risk of miscarriage [5].

1.1.1.2 Low birthweight

Smoking during pregnancy increases the risk of low birthweight. A prospective cohort study of 7098 pregnant women found women who smoked during pregnancy were at an increased risk of having a baby with low birth weight (<2500g) (OR, 1.75; 95% CI, 1.20 to 2.56) compared to women who did not smoke. Often low birthweight is a consequence of pre term delivery or a reduction in fetal growth[6].

1.1.1.3 Preterm delivery

A systematic review with a meta-analysis component (n=20 studies) found a significant association between smoking and preterm delivery (<37 weeks gestation) when compared to women who were not reported smokers (OR, 1.27; 95% CI, 1.21-1.33)[7]. A dose response relationship was identified; compared to non-smokers, 'heavy' smokers (>20 cigarettes per day) had 31% greater odds of preterm birth (OR, 1.31; 95% CI, 1.20 to 1.42), whereas for 'light' smokers (<20 cigarettes per day) the risk was 22% higher (OR, 1.22; 95% CI, 1.13 to 1.32)[7]. However, as the CIs overlap it could be that light and heavy smoking has similarly harmful effects. The duration of smoking in pregnancy was unknown so the impact of this is not measured.

Another study which explored associations between preterm birth (<37 weeks) in women who smoked found that smoking during early pregnancy (<3 months) was associated with an increased risk of preterm birth (OR, 1.30; 95% CI, 1.25 to 1.36)[8]. This study used data from a large birth cohort (n=776,836) and contained information on tobacco use 3 months prior to pregnancy and use at the antenatal appointment (<15 weeks gestation).

1.1.1.4 Fetal Growth Restriction

Maternal smoking is also associated with a reduction in fetal growth[6,9]. Fetal growth restriction results in low birthweight, and can cause the fetus to have a smaller head circumference, reduced abdominal circumference and reduced femur length[10,11]. Findings from a population based cohort study of 7098 pregnant women found that maternal smoking resulted in

reduced femur length from mid pregnancy (-019 mm/week; 95% CI:-0.23 to -0.14) and from 25 weeks onwards smaller head circumferences were seen in the babies of women who smoked during pregnancy (-0.56 mm/week; 95% CI -0.73 to -0.40)[10].

1.1.1.5 Stillbirth

A systematic review and meta-analysis of the risk factors associated with stillbirth found from the 96 observational and case control studies that smoking in early pregnancy was associated with an increased risk of stillbirth compared to women who did not smoke (OR, 1.36; 95% CI, 1.27 to 1.46) [12]. A cohort study of 25,102 singleton pregnancies containing 7503 reported smokers (of >10 cigarettes per day) after the 16th week of pregnancy, found smokers had an increased risk of stillbirth compared to women who were non-smokers (OR, 1.8; 95% CI, 1.2 to 2.8)[13].

1.2 Beneficial effects of quitting smoking during pregnancy

There is evidence to suggest if exposure to tobacco smoke is discontinued during pregnancy then outcomes such as pre term delivery [7,8], still birth[13], fetal growth restriction and[10] low birth weight[11] are reduced and in some cases comparable to women who have never smoked. It remains unclear from the research when the optimal time for quitting smoking during pregnancy is and how this influences pregnancy outcomes. Baba et al found that women who stopped smoking by their first antenatal appointment were at no greater risk of a preterm birth compared to non-

tobacco users[8]. Similarly, women who quit smoking in the first trimester of pregnancy are at no increased risk of stillbirth when compared to non-smokers[13]. However, the exact time when women stopped smoking in the first trimester is unknown, so the point at which exposure may influence pregnancy outcomes could not be determined in both these latter studies.

A study by Jaddoe et al found women who stopped smoking when pregnancy was first known were not at an increased risk of low birth weight compared to non-smokers[11]. Yet, in a different larger cohort study of 18,178 women it found that smoking just before pregnancy (in the 3 months prior) did increase the risk of low birth weight compared to those that were not smoking just before pregnancy (OR, 1.98; 95% CI, 1.62 to 2.41)[14]. This latter study also found that women who quit or reduced their smoking level during pregnancy still had an increased risk compared to those that never smoked, but changes in smoking behaviour were associated with a reduced risk of low birth weight by up to a third compared to continued smokers (OR, 0.65; 95% CI, 0.50 to 0.84)[14]. The exact time when tobacco smoke exposure was reduced or stopped was not reported. The differences in results for these studies could reflect the transient nature of smoking during early pregnancy and differing exposures to environmental tobacco smoke (ETS) which were not accounted for.

A systematic review with meta-analysis found that non-smoking women who were exposed to ETS had infants that weighed 33g less (95% CI 15.7 to 51.3) than non-smoking women who were not exposed to ETS. ETS also increased the risk of low birth weight by as much as 32% (OR, 1.32; 95% CI, 1.07 to 1.63)[15]. These results indicate that any form of exposure to tobacco smoke can have negative health implications on the pregnancy.

1.3 Implications of smoking in pregnancy on the infant

Maternal smoking in pregnancy has been associated with an increased risk of childhood morbidities in the offspring. The findings discussed in this section will concentrate on the effects of smoking in pregnancy and adverse health implications on the infant. However, within many of the studies discussed it is often difficult to separate out the in utero effects of smoking within pregnancy and the effects of ETS on the child after birth, especially as women that smoke during pregnancy are likely to continue to smoke afterwards[16].

1.3.1 Birth Defects

A systemic review of 172 studies found a relationship between birth defects and maternal smoking during pregnancy[17] . This study found positive associations between maternal smoking in pregnancy and birth defects, including cardiovascular and heart defects (OR, 1.09; 95% CI, 1.02 to 1.17), musculoskeletal defects (OR, 1.16; 95% CI, 1.05 to 1.27) and limb reduction defects (OR, 1.26; 95% CI, 1.15 to 1.39). In some cases, maternal smoking was protective of certain defects, such as hypospadias (OR, 0.90; 95% CI, 0.85 to 0.95).

1.3.2 Sudden infant death

A meta-analysis, which looked at 35 case control studies, measured the associations between smoking during pregnancy and postnatally with sudden infant death syndrome; it found that smoking antenatally (OR, 2.25; 95% CI, 2.03 to 2.50) and postnatally (OR, 1.97; 95% CI, 1.77 to

2.19) increased the risk of sudden infant death [18]. There was a dose dependant relationship; infants of women who smoked more than 10 cigarettes per day had an increased risk compared to those smoking less.

1.3.3 Obesity and metabolic disease

A systematic review of the risk factors surrounding child obesity between the ages of 3 and 8 years found mothers who smoked during pregnancy had a 47% increased risk of their child being overweight (95% CI, 1.26 to 1.73, n=7)[19]. Other studies have linked maternal smoking to obesity in offspring, and have also found associations between maternal smoking and type 2 diabetes in offspring [20]. It is speculated that smoking in utero may cause metabolic deregulation, which may lead to obesity and subsequently diabetes [20]. Maternal smoking has not been found to increase the risk of type 1 diabetes in offspring [21]; instead smoking was found to have a protective effect [21,22]. However, these latter associations did not account for when smoking took place in pregnancy, changes over time and the number of cigarettes smoked.

1.3.4 Respiratory and auditory disorder

Maternal smoking, both antenatally and postnatally, has been associated with childhood asthma and other breathing difficulties. A systematic review of 79 prospective studies looking at incidences of asthma and wheezing in relation to antenatal, postnatal and passive smoking found that fetal exposure to tobacco smoke in utero increased the risk of both asthma and wheezing in childhood[23]. The risk of asthma increased by 40% and wheezing by 22% in offspring of smoking mothers; the strongest

association was seen in mothers who smoked in pregnancy and whose child was under the age of 2 years[23].

Smoking in pregnancy has also been linked to ear infections and ear complications in the offspring[24,25]. A cross sectional study found a slight increase in the risk of an ear infection occurrence in the offspring of women who smoked during pregnancy (OR, 1.08; 95% CI, 1.01 to 1.14)[25]. A prospective study by Stathis et al found a dose response relationship between maternal consumption of cigarettes and risk of ear infections[24]. In this study 'light' smokers at the first antenatal appointment (1-9 cigarettes per day) had increased odds of having a child who would get an infection (OR, 1.6; 95% CI, 1.1 to 2.5) and this increased in 'heavy' smokers (>20) (OR, 2.9; 95% CI, 1.3 to 6). Heavy smokers were also more likely to have offspring that were at an increased risk of having ear surgery by the age of five (OR, 2.9; 95% CI, 1.3 to 6.6)[24].

1.3.5 Psychological implications

Maternal smoking has been linked to psychological problems in offspring, such as hyperactivity, attention deficit disorder [26] and conduct problems such as aggressive behaviour[27,28]. A limitation of studies exploring the psychological effects for the offspring of smokers is the difficulty in separating the effects of smoking from potential confounding factors, such as socioeconomic factors and parenting styles. In addition, many studies do not account for changes to smoking patterns during pregnancy, making it difficult to establish if changes to tobacco smoke exposure during pregnancy are related to psychological outcomes.

Psychosocial problems in offspring are associated with other factors aside from smoking during pregnancy such as maternal nutrition, poverty, illness during pregnancy and high maternal psychosocial stress[29]. One study tried to account for potential confounding factors by comparing differentially exposed siblings; it found that maternal smoking was not directly associated with any form of anti-social behaviour[30]. Findings have been inconsistent because another found that smoking was a risk factor for conduct problems in children[31]. This latter study attempted to take into account the effects of parenting on conduct problems, by exploring genetically unrelated mother-child pairs (adopted children). They then compared child conduct problems between children whose biological birth mothers did and did not smoke during pregnancy.

1.3.6 Effects of smoking on family smoking patterns

Adults who smoke are more likely to have children that smoke; findings from a systematic review and meta-analysis involving 58 studies, mainly from the US and Europe, found parental smoking increased the risk of smoking in the offspring as an adolescent [32]. This risk was strongest if the mother was a smoker, whereby the child was more than twice as likely to become a smoker if their mother was a smoker (OR 2.19; 95% CI, 1.73 to 2.79).[14]

1.4 Summary of the harmful effects of smoking on pregnancy and the infant

There is a large body of research, which has investigated the health consequences of maternal smoking during pregnancy. There is a strong causal relationship between smoking and adverse pregnancy outcomes.

There are however, methodological limitations as the studies discussed are mostly based on observational data and many of the studies rely on maternal self-report of smoke exposure that may be subject to either underreporting or recall bias. While these studies have accounted for multiple potential confounders, there still may be factors that are unaccounted for in the multivariate analyses that may explain the differing outcomes between smokers and non-smokers[33]. However, despite some limitations, evidence consistently shows associations between tobacco exposure and adverse maternal and fetal health risks; often a biological gradient exists and greater exposure to tobacco results in increased health risks.

1.5 Prevalence and smoking patterns during pregnancy

Despite smoking in pregnancy being a major health risk, many women continue to smoke during pregnancy. In order to assess the magnitude of the problem the following section will discuss the data available on the current prevalence of smoking in pregnancy. It will also review both international and national studies that have aimed to describe smoking trends and smoking behaviours during pregnancy.

1.5.1 Current prevalence of smoking in pregnancy

Data from the Millennium Cohort Survey (MCS), a UK study which disproportionally sampled to ensure that there was adequate representation from all four UK countries, underprivileged areas and areas with high levels of ethnic minorities, showed that 35.3% of UK women smoked at some point during pregnancy, and 28% of women were smoking at 9 months after having their baby [34]. However, this data was

collected in 2001 and may be outdated. The Infant Feeding Survey (IFS), a UK study carried out every 5 years using a representative sample of mothers selected from the birth register, aims to describe data on breastfeeding, smoking and socio-economic factors. The survey was conducted when the baby was between 4-10 weeks of age and collected information on smoking before, during and after pregnancy. The findings from the most recent 2010 IFS show that 26% of women smoked at some point during pregnancy and 12% of women continued to smoke throughout [35]. Compared to results from the 2005 IFS, which collected comparable data on smoking, the smoking rate in pregnancy is declining; in 2005 33% of women smoked at some point during pregnancy and 17% smoked all the way through [35]. However, despite the decrease, the number of women smoking in pregnancy remains high.

In England, the current total equates to approximately 255,000 infants being exposed to tobacco smoke annually in utero, and 144,500 continuously so [35,36]. Routinely reported data conducted in England called the Smoking Status at Time of Delivery (SSTOD) recorded information on the number of women smoking at time of delivery. This report found that between 2014-2015, 11.4% of women were smoking at delivery, which is comparable to the IFS[37]. Reducing smoking in pregnancy is an NHS priority; the Department of Health stated the target prevalence for 2015 is 11% smoking rate at delivery [38].

1.5.1.1 Accuracy of smoking prevalence

Large cohort studies and data sets such as the IFS, SSTOD and MCS measure smoking in pregnancy using maternal self-report and is not

chemically validated. A limitation of self-report could be that women underestimate their level of smoking, or do not disclose smoking in pregnancy. As these cohort studies do not involve a smoking intervention where there is an expectation for women to quit smoking, it may be less likely women would deliberately not disclose their smoking status accurately[39]. Data for the IFS was collected when the baby was between 4-6 weeks of age and the MCS approximately 9 months after birth, therefore responses may be subject to recall bias. However, despite some limitations these large cohort surveys offer estimations on smoking prevalence based on representative samples of women within the UK.

Retrospective studies that have biochemically validated self-reported smoking have been able to provide some insight into the rates of non-disclosure of smoking in pregnancy. A US study compared self-reported smoking at the end of pregnancy with cotinine levels from saliva samples taken at a similar time from a group of women who were part of a smoking cessation trial. Despite the women being aware that self-reports on smoking were going to be biochemically validated, of the 197 reported non-smokers there were 28 women who had cotinine levels above 24ng/ml, which was a level the study defined as indicative of active smoking[40]. In a retrospective Scottish cohort study, routine disclosure of self-reported smoking status in the first trimester was compared with cotinine levels in blood samples taken as part of routine screening blood tests in the second trimester of pregnancy. Analysis of cotinine levels in blood samples indicated that 30% of women were smokers, yet only 24.1% self-reported smoking[41]. However, the discrepancy may not be due to non-disclosure and could be that women who had quit in the first

trimester had relapsed by the time the blood samples were taken in the second trimester.

A study set in New Zealand compared cotinine levels from routine screening bloods taken in pregnancy in the first and third trimester with self-reported questionnaire data about smoking in pregnancy, which was collected retrospectively when the baby was between 4-8 weeks of age. They found that women under-reported smoking; cotinine levels in the first trimester suggested 31.3% were smoking, and 27.7% in the third trimester, however self-reported levels were 19.2% and 15.7% respectively. Of the women who did report smoking, they often under-reported the amount of cigarettes smoked, particularly women who were heavy smokers (≥10 cigarettes per day)[42]. A limitation of the latter study is that women were questioned retrospectively and findings could be subject to recall bias , so there may be systematic differences in the women who could accurately recall their smoking status or level.

1.5.1.2 Reasons for non-disclosure

Public perceptions of smoking in pregnancy are negative, and it has been hypothesized that some pregnant women conceal smoking during pregnancy in order to avoid this negativity [43]. For example, a study that involved 529 psychology students rating vignettes of pregnant smokers and non-pregnant smokers found that participants held stronger judgemental views about pregnant smokers compared to the non-pregnant smokers. Pregnant smokers were rated as being ignorant, selfish and unhealthy [43]. This study was based in Australia and set among a group of

non-smokers from affluent backgrounds; therefore these participants may not represent the views of wider audiences.

A qualitative study set in Australia, which interviewed 11 women who had continued to smoke during their most recent pregnancy (within the previous 2 years), found that women were aware of negative societal attitudes towards smoking in pregnancy. Several women reported they had concealed their smoking in a range of contexts, including concealment from health care providers, and made efforts not to smoke visibly when in view of the general public. Their reasons for concealment were to avoid social stigma, and the feelings of shame, judgement and disapproval from other people [44]. The social stigma they experienced sometimes meant they did not disclose smoking to health professionals and the consequences of concealment often created a barrier to gaining access to treatment for smoking cessation [44].

1.5.2 Smoking behaviours

Pregnancy is regarded as a 'window of opportunity' to quit smoking; women may experience higher levels of social and family support to quit [45] and develop a strong desire to seek a healthier lifestyle for the benefit of the baby [46]. Pregnant smokers are most likely to make their first quit attempt in the first trimester of pregnancy, soon after becoming aware of their pregnancy[39,47-49]. Evidence from questionnaire data from a UK population based cohort study, which followed pregnant smokers at regular intervals throughout pregnancy and following birth, showed that while many pregnant smokers quit temporarily during pregnancy they often

relapsed later on in pregnancy and quit attempts were made throughout pregnancy[49].

Fluctuations in smoking behaviours has been identified in a US study which used data from two independent studies; one study, which was a nationally representative study, collected information on fluctuations in smoking (n=1426) and the second was a small clinic based sample (n=90) which measured the number of cigarettes smoked per day during pregnancy. Data from the large retrospective study found that of 540 previous smokers (had quit for >1 week) who reported not to be smoking at the start of pregnancy, 237 (44%) had relapsed during pregnancy and, 88 of these women then went on to make another quit attempt later on in pregnancy [47]. The smaller prospective study found the level at which women smoked during pregnancy varied between the first trimester and third trimester. The latter study was small and most of the women were working class and white, and so it is not known how generalizable these findings are. However, a larger study that explored self-reported smoking levels and patterns across pregnancy, confirmed using cotinine assays, found that smoking patterns and behaviour fluctuated during pregnancy[39]. A total of 8% of women who reported 'ever smoked, but not now' at baseline had relapsed and were smoking at the time of their last antenatal appointment and 40% of the women who reported being light smokers (1-5 cigarettes per day) at baseline were not smoking at the last visit. Women who reported being 'very heavy smokers' (>16 cigarettes per day) at baseline all reported a reduction in their cigarette intake by the final visit.

1.5.3 Summary

Given the harm smoking in pregnancy causes, current levels of smoking in pregnancy are too high. Of the studies, that report smoking prevalence many rely on self-reported data and are not biochemically validated. However, despite some limitations they provide an approximation of smoking patterns in pregnancy. Evidence has also shown that smoking behaviour fluctuates throughout pregnancy; not only do women make various quit attempts in pregnancy, but the amount they smoke varies throughout pregnancy too. These fluctuations seem to be unique to pregnancy, as non-pregnant smokers tend to have relatively stable smoking behaviour[50].

1.6 Characteristics of pregnant smokers

Smoking has been referred to as a leading cause of health inequality between the rich and poor [51]. Literature from non-pregnant populations has shown strong associations between smoking and social class; a disproportionate number of smokers are from lower social classes. Women who smoke during pregnancy similarly tend to be from lower social classes; the characteristics of pregnant smokers will be discussed in this section.

Large nationally representative cohort studies such as the IFS and MCS have found associations between smoking in pregnancy and socio-economic status. Pregnant smokers are more likely to be socially disadvantaged compared to non-smokers; mothers in routine and manual occupations are more likely to smoke during pregnancy compared to mothers in managerial and professional occupations [35]. Pregnant smokers are less likely to have educational qualifications above GCSEs in

comparison with non-smokers [34,35]. Maternal age has also been found to be associated with smoking in pregnancy with women less than 20 years being four times more likely to smoke than mothers above the age of 35.

Socio-economic differences have been identified between women who quit before or during pregnancy and those that continue to smoke. A cross sectional study, by Picket et al, used data from the MCS and compared the characteristics of pregnant women who had never smoked with women who had either quit during pregnancy (91% of quitters gave up in the first trimester), were light smokers (<10 cigarettes per day) or were heavy smokers (≥10) during pregnancy[52]. Using logistic regression models they found systematic differences between women depending on their smoking status. Women who continued to smoke during pregnancy were from lower socio-economic groups (as classified by the national statistics socio-economic classification) compared to women who either quit smoking or who were non-smokers[52]. The number of cigarettes smoked per day was also related to socio-economic factors, such that one in five single women were heavy smokers, whilst married women were least likely to smoke.

The aforementioned study by Pickett et al also looked at psychosocial characteristics by smoking category. Smoking in pregnancy was associated with increased interpersonal problems with family, friends or peers, quitters (RR 1.27; 95% CI, 1.21 to 1.33), light smokers (RR, 1.42; 95% CI, 1.36 to 1.49) and heavy smokers (RR, 1.67; 95% CI, 1.57 to 1.78)). Similar associations were found with levels of adaptive functioning such as low self-esteem and health related behaviour problems such as unplanned pregnancy; women who smoked were more likely to have an increased risk of having these issues[52]. A limitation of this study is that

data were collected 9 months following the birth of their baby, and therefore subject to recall bias. In addition, information on cigarette smoking, and mental wellbeing were self-reported so there is the possibility that women may have not accurately disclosed this information; however, the MSC was not an interventional study so non-disclosure may be less likely.

In a recent Nottinghamshire prospective cohort study, which explored smoking patterns throughout pregnancy and postnatally, the characteristics of women who smoked during pregnancy reaffirmed the socio-economic gradients observed in previous national cohort studies[53]. This local study also compared the characteristics of pregnant smokers who stopped smoking before or on finding out they were pregnant with those that continued to smoke after finding out they were pregnant. Significant socio-economic differences were found between ex and current smokers, with current smokers tending to be younger, less likely to have formal educational qualifications, less likely to own their own homes and less likely to be in current employment compared to women who quit before or soon after finding out they were pregnant [53]. However, as this study took place in a local setting these results may not be generalizable to wider populations.

Overall, evidence has shown that smoking in pregnancy is a significant public health issue and a source of great inequality. Pregnant smokers are likely to be of lower socio-economic status, younger and more likely to have psychosocial issues compared to non-smoking women. The effect of smoking in pregnancy results in the offspring being exposed to health inequality even before birth. An understanding of the characteristics of

women who smoke in pregnancy may help to identify how health inequality is linked to social inequality, and vice versa. This understanding may help direct smoking cessation interventions to target certain groups, and individualise the support based on specific needs.

1.6.1 Relapse after pregnancy

Many women who stop smoking in pregnancy restart after having their baby[54,55]. Data from the MCS, found that 57% of women who had quit smoking during pregnancy had relapsed within 9 months of giving birth[55]. Similar findings were reported in a Polish study, which found 50% of women were smoking again within a year after giving birth[54]. Socio demographic factors have been associated with relapse, with women from deprived social groups being more likely to relapse[55-57]. These factors include having other smokers in the household[57,58], living in an urban area and having more than three children [57]. Women who breastfeed are less likely to relapse postnatally compared to women who bottle-feed[56,57]. There is some evidence that maternal weight concerns postnatally can also influence smoking relapse, with women using cigarettes as a way to supress their appetite and control weight[59].

1.7 Smoking cessation interventions in pregnancy

There are large numbers of women who quit smoking when they become pregnant, and many do so without receiving support from health professionals. However, many women cannot quit alone and require smoking cessation support. This section will discuss the current pathways for offering smoking cessation support to pregnant women and will then discuss smoking cessation interventions and their effectiveness.

Pharmacological interventions will be referred to briefly in this section, but will be discussed in more detail in later sections.

The National Institute for Health and Care Excellence (NICE) recommend that all pregnant women are routinely asked about their smoking at the first appointment known as the 'booking appointment' which is usually with a midwife [60]. However, research has shown that many pregnant smokers decide to quit smoking without using interventions, and stop smoking after finding out they are pregnant, before receiving advice or an intervention from a healthcare provider [46]. A large retrospective cohort study found that 71% of women reported quitting in the first trimester of pregnancy, with most quitting upon finding out they had become pregnant [47]. Literature describing the characteristics of spontaneous quitters found they were more likely to be having their first child, to be less nicotine dependant, more highly educated, have a higher income and to be married or have a partner, when compared to pregnant smokers who do not quit [46,47]. The latest NICE antenatal guidelines advise that women are asked about their smoking status regularly during pregnancy [60]. Therefore women who may have initially quit but subsequently relapse will still be identified and offered smoking cessation support during their pregnancy.

There are a variety of smoking cessation interventions available to assist pregnant women with quitting smoking; these include counselling, health education and self-help, health professional feedback, incentives, social support, and use of nicotine replacement therapy (NRT)[61]. A Cochrane review of the psychosocial interventions to aid smoking cessation during pregnancy found behavioural interventions to be effective at increasing smoking cessation among pregnant women, yet recognised there are still

significant numbers of women who cannot quit with behavioural support alone [62]. In this review, financial incentive based interventions appeared most successful at achieving smoking cessation. Yet, only four incentive studies were included and all were US based studies with small sample sizes. However, a more recent large Randomised Control Trial (RCT) (n=612) set in Glasgow found that incentivising quit attempts during early pregnancy (<24 weeks gestation) are effective at reducing smoking rates; the study reported quit rates of 8.6% in the control and 22.5% in the intervention group at 34 to 38 weeks gestation [63].

There is mixed evidence as to whether social support such as peer or partner support influences smoking cessation, and behavioural support has been found only to be effective in combination with other strategies [62]. A total of 27 studies in a Cochrane review investigated the effect of counselling interventions; pooled together these studies had a significant effect (RR, 1.44; 95% CI, 1.19 to 1.75) compared to usual care (brief information/advise to quit). However, there was no evidence to suggest this method was successful at achieving long-term abstinence (>17 months postnatal). Health educational feedback, which involved providing women with educational material such as self-help manuals or a text messaging service, was not significantly associated with quitting; however, there was some evidence to show that feedback interventions and peer support interventions influenced smoking cessation[62].

In England, women who smoke in pregnancy can be referred to specialist stop smoking services in order to receive cessation support. These services offer a wide range of psychological support to assist pregnant women in quit attempts. Data from NHS Stop Smoking Services (SSS) gives an

indication as to the numbers of women who seek professional support to assist a quit attempt. Between April 2014 and March 2015, 18,887 pregnant women set a quit date with SSS[64]. This figure has decreased from the previous year, when 19,833 pregnant women accessed smoking cessation services and set a quit date [64]. Of the women that attempted [65]to quit smoking through SSS, only 47% managed to remain abstinent at the 4 week follow up [64]. The decline in the number of women who are accessing SSS could be a result of reducing smoking rates in pregnancy. SSTOD data has shown a decline in smoking rates at delivery between 2013-2014 (12%) and 2014-2015 (11.4%)[37]. Another explanation could be that pregnant women are using novel forms of smoking cessation devices such as electronic cigarettes rather than accessing more traditional forms of support through SSS. However as the prevalence of electronic cigarette use during pregnancy is currently unknown this is just speculation.

As well as psychological support women may be offered pharmacological treatments such as nicotine replacement therapy (NRT) in order to assist a quit attempt. NICE guidance on smoking cessation support in pregnancy recommends the use of NRT once the woman has been informed of the risks and benefits of using NRT, and where smoking cessation with behavioural support alone has failed [60,66]. The following sections will aim to describe the mechanisms of NRT and its use and efficacy in both non-pregnant and pregnant populations.

1.8 General description of Nicotine Replacement Therapy (NRT)

NRT is used by both pregnant and non-pregnant smokers to aid quit attempts. This section will provide an overview of the pharmacology of NRT, and will focus primarily on its effectiveness in non-pregnant populations and the characteristics of users in non-pregnant populations, as later sections will specifically discuss NRT in pregnancy.

1.8.1 Overview of NRT

NRT provides a therapeutic dose of nicotine to help with relief of withdrawal symptoms from smoking [67]; it is usually prescribed alongside behavioural support and is available over the counter or on prescription [68]. NRT is available in the form of transdermal patches that provide a long acting supply of nicotine. The patches are available to smokers to wear for either 16 or 24 hours, and are available in varying doses. It is recommended that smokers who are highly addicted to nicotine should use both a long acting NRT product (patches) alongside a short acting form of NRT, for example gum, spray and lozenges [68]. The short acting products provide a quick releasing dose of nicotine to assist with acute nicotine withdrawal symptoms, while patches supply a continuous consistent dose of nicotine. The side effects from using NRT can be similar to the side effects of nicotine withdrawal, these include sleep disturbance, irritability, weight gain and drowsiness [68]. In order for NRT to be effective it is thought that cotinine levels achieved from using NRT might need to be similar to those achieved by smoking[67,69]. Table 1 describes how NRT is recommend for use in non-pregnant smokers who access stop smoking services[70].

Table 2 Recommended NRT use in non-pregnant populations **Error! Reference source not found.**

Information obtained from National Centre for Smoking Cessation and Training[70] To note there could be individual differences in the products and nicotine doses services offer[71].

1.8.2 Efficacy of NRT in non-pregnant smokers

A Cochrane systematic review of RCTs exploring the effectiveness of NRT for smoking cessation in non-pregnant smokers found that NRT significantly increases the chances of quitting for 6 months or longer (RR, 1.58; 95% CI, 1.50 to 1.66)[72]. Participants who used a combination of NRT products, for example using short acting products (nicotine gum, inhaler or spray) alongside a longer acting product (patch) had greater rates of smoking cessation when compared to just using a single form of NRT (RR, 1.35; 95% CI, 1.11 to 1.63). It is unknown whether this is because of increased nicotine substitution or behavioural effects of having two sources of NRT. There is borderline evidence to suggest using higher dose patches was more effective than standard doses[72]. There is no evidence to suggest longer duration of NRT use (>8 weeks) is more effective than shorter usage (< 8 weeks) nor is there evidence to support that tapering the amount of NRT used towards the end of NRT treatment is better than an abrupt withdrawal[72].

NRT can be obtained directly from a retailer 'over the counter' (OTC) or on prescription. Evidence has shown that OTC NRT increases smoking cessation compared to not using NRT at all [73,74]. A meta-analysis (11 studies) compared OTC NRT with OTC placebo and OTC NRT with a prescription. This analysis found that OTC NRT produces quit rates at 6 months similar to those when NRT is provided on prescription [74] (OR, 1.4;

95% CI, 0.6 to 3.3); the CI overlap indicating no evidence of an effect. The meta-analysis only consisted of four studies and the CI are wide which suggest that there may not have been a large enough sample size to show an effect and so more studies are necessary to determine the effects of OTC NRT. Studies exploring OTC NRT verses OTC placebo found that OTC NRT was more effective (OR, 2.5; 95% CI, 1.8 to 3.6). However, in this analysis most studies involved nicotine patches, so it is unknown if similar findings would be found with other NRT products.

There is limited evidence about the level of nicotine substitution required to increase the effectiveness of NRT; one RCT exploring the effectiveness of NRT found that after 1 week on nicotine patches (15ng/16hr) the nicotine substitution level was 56% compared to baseline smoking [75] yet this study did not explore how nicotine substitution influenced quit outcomes. The rate at which smokers break down and metabolise nicotine can vary between individuals and may influence how they respond to NRT. One study found that slower metabolisers respond better to NRT compared to faster metabolisers of nicotine; fast metabolisers are more likely to have better cessation outcomes using other smoking cessation medications such as varenicline[76] (See section 1.11 for information on nicotine metabolism). This may be because nicotine substitution supplied by NRT is higher for slower metabolisers, but again further research is required to draw any firm conclusions.

1.8.3 Characteristics of non-pregnant smokers who use NRT

In the UK, survey data from stop smoking services suggests that compared to smokers who do not use NRT, users are most likely to be middle aged, have a high nicotine dependence score (as measured by the Fagerstrom test for nicotine dependence) and are more likely to be from lower socioeconomic groups[77,78]. Using data from a cross sectional telephone survey and a cohort tracking survey of adult cigarette smokers within the same communities, a US study was able to determine characteristics of NRT patch users compared to smokers who do not use NRT[79]. The study found women were more likely to report using NRT patches than men (OR 1.32 (95% CI 1.15-1.52), and participants who were either more dependant, had a strong desire to quit, smoked more heavily, and within 30 minutes after waking were more likely to use NRT. Smokers with higher incomes were also more likely to report using patches (OR, 1.88; 95% CI, 1.42-2.50). Direct comparisons with the UK are difficult due to variations in the US payments for healthcare and health insurance[79]. This study was subject to non-response bias, as non-responders tended to be younger, single and have fewer years in education than those that responded. However, similar characteristics of NRT users have been described in another US survey (n=6,839) where users tended to be heavier smokers and had higher measures of dependence[80].

1.8.3.1 Characteristics of long term NRT users

One study explored the influences on long-term NRT use (using NRT for 1 year or over) among 1,518 smokers who were prescribed NRT from a smoking cessation services in East London[77]. This questionnaire-based

study found long-term users of NRT were more likely have a greater nicotine dependency compared to short term users[77]. This was a local study and may not be representative of NRT users nationally. However, a larger survey based study (n=9,224) set in England which explored the characteristics of long term NRT users (>3 months) found similar results[81]. Long-term users of NRT were more likely to have a stronger addiction to nicotine compared to short-term users; they also tended to be older, and more likely to be in non-manual occupations. Although these studies provide insight into the characteristics of users of NRT, they both rely on self-reported use of NRT and neither account for adherence and compliance to treatment regimens.

1.8.4 Summary

NRT is available in a variety of forms and strengths. It is an effective aid to smoking cessation for non-pregnant smokers. The characteristics of NRT users are difficult to describe as smokers obtain NRT both 'over the counter' and on prescription. Few studies have explored the characteristics of smokers who use NRT; of those available, they have found that users are more likely to be strongly addicted to nicotine. In UK studies, longer term NRT users are more likely to be from higher socioeconomic groups, this may be because NRT after a certain period of time, usually after 8 to 10 weeks, is no longer prescribed for free and smokers are required to pay prescription charges or purchase NRT from retailers.

1.9 NRT in pregnancy

This section will describe current evidence on the prevalence of NRT use during pregnancy, review the efficacy of using NRT from randomised

control trials (RCTs), and will describe the current evidence about the level of nicotine substitution provided by NRT during pregnancy. Finally, this section will review the current consensus about using NRT during pregnancy based on the evidence of its safety and effectiveness during pregnancy.

1.9.1 Prevalence of NRT use in pregnancy

NRT is available to pregnant women in the UK to support quit attempts, and can be obtained from the general practice (GP), SSS or 'over the counter'. Data on the number of pregnant women who buy 'over the counter' NRT is difficult to obtain, but there is data available which provides insight into the numbers of pregnant women obtaining NRT by other means. A survey of 141 stop smoking services for pregnant women conducted in England between April 2010 and March 2011 found of the 121 services that responded, that they all offered NRT to pregnant women. Most services (86%) offered dual therapy NRT, which involves prescribing a combination of short and long acting NRT product. One in 10 services offered NRT without behavioural support. Transdermal patches were most commonly prescribed (93%), followed by nicotine inhalators (87%)[71].

Pregnant women may also access pharmacological treatment via their GP. Data available from GP records show that 2% of all pregnant women and 11% of all pregnant smokers are prescribed NRT by their GP during pregnancy[82]. As with data from the SSS it does not describe whether NRT was used after being prescribed, although most prescriptions were only for a period of 2 weeks suggesting women did not continue treatment beyond. Unpublished data from a longitudinal cohort study set in Nottingham which

aimed to describe smoking patterns during pregnancy[53] found that 89 (11%) of the 850 participants, who were between 8-26 weeks pregnant, reported they had tried using NRT as an aid to stopping smoking during their current pregnancy. Although this latter study was relatively small and local, the findings do give an indication of the percentage of women who try using NRT in early pregnancy.

1.9.2 Efficacy of NRT in pregnancy

A Cochrane systematic review of six randomised control trials exploring the effectiveness of NRT in pregnant women concluded that there is little evidence to suggest NRT is effective at increasing smoking cessation in pregnant smokers (RR, 1.3; 95% CI, 0.93 to 1.91)[83]. The effect size is within the confidence intervals of the Cochrane review exploring NRT in non-pregnant populations, which did show NRT to be effective[84]. The wide confidence intervals suggest there was insufficient power to determine whether NRT is effective during pregnancy. Therefore, it could be that significant effect sizes may be observed with sufficient power. Four studies in the review explored the effectiveness of nicotine patches. One of these studies explored the effectiveness of 15mg/16hr NRT patches (n=521) compared to placebo patches (n=529) in pregnant women recruited between 12-24 weeks gestation. Although it did not find NRT to be effective at delivery, it was found to be effective in early pregnancy, almost doubling cessation rates in women at approximately 20 weeks gestation (OR, 2.05; 95% CI, 1.46 to 2.88) [85].

One study included in the Cochrane review explored the use of nicotine gum (2mg) (n=100) compared to placebo gum (n=94) in pregnant women

recruited in the second trimester. NRT gum was not effective in smoking cessation (13% quit rates in NRT group and 9.6% placebo at 6 weeks post quit date, p=0.45). In this study participants were able to vary the amount of gum they consumed each day, therefore participants had differing intakes of nicotine but cotinine levels were measured to ensure levels did not exceed baseline levels by more than 50%[86]. Another study included in the Cochrane review compared NRT and cognitive behavioural therapy (CBT) with CBT only, and did find NRT was effective[87]. NRT use was associated with increased smoking abstinence at 7 weeks from quit date (p=0.005) and at 38 weeks gestation (p=0.008), although it was not effective postpartum. In this study participants were able to change the type of NRT product (patches, lozenges, gum) during the study and the patch dosage was individualised depending on baseline smoking levels. Women who smoked more than 15 cigarettes daily were given higher dose patches (21mg/16hr). As differing nicotine products were used and some participants used varying combinations it is unclear what dosages of NRT were taken by participants in order to be effective. Further still, adherence to NRT was low, with the mean number of days patches, gum or lozenges were used being 23 days, 8 days and 4 days respectively, out of a potential 6 weeks of treatment.

A recent RCT not included in the Cochrane review, explored the effectiveness of NRT in 402 pregnant women in their second trimester. Women were given NRT patches (n=203) and placebo patches (n=199) in doses that were individually adjusted in an attempt to provide a 100% nicotine substitution rate. Despite the patches being adjusted to approximate baseline nicotine levels it did not find NRT was effective; 5.5% of women in the patch group and 5.1% in the placebo were smoking

abstinent just before delivery [88]. Self-reported compliance of NRT was 85%, which compared to studies included in the Cochrane review is higher[83]. This study was conducted in France in a population of women that were nicotine dependant; therefore they may not be generalizable to other populations of smokers. In addition, NRT was given in the second trimester so the effects of giving NRT to women in the first trimester of pregnancy have not been explored.

Observational evidence has shown that, during pregnancy, using a combination of long and short term acting NRT (patch and a faster acting form) is associated with higher short term quit rates compared to those attributable to using either no medication or single form NRT [89]. However, to date no randomised control trials have investigated this, so further research is required on the effectiveness of higher dose NRT.

A systematic review that investigated the predictors of smoking cessation during pregnancy found that factors such as having a partner who smoked and having higher levels of nicotine dependence were associated with lower levels of smoking cessation[90]. However, there is limited evidence about the factors that might influence pregnant women's success in quitting smoking when using NRT. One study, which used data from a large RCT, found that at one month post quit, smoking cessation was higher among women who had completed full time education and in women who had lower baseline cotinine levels taken at baseline when smoking[91]. Kwok et al found that cotinine measures are highly correlated with measures of nicotine dependency during pregnancy[92] and nicotine metabolism has been found to be inversely related to smoking cessation success[93]. Therefore, NRT may

be less effective in pregnant women with higher levels of nicotine dependence or with higher rates of nicotine metabolism.

1.9.3 Nicotine substitution from NRT

The aim of NRT is to deliver nicotine to help alleviate withdrawal symptoms, and the dose should be provided at the lowest level to assist with cravings [94] so that nicotine withdrawals can be alleviated while exposing the fetus to the lowest amounts of nicotine. The way in which nicotine is delivered varies between smoking and using NRT. After smoking cigarettes nicotine is absorbed into the pulmonary venous circulation and reaches the arteries of the brain within 10 to 20 seconds [95]Nicotine delivery from using NRT is absorbed into the systemic venous circulation system and reaches the brain at a much slower rate and lower concentration. Therefore, nicotine is delivered differently when using NRT, and smokers do not get the same intensity of reward compared to smoking a cigarette, even if venous concentrations are matched[96].

There are few studies which specifically measure paired differences in cotinine levels while smoking with those generated while using NRT and remaining abstinent from smoking in pregnancy. No studies have looked at nicotine substitution from using NRT in pregnancy, and how the level of substitution relates to quit outcomes. Some studies have found that using NRT patches for at least 24 hours [97] or 8 hours [98] results in nicotine levels similar to smoking. Conversely, other studies have found nicotine and cotinine levels were lower when using NRT patches [99] and nicotine gum compared to levels while smoking[100]. All of these studies were laboratory based and usually required participants to be hospitalised. Although

informative, the results are unlikely to accurately represent cotinine levels that are achieved by pregnant women using NRT in routine clinical practice.

RCTs in community settings that replicate how NRT is used by pregnant women in routine practice, have found that cotinine levels when using NRT are lower than levels when smoking measured at baseline[86,101]. However, where nicotine comparisons between baseline smoking and NRT have been made, continued smoking and NRT adherence is often not accurately accounted for. Therefore, it remains unknown what level of nicotine substitution is provided by the NRT. Understanding the degree of nicotine substitution supplied by NRT products in pregnancy is necessary in order to ensure NRT alleviates nicotine withdrawal symptoms, whilst avoiding nicotine levels that are greater than those from smoking.

A study conducted during pregnancy, which aimed to explore the effectiveness of NRT, individualised the dose of nicotine in the NRT to ensure that the nicotine substitution level was comparable to nicotine levels when participants smoked at baseline. The study found that women between 12 and 20 weeks gestation required between 10-30mg/16hrs of nicotine per day in order to meet their substitution level. Although this study did not find individualising doses to be effective at smoking cessation, it does provide insight into the level of nicotine in a patch that is required to approximate the nicotine level reached from smoking. The study found that the mean level of nicotine required in the patch was 18mg/16hrs. A total of 25% of participants required higher doses between 25-30mg/hrs to achieve adequate substitution [88]. Cotinine levels achieved from smoking were 119ng/ml, and were 108ng/ml at 2 weeks on NRT and 80ng/ml at 8 weeks quit on NRT.

1.10 Safety of nicotine in pregnancy

This section will discuss the evidence regarding the safety of using nicotine during pregnancy. Many of the studies discussed involve exploring the effects of 'pure' nicotine on the fetus within non-human animal studies; this is due to the ethical considerations of experimenting on human subjects. Animal studies have provided the opportunity to explore the short and long-term effects of exposure to nicotine on the animal fetus following continuous infusions of nicotine. Human based studies, which are mainly observational studies, have explored the safety of NRT and snus in the human pregnancy and offspring. NRT and snus provide an opportunity to explore the possible effects of nicotine on the human pregnancy without the mother being exposed to products of combustion from smoking and many of the other chemicals found in cigarettes. Therefore, these studies provide the closest opportunity to observe the effects of nicotine on the human pregnancy.

1.10.1 Non-human animal studies

Animal studies have shown that nicotine has physiological effects on both the mother and fetus. Animal studies offer the opportunity to explore the isolated effects of nicotine on the fetus without exposure to other chemicals which are present in cigarettes. These studies offer the ability to carry out strict, long processes under isolated conditions that would not be ethically possible in human based studies. The benefits of such conditions is that the effects of nicotine can be analysed without being undermined by potential environmental confounders that influence human studies, for example socio-economic status, weight and access to antenatal care [102].

Pregnant ewes, after being given intravenous doses of nicotine at a rate of 1.0-1.5ng/min for 10 minutes, experienced a 44% decrease of uterine blood flow lasting for up to 15 minutes after the nicotine infusion was terminated. They also experienced increased vascular resistance. However, this did not occur when lower dose nicotine infusions (0.5ng/ml) were administered [103]. Compared to the average 1.04mg of nicotine yielded from a cigarette [104], these levels are higher than a typical pregnant woman may intake from smoking and the exact level of nicotine required to cause an effect is unknown. However, they do indicate that nicotine can influence the blood flow between fetus and the mother.

Nicotine has been associated with alterations in lung development of the monkey fetus. A study involving monkeys who were administered high doses of nicotine via intravenous osmotic infusion (equivalent to >1 pack of cigarettes per day) throughout pregnancy found that the thickness of the pulmonary wall vessel in the developing fetus was increased. The authors speculated that this may explain higher rates of persistent pulmonary hypertension in the fetus of mothers who smoke[105]. Nicotine has also been shown to impair nerve generation and maturation and to cause neuronal damage in rodents [106-108], which may be result in long term alterations in fetal development[107].

Longer-term effects of nicotine exposure in utero have been found in studies involving mice and rats where nicotine exposure has been associated with increased levels of attention and memory deficits[109-111]. A study that looked at the effect of consuming tobacco alkaloid extracts in pregnancy on the offspring of rats found that it delayed the development of

the behavioural patterns and motoring skills of the offspring[111]. Two reviews exploring the risks of nicotine in pregnancy based on animal and human literature concluded nicotine is a toxic chemical and may be responsible for a multitude of detrimental effects to the fetus both neurologically and behaviourally[102,112].

There are some limitations to the animal studies. Animal studies are set in controlled experimental environments where they are often infused with high doses of nicotine in short periods. These conditions do not replicate the peaks and troughs of plasma nicotine seen from cigarette smoking. However, the animal studies may be more representative of the nicotine concentrations from continued patch use as they deliver a constant amount of nicotine resembling the actions of a nicotine patch [1,102]. It is also difficult to compare animals and humans due to differing abilities to metabolise toxins, for example rodents metabolise nicotine at a faster rate than humans[113]. Therefore, some caution should be taken when making comparisons with humans.

1.10.2 Human studies

1.10.2.1 Safety of using NRT during pregnancy

1.10.2.1.1 Laboratory settings

A few studies have reported the acute side effects of using NRT in pregnancy on the fetus and mother in a laboratory setting. One study attempted to determine if NRT compromised the fetus by monitoring 21 women while wearing 22mg/24hrs nicotine patches for 4 days in a laboratory setting. There was not a significant association between

maternal patch use and fetal cardiovascular effects[97]. A study that measured the fetal and maternal effects of NRT in six women wearing a nicotine patch over 8 hours also did not report cardiovascular changes in the fetus, and the fetal heart rate remained stable. Patch use did have a minor effect on the mother as there was an initial reduction in maternal pulse after discontinuing smoking at baseline, but after applying the patch there was a gradual increase in maternal heart rate[114].

1.10.2.2 Randomised Control Trials and Cohort Studies

Evidence from a Cochrane review exploring the efficacy and safety of NRT from clinical trials found that birth outcomes tended to be better in the NRT groups compared to placebo groups or those continuing smoking, yet these were not statistically significant when all the studies were combined. The review concluded there was insufficient evidence to ascertain whether NRT is either effective or safe in pregnancy[83]. Evidence from a large double blinded RCT reported that the only adverse birth outcome significantly associated with using NRT was an increased risk of caesarean section birth compared to the placebo group (OR, 1.45; 95% CI, 1.05 to 2.01)[85]. Most other RCTs have reported better outcomes in participants allocated to the NRT group, and in two placebo RCTs, participants in the NRT group had higher birth weight babies compared to the placebo group [86,101]. One of these explored nicotine gum, and found NRT participants had a mean birth weight of 3,287g which was higher compared to the placebo group 2,950g ($p<0.001$)[86]. A study involving participants using 15mg/16hr NRT patches found women in the NRT group also had higher birth weight babies compared to placebo (186g, 95% CI; 35g to 336g)[101]. Yet a limitation of all

these RCTs is that adherence was low making it difficult to determine the safety implications.

One placebo-controlled trial also took regular blood pressure measurements from participants who had been given individually dosed nicotine patches throughout pregnancy. They found that women using the nicotine patch had a very small but significant increase in diastolic blood pressure at the end of pregnancy of 0.02mmHg per day but there were no changes in the placebo group (p=0.02) [88]. Research from a Danish birth cohort found a slightly higher risk of congenital malformation in the offspring of non-smokers who used NRT during pregnancy; the rate prevalence ratio was 1.61 (95% CI, 1.01 to 2.58) compared to non-smoking mothers who did not use nicotine products. Comparisons between non-smoking women who did not use nicotine products and women who smoked during pregnancy found there was no difference in the risk of malformation[115].

There is little information available on the longer-term safety of the offspring of women who have used NRT during pregnancy. However, one RCT exploring the effectiveness of NRT during pregnancy followed women up 2 years after the study was completed. This study found that infants born to women in the NRT group were more likely to have survived without having developmental and behavioural problems or disability compared to infants born to women in the placebo group (OR 1.4; 95% CI, 1.05 to 1.86, p=0.023)[116]. However, this study relied on maternal self-reported responses to survey questions.

1.10.2.3 Safety of Snus use during pregnancy

Snus (Swedish snuff; a moist, powdered, smokeless tobacco) is a moist ground form of tobacco that is placed under the upper lip in the mouth. Snus contains lower levels of toxins compared to snuff products (fine ground tobacco, usually inhaled nasally) and unlike cigarette smoking there are no products of combustion, so the user avoids inhalation of some of the chemicals in smoked tobacco[117]. Snus use has been associated with increased risk of oral cancers[118], yet the overall health effects of using snus compared to smoking are lower in non-pregnant tobacco users[117]. Epidemiological studies exploring the safety of Snus during pregnancy may help to offer some insight into the isolated effects of nicotine on the human pregnancy.

A study using data from a large birth cohort (n=776,836) found that Swedish snuff use in early pregnancy was associated with increased risk of preterm birth (OR, 1.29; 95% CI, 1.17 to 1.43)[8]. However, women who stopped using snus by their first antenatal appointment were at no greater risk of a preterm birth compared to non-tobacco users; (OR, 0.92; 95% CI, 0.84 to 1.01). Another study found that using snus during pregnancy increased the risk of stillbirth compared to non-tobacco users (OR, 1.43; 95% CI, 1.02 to 1.99). Although women who stopped using snus in early pregnancy had no increased risk compared to non-tobacco users [119].

Data from the Swedish Birth registry (1999-2000), which contained information on self-reported tobacco use during pregnancy, found that snus users (n=789) had an increased risk of reduced birth weight compared to non-tobacco users (11,494). Birth weight was reduced by 39g in women who reported using snus (95% CI, -6 to -72 g) although

compared to tobacco smokers (11,240) (>10 per day) the effect on birth weight was less (-190g, 95% CI,-178 to -202g) [120]. Using the same Swedish birth register over a 5 year period (2001-2006) it was found snus use was significantly associated with preterm birth (OR, 1.27; 95% CI, 1.14 to 1.41) [121].

A study that examined the effects of snus use on birth weight using a quasi–experimental sibling design, compared the birth weight of siblings whose mothers had used snus in one pregnancy but not the other, and this enabled the researchers to account for unmeasured familial confounding. The study found snus use was not significantly associated with birth weight, suggesting that the risks of snus use on birth weight may not be as strong as previously thought after taking into account unmeasured familial factors [122]. This study relied on maternal self-report of snus use. There was no information collected about the father of the offspring, and some siblings may have had different fathers with differing genetics that could also influence birth weight.

A population based cohort study using data from the Swedish birth register compared the risk of oral cleft malformation with snus use and smoking in pregnancy; women who self-reported using snus in pregnancy had a 48% increased risk of having a baby with a cleft malformation compared to women who did not report using any tobacco products during pregnancy[123]. This risk was higher compared to women who continued to smoke in pregnancy; smokers had a 19% increased risk of oral cleft malformation compared to non-tobacco users. The Swedish birth register did not collect information about the frequency and dosage of snus consumption, so a dose dependant relationship was not explored.

1.10.2.4 Safety of Electronic cigarette use

It is estimated that 2.6 million people use an electronic cigarette in Great Britain[124]. Electronic cigarettes deliver nicotine in the absence of many of the harmful chemicals associated with tobacco smoking[125]. As electronic cigarette use is a recent phenomenon, the evidence about their safety is limited. However, studies conducted so far does suggest that electronic cigarette use exposes smokers to lower levels of toxic chemicals compared to cigarette smoking. Clinical trials that have evaluated the short-term effects of electronic cigarettes have shown some mild effects on the cardiovascular and respiratory system, yet these were far lower than the effects of smoking[126]. During pregnancy, the prevalence of electronic cigarette use is unknown and the safety of using electronic cigarettes in pregnancy is unknown. Evidence about the safety of electronic cigarettes can only be drawn from studies conducted outside of pregnancy which have shown they are safer than continued smoking[127]. There is a potential role for electronic cigarettes in pregnancy as a form of smoking cessation but there use in pregnancy should be approached with caution[70] as they are likely to contain nicotine, which as shown in section 1.10.1 can have detrimental effects on fetal development in animal studies [102].

1.10.3 Summary of the safety of nicotine in pregnancy

Animal studies have provided a unique opportunity to measure the effects of nicotine in pregnancy and have found that nicotine has adverse effects on the fetus by causing; reduced placental blood flow, alterations in fetal development and cognitive impairment in the animal offspring. Direct

comparisons with human studies are problematic as during animal studies nicotine is often administered via continuous infusions and at higher levels than humans would intake, so the delivery of nicotine does not mimic the normal peaks of plasma nicotine seen in smokers. In addition, there are variations in metabolism between animals and humans making comparisons difficult.

The isolated effects of nicotine has not been explored in human studies. Evidence about the safety of nicotine during pregnancy often comes from studies that have explored the effects of snus or NRT use. Snus nor NRT products contain 'pure' nicotine however unlike cigarettes, they do not contain the thousands of chemical products, carbon monoxide, metals and other products of combustion[128]. Snus studies indicate nicotine can still be harmful to the fetus, but often have smaller effects on birth outcomes compared to smoking. RCTs and laboratory studies that have studied NRT use in pregnancy have not shown any significant risk to the fetus. As there is no evidence to suggest that using NRT is more harmful than smoking[129] NICE guidelines currently advise that NRT can be used for smoking cessation attempts in combination with psychological interventions[66].

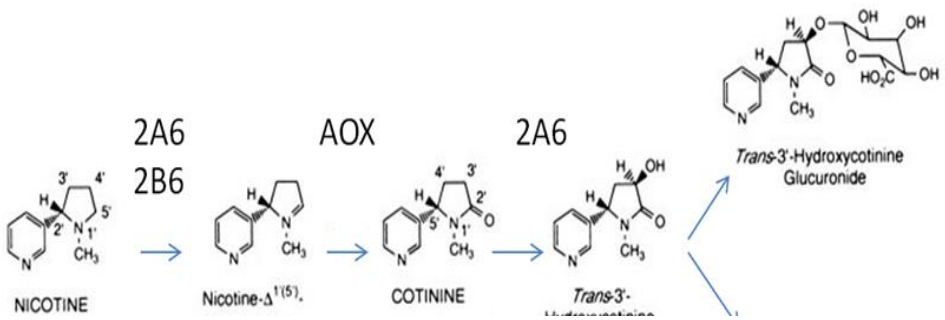
1.11 Nicotine metabolism

This section will discuss the mechanisms of nicotine metabolism and the methods of measuring nicotine metabolism before discussing factors that can potentially influence nicotine metabolism. The evidence discussed in this section mainly involves non-pregnant populations. Later sections will review evidence related to pregnancy. Understanding nicotine metabolism and the factors that influence metabolism may aid understanding of the

differences in the effectiveness of NRT between populations. This could include assisting with the accurate biochemical assessment of nicotine exposure and aid the development of pharmacological treatment for differing populations to achieve better smoking cessation outcomes.

Nicotine is primarily metabolized by the liver through the hepatic cytochrome CYP2A6; approximately 70-80% of nicotine is metabolised via this pathway [130]. The primary metabolite of nicotine is cotinine (COT) which is then converted to trans-3'-hydroxycotinine (3HC) [131] (Figure 1). Nicotine metabolism is usually measured by proxy by measuring the ratio of cotinine to nicotine or 3HC to cotinine. This ratio is a phenotypic measure of the speed of nicotine metabolism, and represents one stage of the process of nicotine metabolism known as the nicotine metabolite ratio (NMR)[130,131]. The greater the ratio the faster the clearance of nicotine[132]. There are a number of other metabolites produced from nicotine such as nicotine N'-oxide and nicotine glucuronide[1]. Nicotine is also cleared none metabolically and is excreted by the renal system, and this is not measured by the NMR[131]. Nicotine has a relatively short half-life of approximately 2 hours, whereas cotinine has a longer half-life of 16 hours[1,133] so cotinine is often used in pragmatic studies as it is more stable and less likely to be influenced by recent smoking. The 3HC to cotinine ratio can be ascertained effectively using non-invasive methods through saliva, blood and urine samples[134,135]. Each 100ng/ml of cotinine measured at steady state represents a daily intake of 8mg nicotine, which is the equivalent to 8 cigarettes[136]. However, individuals may differ in how they metabolise nicotine which can limit the ability of such measurements to detect accurate exposure [136].

Figure 1 Hukkanen Nicotine Metabolism Pathway



Adapted from Hukkanen et al. 2005. 2A6 = P450 CYP 2A6, 2B6 = P450 CYP 2B6, AOX = Aldehyde Oxidase [1].

1.11.1 Influences on nicotine metabolism

The rate at which nicotine is metabolised may differ depending on individual, environmental and genetic predispositions[130,131,137]. The impact of genetics, age, weight, gender, ethnicity, nicotine dependence, cigarette consumption and grapefruit consumption on nicotine metabolism is discussed below.

1.11.1.1 Genetic factors

There are inter-individual differences in the rate in which nicotine is metabolised[137,138]. Some individuals may lack genes that assist with cotinine formation[139] and inter-individual differences have been associated with CYP2A6 genetic polymorphisms. One study observed nicotine to cotinine formation in 10 participants, who after abstaining from smoking were given a cigarette and later nicotine gum; one participant had low cotinine formation, and on examination of their DNA found that an allele of

the CYP2A6 gene had been deleted[138]. A follow up study which used a larger sample size, administered nicotine gum to 92 healthy non-smoking participants (at least in the last 2 weeks) as determined by cotinine levels. After participants had chewed the gum, the nicotine to cotinine ratio was measured, and participants' DNA was recorded to collect the genotype of the CYP2A6 activity, which is responsible for metabolism[137]. The study found a large variation in metabolism of nicotine and this was partly explained by genetic polymorphisms in the CYP2A6 gene.

1.11.1.2 Age

There is limited and conflicting pharmacokinetic evidence about the effects of age on nicotine metabolism. A study which measured the ratio of 3HC/COT using plasma samples in 543 smokers aged between 25-65 years, found age was significantly associated with the rate at which nicotine was metabolised; for every 5 year increase in age metabolism increased by 2% (95% CI, 0.5% to 4%, p 0.02) [140]. However, another study which compared nicotine metabolism between two groups of participants aged between 22-43 years (n=20) and 65-76 years (n=23) found the older age category had a lower rate of nicotine metabolism, with nicotine total clearance being 23% less than those in the younger category (p=0.0005) [141]. An in vitro study, which looked at the role of CYP2A6 in the metabolism of nicotine by the human liver, did not find a correlation between cotinine formation and age[142].

1.11.1.3 Body weight

There is limited research as to how body weight influences nicotine metabolism. One study measured the ratio of 3HC to COT in ad libitum and

reducing smokers and found that participants with a lower body mass index (BMI) were more likely to have a higher rate of nicotine metabolism (r=-0.27; $p<0.01$)[143]. However, in another study higher body weight was associated with a faster nicotine clearance. This study applied nicotine patches in normal sized male participants (n=13), obese males (n=13) and normal sized women (n=13). After remaining abstinent from smoking for 24 hours, participants applied a nicotine patch to wear for 24 hours. The study found that nicotine maximum plasma concentration were lower in obese men compared to normal sized men ($p=0.002$)[144]. Women in this study had a higher nicotine metabolism compared to both obese and normal sized men.

1.11.1.4 Gender

Women appear to have a faster nicotine metabolism compared to men[140,145-147]. A UK study involving 545 current smokers who completed surveys about their current smoking and provided blood samples, found men had a 15% lower mean nicotine metabolic ratio compared to women ($p<0.001$) [140]. It is hypothesized that female sex hormones accelerate nicotine metabolism[146,148].

Some studies have measured hormonal contraceptive medication and explored how this influences nicotine metabolism. A US twin study of 294 participants who received infusions of deuterium labelled nicotine and cotinine found nicotine metabolism to be faster in women compared to men ($p<0.001$). A total of 25.6% of women in the sample reported using contraception and women who took oral contraceptives had a faster nicotine metabolism compared to women not using oral contraceptives[146].

The same study aimed to determine if the type of hormones within the contraception influenced nicotine metabolism, and they found that women using oestrogen only contraceptives metabolized nicotine faster than those on progesterone based contraceptives. However, there were only 10 women in the oestrogen group, therefore these findings are limited by the small sample size[146]. The study also found that premenopausal women metabolised nicotine faster than men, again suggesting sex hormones have a significant role in the metabolism of nicotine.

In contrast to adult findings, a study involving 159 adolescent participants (13-17 years of age) who received deuterium labelled cotinine did not find significant differences in nicotine metabolism between sexes(p=0.70). This study also did not find a difference between adolescent females on oestrogen contraceptives (p=0.24) or progesterone contraceptives (p=0.45) compared to adolescent females using no contraceptives [149]. However, another study exploring nicotine metabolism in 120 adolescents (13-17 years of age) did find that the plasma 3HC/COT ratio was higher for females than males (0.317 and 0.253 respectively, p=0.025). Females using hormonal contraceptives had a higher nicotine metabolism compared to females who were not, although the type of contraception was not explored[148]. Overall there appears to be significant differences in nicotine metabolism between men and women. Sex hormones may increase CYP2A6 activity, particularly in women on the oestrogen contraceptive pill.

1.11.1.5 Ethnicity

Ethnicity has been found to be associated with nicotine metabolism. A study comparing nicotine metabolism in participants after receiving

intravenous infusions of nicotine and cotinine found that the total and non-renal clearance of cotinine was faster in whites; non-renal clearance was 41.2mL/min in white participants and 34.1mL/min in black participants ($p=0.05$)[150]. A study exploring the effects of gender and ethnicity found nicotine metabolism in white adolescents was faster, as measured by the ratio of 3HC/COT, (mean ratio= 0.20), than that in Black/African Americans (mean ratio 0.14, $p=0.01$) and Asians (mean ratio 0.11, $p=0.01$)[149]. These participants were given 2mg of oral deuterium labelled cotinine and then metabolism was measured using the 3HC/COT ratio. Chinese American smokers have also been identified as having differing metabolic rates when compared to white American smokers; after receiving deuterium-labelled nicotine and cotinine, serum blood levels showed that Chinese American smokers had a lower cotinine metabolic clearance than both 'whites' and Latinos[151].

1.11.1.6 Nicotine dependence

A systematic review of cigarette dependence and NMR found no association between the result of questionnaires measuring dependence such as the Fagerstrom test and nicotine metabolism. Nicotine cravings were also not found to be associated with nicotine metabolism[131]. However, the review did find evidence that showed nicotine metabolism was related to how smokers responded to stop smoking medications, in particular bupropion and NRT. The review found slower metabolisers were more likely to be abstinent at the end of treatment courses compared to faster metabolisers. The authors speculated that slower metabolisers who respond better to NRT may receive therapeutic levels of nicotine when using NRT compared to faster metabolisers[131].

Although, many studies have not found metabolism to be associated with addiction, one study did find that self-reported levels of addiction was associated with NMR in adolescent smokers[152]. In 20 adolescent smokers who refrained from smoking for 24 hours they found that participants with a higher nicotine metabolism had increased levels of withdrawal symptoms. A study measuring the withdrawal symptoms of 11 smokers who abstained from smoking for 4 hours was able to measure early withdrawal symptoms[132]. Participants completed the Wisconsin Smoking Withdrawal Scale, which measures factors such as anxiety, stress, anger and cravings. The study found that faster metabolisers were more likely to experience increased levels of anxiety, but less likely to experience cravings in very early withdrawal [132].

1.11.1.7 Nicotine consumption

A systematic review of NMR and its relationship to nicotine dependence and success in stopping smoking found that there was an overall positive association between cigarettes smoked per day and NMR. A total of nine out of 15 studies found this association, but the overall association was weak, the correlation coefficient (r) was between 0.1-0.19[131]. However, the number of cigarettes smoked does not always correlate to the level of nicotine generated from smoking due to the differences in individual smoking topography. For example, smokers may vary in the amount of nicotine absorbed through smoking, due to differences in puffs smoked and depth of inhalation [153]. A study which found that Chinese American smokers were slower metabolisers compared to white American smokers, also found an association between slower metabolism and reduced nicotine

intake per cigarette[151]. Participants who consumed the highest level of nicotine per day also had the highest nicotine clearance (r=0.41, p=<0.0001).

The review also investigated the relationship between nicotine metabolism, quit attempts and smoking cessation outcomes. Five studies reported the outcomes of quit attempts from smokers using pharmacological methods; smokers with a slower nicotine metabolism were found to be more likely to be abstinent at the end of nicotine patch use compared to faster metabolisers. It is hypothesised that slower metabolisers respond better to NRT patches as they receive higher and more therapeutic levels of nicotine compared to faster metabolisers[154].

1.11.1.8 Grapefruit consumption

Grapefruit juice, when consumed in large quantities (1 litre) has been found to inhibit CYP2A6 activity in the metabolism of coumarin, a chemical compound metabolised by the same enzyme as nicotine[155]. Grapefruit consumption has also been shown to affect the metabolism of methadone; a study involving eight patients on a drug treatment programme being administered grapefruit over 5 days found it significantly reduced metabolism of methadone via the CYP3A4 pathway [156]. A review of the effects of grapefruit in drugs metabolised by the CYP3A4 pathway concluded grapefruit juice even in small quantities (200ml) had the potential to affect drug metabolism[157]. Although there is significant evidence about the effects of grapefruit juice on a variety of drugs metabolised by similar cytochromes to nicotine, there is limited evidence of what the effects are for nicotine metabolism. A study which aimed to

determine the effects of grapefruit on nicotine metabolism in 10 non-smoking participants who were given NRT, found that after consuming grapefruit juice, nicotine and cotinine metabolism was inhibited and there was decreased clearance of nicotine (p=0.043) and cotinine (p=0.016) [158]. However, for metabolism to be altered a large quantity of grapefruit juice (1 litre) was required to be consumed.

1.11.1.9 Summary

This section has described the mechanism of nicotine metabolism and has highlighted factors that can potentially influence nicotine metabolism. A large study which investigated many of these factors concluded that genetic predisposition was the most influential factor on nicotine metabolism with other factors only accounting for 7.6% of the total variation[159]. Although the studies discussed in this section only involve non-pregnant populations, theoretically many of these factors are likely to influence nicotine metabolism in pregnancy too. Section 1.13 will discuss nicotine metabolism in pregnancy in more detail.

1.12 Drug metabolism in pregnancy

Understanding the pharmacokinetics of drugs specific to pregnancy is important to understand the health effects on the pregnancy and the fetus, and to ensure that accurate levels of drug dosages are prescribed in pregnancy. In section 1.11 the pharmacokinetics of nicotine metabolism were explored, yet all of the data were based on non-pregnant populations. There are limited studies on the pharmacokinetics of nicotine metabolism in pregnancy, so this section will draw upon studies surrounding the pharmacokinetics of drugs that are metabolised by similar cytochrome

P450 isoenzymes to nicotine, as these may aid the understanding of nicotine metabolism during pregnancy.

The pharmacokinetics of drugs in pregnancy is complex; pregnancy can influence the absorption, distribution, metabolism of a drug[160]. In order to ensure drug doses are appropriate for pregnant populations it is first necessary to understand metabolic changes in pregnancy. The data on alterations of drug metabolism during pregnancy are limited and this is reflected in the drug doses and guidelines used for pregnancy, which often involve reduced dosages compared to non-pregnant populations [161]. Some drugs are metabolised more slowly during pregnancy, such as caffeine where the CYP1A2 enzyme responsible for its metabolism, a variant of cytochrome P450, decreases in activity within the antenatal period compared to postpartum[162]. As a result women are encouraged to drink less caffeine during pregnancy[60]. Other drugs catalysed by cytochrome P450 isoenzymes, include methadone, labetalol, lamotrigine and morphine, and the metabolism of these drugs has also been found to alter during pregnancy[163]. A study exploring methadone metabolism in nine women undergoing a methadone treatment programme found the clearance was faster during pregnancy compared to postpartum and that the alterations in methadone metabolism can last until 2 months post-delivery, despite medication levels staying the same [164]. A study which reviewed the effects of pregnancy on the metabolism of anticonvulsants and analgesic medications found mixed evidence about an association with antihypertensive treatments and there was no consistent evidence about the clearance of analgesic medications [161].

Overall, there is evidence to show that pregnancy affects the pharmacokinetics of certain drugs metabolised by P450 enzymes similar to those responsible for nicotine metabolism. Consequently, drug guidelines may need altering to reflect metabolic changes in pregnancy[163]. However, further research is required to determine the exact time in pregnancy when changes in metabolism occur, the gestation at which metabolic changes are highest and when metabolism returns to a pre-pregnancy level.

1.13 Nicotine metabolism in pregnancy

Metabolism of nicotine is thought to increase during pregnancy, yet fluctuations in nicotine metabolism are not apparent in non-pregnant populations[143,165] indicating that metabolic changes are unique to pregnancy. A study involving 10 pregnant smokers compared the metabolic clearance of nicotine and cotinine between 16-37 weeks gestation, with 12-week postpartum levels[166]. The participants were given intravenous infusions of deuterium labelled nicotine and cotinine simultaneously, the dose being equivalent to two cigarettes. Following the infusion, blood samples were taken regularly to measure plasma nicotine and cotinine levels. The study found that metabolic clearance of cotinine increased significantly during pregnancy compared to non-pregnant levels, with a reported 60% increase in nicotine clearance and 140% increase in cotinine clearance, obtained by both plasma and urinary measurement[166]. Five participants received two infusions during pregnancy. There was no significant trend seen in changes in metabolism with advancing gestation, however only three women had infusions between the second and third trimester and the remaining two women received both antenatal infusions

in the third trimester. Therefore, given the small sample size it remains unknown exactly when metabolic changes occur during pregnancy.

Increased cotinine clearance during pregnancy compared to postpartum levels has been detected in studies using maternal saliva, blood, urine[97,134,167] and hair samples[168,169]. A comparison of plasma cotinine concentration from ad libitum smoking in pregnancy and postnatally found that cotinine levels were lower in pregnancy (119ng/ml) compared to postnatal levels (202ng/ml) despite no significant changes in the nicotine they received from smoking cigarettes [166]. A study comparing saliva cotinine levels in 42 smokers in their third trimester with levels at 1 month postnatally found that cotinine levels were 7.3ng/ml less per cigarette in the antenatal period compared to postnatal samples ($p<0.001$)[167]. A combination of an increased number of metabolic enzymes such as the CYP2A6 enzyme and increased liver blood flow are potential factors responsible for alterations of nicotine metabolism in pregnancy[170].

A study that measured steady state cotinine levels after using NRT patches in abstinent pregnant smokers, found levels were comparable with the nicotine and cotinine levels in non-pregnant smokers[97]. In this study, 21 pregnant women in their third trimester of pregnancy were hospitalised for 4 days and a nicotine patch applied (22mg/16hr), after which blood samples were taken to measure nicotine and cotinine levels. In order for comparisons to be made with non-pregnant populations, the study included five non-pregnant women who were given the same treatment as the pregnant participants. Compared to the non-pregnant women, the pregnant women had lower morning cotinine measurement on day four of the inpatient study ($p=0.01$), but there were no other differences in the

cotinine or nicotine levels between the two groups. Nicotine and cotinine levels while both groups of participants were smoking found lower levels in the pregnant group compared to the non-pregnant group. This may be explained by pregnant women smoking fewer cigarettes per day (20.5/day) compared to the non-pregnant women (24.6/day). Although this study did not indicate nicotine metabolism differed in pregnancy, the comparison group of non-smokers only contained five participants and it is possible that, in pregnancy, use of NRT for longer than 8 hours is required before stable cotinine levels are generated.

1.14 Adherence to NRT

This section will begin by defining what adherence is, before describing how smokers adhere to NRT based on findings from studies set in routine practice and clinical trials. Adherence to taking NRT has been associated with increased effectiveness of NRT in achieving smoking cessation; the longer NRT is used and adhered to the more likely the quit attempt will be successful [171,172]. Studies discussed in this section are based on non-pregnant populations, setting a wider context about adherence among smokers using NRT, and section 1.15 will concentrate on adherence specifically in pregnancy.

1.14.1 Definition of adherence

Adherence can be defined as the degree to which the individual conforms to using medication as it is prescribed, including the timing, dosage and frequency of using treatment [173]; low adherence includes early discontinuation of treatment [154]. Adherence can also relate to the uptake of medication. Poor medication adherence may be either intentional or

unintentional, with the former being where the person weighs up the risk and benefits of treatment and makes an informed choice, and the latter where the person forgets to adhere to treatment and is not careful to administer medication as prescribed [174]. This section will aim to describe adherence to NRT among smokers who have been prescribed or commenced using NRT.

1.14.2 Measuring adherence

Measuring adherence to NRT is complex as the reporting of adherence varies between studies and often there is little detailed description as to how adherence was reported. Adherence has been measured in a variety of ways; counting the number of days NRT has been used, reporting the percentage of days the smoker used NRT and reporting whether the participant has used 'high' or 'low' amounts of NRT[175,176]. Variations in the reporting of adherence can make comparisons between studies difficult, especially when studies have used differing types of NRT products. Shiffman et al suggests an accurate way of reporting adherence would be to detail the proportion of NRT prescribed which is used and present this information in terms of the nicotine dose consumed. This measurement would enable a greater understanding about the optimum dose of nicotine required to achieve abstinence[177]. However, few studies provide this information and adherence is often measured using self-report information and sometimes retrospectively, so this could influence the accuracy, as participants may not always recall or give truthful information. In addition, adherence data is often collected from smokers who have relapsed, yet by including these participants it may overestimate low adherence, as participants are often told to stop using NRT in the event of a relapse[73,175].

1.14.3 Adherence in non-pregnant smokers

The reported levels of adherence to NRT among non-pregnant smokers varies between studies from 37% to 94.5%[175]. As mentioned previously it is difficult to compare adherence levels between studies, as there is not a set criterion to measure NRT[176]. Data from the International Tobacco Control Four-Country Survey (Canada, United Kingdom, United States and Australia), which is an annual cohort survey found that of the 981 participants who used NRT, 71.4% discontinued treatment prematurely (<8 weeks)[178]. There were no differences in adherence according to whether smokers purchased NRT 'over the counter' or were given a prescription[178]. The timing of when NRT adherence is measured during a quit attempt influences the adherence results, for example in an RCT involving adolescent smokers it was found that 78.4% of participants allocated to the NRT arm reported wearing a patch everyday between baseline and 1 month[179]. However, these findings are difficult to compare with studies that have measured NRT adherence after longer periods of use, as adherence rates are likely to decline over time.

Marteae et al found in adult participants who were given dual therapy NRT in doses based on their gene variant and nicotine dependence score that adherence levels were similar; rates of adherence at the 4 week follow up were measured by the proportion of NRT prescribed compared with self-reported consumption[180]. Participants in the genotype group had an adherence rate at 28 days of 68.6% and in the phenotype group 63.5%[180].

In these studies adherence is based on self-report and information about the total nicotine dose participants received remains unknown.

A study involving cardiovascular patients found only 37% (n=62) adhered to the treatment timeframe (7-8 weeks) of which 50% smoked while using a patch[181]. Similar low rates of adherence have been reported in a study by Cooper et al which involved the use of nicotine patches; data from this RCT found that only 26.6% (n=196) of women and 32.2% (n=289) of men reported to have used all of the patches supplied in the 6 week study[182]. Also in this study it was found that females were less likely to adhere (measured by the number of patches worn in 6 weeks), which the authors hypothesized as a reason why they were thus less likely to quit smoking compared to males (OR 0.69 (95% CI, 0.51 to 0.92 p= 0.012). Yet as mentioned previously participants who relapse tend to stop using NRT and so this could explain the particularly low adherence rates seen in the study. Studies which have attempted to address reverse causation have found adherence levels are higher in those that abstinent from smoking[175].

Some studies collected information from participants about their reasons for low adherence or early discontinuation of treatment. Factors such as feeling that NRT was not necessary or that it did not help their quit attempt were reported[178,183]. Other responses included reports of negative side effects of using the medication, and in some cases this was the primary reason for low adherence[178,184]. Practical and logistical issues such as running out of NRT medication were also reported as a reason for low adherence[178].

1.15 Adherence to NRT in pregnancy

1.15.1 Adherence levels

The majority of trials that have investigated the effectiveness of NRT in pregnancy have reported low levels of adherence[83]. However adherence was often measured at different points after commencing NRT, used differing criteria to measure adherence and was based on self-reported information, all of which make it difficult to establish what adherence levels are during pregnancy. There is also little evidence about the variation of adherence to the differing types NRT products. Low adherence levels have made it difficult to determine the effectiveness of NRT as the full effect of using NRT as a treatment may not be accurately explored [85,101,185,186]. Six studies that were included in a Cochrane review of pharmacological interventions during pregnancy measured adherence to NRT. Pollak et al found that in the 72 women who were given NRT patches to use for 40 days, the actual mean number of days patches were used was 23.4 days. Women who were prescribed nicotine gum only used this for 8 days, out of a total of 18, and women prescribed lozenges used them for a mean total of 4 days out of a possible 19 days. Overall, in this study, 24% of participants did not use their allocated NRT at all, and of the 76% of participants who reported using NRT at some point the majority had low adherence[87]. In this study 19% of women swapped one form of NRT product for another, and it is unclear how this is reflected in the self-reported measures of adherence.

A study by Wisborg et al, which prescribed patches to pregnant women for 11 weeks, found that in the NRT group only 17% of participants used the 15mg/16hr patches for the prescribed time (8 weeks) and 11% for the

subsequent 10mg/16hr patches (3 weeks)[101]. Out of a total of 11 weeks (77 days) the median number of days a patch was worn was 14 (range 0-77 days) in the NRT group. In a study where 17 women were allocated to the NRT arm, only four participants completed the 12-week course of treatment; three used NRT for up to 3 weeks and 10 participants discontinued treatment in the first week[186]. In some cases women using NRT patches have reported using them intermittently throughout the trial; in one study, 4 women never used their patches or only a few times, 10 used the patches for 6 weeks or less out of a total of 12 weeks and the remaining 11 reported using the patches intermittently[187].

Adherence tends to reduce with the length of time participants use NRT. A study exploring the effectiveness of nicotine gum found adherence at the first follow up visit (3-7 days) after quitting was 90%, however levels decreased by the final visit (32-34 weeks gestation) and only 30% of women in the NRT gum group reported being compliant [86]. As time progressed the number of women adhering fell. Another study exploring the effectiveness of patches found that only 7.2% of women in the NRT group used patches for longer than 1 month[85]. However, all studies presenting information about nicotine adherence both during and outside pregnancy report little information about the total dose of nicotine participants consumed. Increasing understanding about the nicotine dosages consumed during a quit attempt would enable a greater understanding about the level of adherence and nicotine substitution required to optimize smoking cessation[175].

The most recent trial exploring the effectiveness of NRT in pregnancy has reported the highest levels of adherence yet[88]. In this study by Berlin et al,

adherence was measured using maternal self-report of the number of patches used between each visi t which was then divided by the amount of NRT prescribed. A compliance record was recorded for 307/402 (76%) of participants. It found that 85% of participants in the NRT group adhered to treatment for a median of 105 days [88]. Attempts were made to validate self-reports by asking women to return the packaging of the patches, but as with other RCTs which took similar validation methods, this proved impractical. It is unknown why adherence levels in this study were higher than in previous studies; this study was set in France and it could be that there is some variation between the populations of pregnant smokers in France compared to the UK or the US where the majority of previous studies have taken place. Women were advised not to smoke while using NRT but were allowed to stay in the study even if they reported continued smoking, so this could also explain the higher than usual adherence levels reported.

1.15.2 Physiological explanations for low adherence

As discussed previously, nicotine metabolism increases during pregnancy[166]. It has been hypothesised that increased metabolism may explain low adherence rates, as the dose of nicotine supplied in standard dose NRT may be insufficient in pregnancy to alleviate withdrawal symptoms associated with smoking cessation[85]. There is mixed evidence about the level of nicotine substitution provided from standard dose NRT in pregnancy [97-99] and no evidence as to how substitution may change with advancing gestation. However, inadequate nicotine substitution when using NRT could theoretically explain why adherence is low, and why pregnant women who attempt to stop using NRT then re-start smoking.

1.15.3 Side effects of using NRT

In RCTs exploring the effectiveness of NRT in pregnancy, most have collected information about the side effects experienced by participants. Some studies have collected information about how side effects experienced after using NRT may result in early discontinuation of NRT. In one study, exploring the effectiveness of nicotine gum, 15% (n=14) of the participants reported discontinuing treatment due to side effects[86]. Data from a large RCT exploring the effectiveness of nicotine patches during pregnancy has reported on adverse events that were given as reasons for permanently discontinuing NRT[188]. Out of 521 women allocated to the NRT group, 33 reported to experience application site reactions, 7 reported nausea and 5 women experienced dizziness after using NRT. Although such adverse events were less frequently reported in the placebo group, similar side effects were reported[188]. A qualitative study conducted in the UK which explored pregnant smokers views on using NRT found women who had previously used NRT disliked the marks that the patches left, and that being able to find an a unobtrusive place on the body to wear the patches was an important factor influencing NRT use[189].

1.15.4 Acceptability and attitudes to using NRT among pregnant women

In non-pregnant smokers, pre-existing attitudes and views towards NRT can affect how smokers view and use smoking cessation treatments[190]. Yet few studies have described quantitatively or qualitatively the views and attitudes of pregnant women before or after using NRT as part of a quit attempt. Data from UK SSS indicate that NRT is widely accepted as a

method of supporting pregnant women with stopping smoking. Unpublished data from a survey from 38 out of 48 SSS [71] found that between 2010/2011 35 services prescribed NRT to over 50% of the pregnant women that attended their service. Of these services, 19 provided NRT to 90% of women who attended [191]. Although this data provides some insight about the acceptability of NRT, few studies have explored in-depth the views of pregnant women about using NRT. An Australian study, which used focus groups to try and determine attitudes towards nicotine patch use for smoking cessation attempts, found that pregnant women were generally positive about the idea of using patches[192]. However, women did raise concerns about the efficacy of using patches and the cost, and some believed smoking cessation involved habitual changes rather than nicotine withdrawal[192]. As this study was Australian it may not be comparable to other countries, and at the time interviews were carried out NRT was not free or licensed for use in pregnancy in Australia, thus this may have influenced the views of women, limiting the generalizability of these findings.

Studies set in the UK, where NRT is available at no cost to women and licensed for use, have also found that women were positive about the idea of using NRT to support a quit attempt [193,194]. A cross sectional study which surveyed 150 pregnant women from a large UK London hospital who had registered as smokers at their antenatal appointment. The study found that nearly half (45%) expressed an interest in using NRT as part of a smoking cessation attempt [193] and interest was highest in women who reported smoking higher number of cigarettes per day. Another UK based study which involved surveying 145 women in their third trimester of pregnancy found that 68% of pregnant smokers would use a nicotine patch if it was

offered to them[194]. These studies indicate that pregnant women are open and positive about the idea of taking NRT; however, these surveys are based on women who agreed to take part in research, who may be highly motivated and not be representative of the pregnant smoking population.

One study interviewed 10 women about their views of the advice and support given during their quit attempt which involved using NRT in pregnancy[189]. Eight of the 10 women had used patches and two used gum with the usage varying between 1 to 180 days. The study found that women perceived using NRT positively but felt that smoking cessation support from a midwife was most important. Four of the 10 women had relapsed and were smoking at the time of the interviews. Although this study provided some information about attitudes towards using NRT and views about smoking cessation advice, it did not elicit specific reasons for discontinuing NRT. Also, some of the interviews took place after pregnancy and so the interviews were subject to recall bias and may not have captured experiences close to the time when NRT was used.

Questionnaire data from pregnant smokers allocated to the NRT group of an RCT found that factors such as negative side effects and wanting to test one's own ability to stay abstinent were reasons for not adhering to NRT[185]. Women also reported that they did not find NRT useful or used NRT incorrectly as reasons for why they did not adhere[185]. A review of the literature which has explored pregnant women's views about using NRT in pregnancy found that there was limited information about this topic[195]. This review showed some evidence that women thought NRT would aid nicotine withdrawal symptoms, but conversely some women believed stopping

smoking was a psychological problem requiring behaviour change, and believed that NRT could help little with this[195].

1.15.5 Acceptability of other medications in pregnancy

Since there is limited literature which has explored experiences of using NRT in pregnancy, literature that has explored the experiences of pregnant women using other forms of medication may add further understanding to the potential issues involved. One study, which used questionnaires to explore adherence to treatment for ulcerative colitis during pregnancy, found that women were concerned about the effects of the medication on fertility and the fetus during pregnancy[196]. Another study, exploring medical treatment among women with Crohn's disease, found similar concerns among participants. Adherence is fairly high among pregnant women taking Crohn's medication but the main reason for not adhering was fear of the effects on the fetus[197]. An in-depth understanding of the beliefs of pregnant women who have used NRT is lacking.

A study by Olesen et al looked at adherence to prescription medication during pregnancy using participants from a Danish birth cohort, which is a health interview survey. This study measured adherence by matching prescription data records with self-reported information about drug use in the same participants. Prescription data was available for 2,041 women who took part in the birth cohort. The study found that women reported only using 43% of the drugs prescribed in pregnancy[198]. Of the drugs that were used it is not known whether they were used for the prescribed length of time or whether the correct doses were used. Drugs which

treated chronic diseases such as insulin dependent diabetes had a higher level of compliance than shorter term medications such antacids and antibiotics[198].

These studies offer some insight into compliance and the potential reservations pregnant women have towards taking medication during pregnancy; these reservations may be indicative of how women feel about using NRT, although this has not been explored and these issues remain unknown.

1.16 Summary of literature

This literature review has highlighted the dangers of smoking in pregnancy and that smoking in pregnancy is a significant public health issue. Pregnancy can provide a unique opportunity for women to quit smoking, however many women cannot quit alone or with behavioural support. In non-pregnant populations, NRT is effective at increasing smoking cessation outcomes, but it has not been proven effective in pregnancy. It is currently unknown why this is. This review has highlighted that increased nicotine metabolism during pregnancy may mean the level of nicotine supplied by NRT is insufficient to alleviate nicotine withdrawal symptoms. However, little is known about the level of nicotine substitution provided by NRT or whether increased nicotine substitution improves smoking cessation outcomes during pregnancy. In addition, no studies have yet to describe the pattern of nicotine metabolism during pregnancy, and so it is currently unknown when metabolism may increase during pregnancy or the magnitude of this change.

The review had also highlighted that there is little understanding about adherence towards NRT treatment during pregnancy. Despite adherence to NRT being low in the majority of trials exploring the effectiveness of NRT, at present there is limited understanding about the views of pregnant women who either discontinue NRT treatment early or do not adhere to treatment. This thesis will hope to address some of the gaps in research surrounding NRT use during pregnancy, by seeking further understanding about nicotine substitution, changes in nicotine metabolism during pregnancy and will learn further about the experiences of pregnant women who use NRT. This increased understanding could help to develop innovative ways to use NRT that may provide a more effective way of helping pregnant women quit smoking.

1.17 Aims and objectives

The principal aim of this thesis is to understand why NRT may not be effective during pregnancy. This will include understanding pregnant women's reasons for discontinuing treatment, and determining how changes in nicotine metabolism varies across pregnancy. The objectives are:

- To investigate the level of nicotine substitution provided by NRT during pregnancy, by comparing 'pre-treatment' (whilst smoking) cotinine levels with cotinine levels when using 'standard' dose NRT (Chapter Two).

- To describe pregnant women's nicotine metabolite ratio (NMR) at different times during pregnancy and to investigate changes in individual's NMR as pregnancy progresses (Chapter Three).

- To describe women's perceptions as to why they discontinue or do not adhere to NRT treatment during a quit attempt in pregnancy (Chapter Four).

2 Chapter Two: Comparison of cotinine levels in pregnant women whilst smoking and when using nicotine replacement therapy

2.1 Introduction

As highlighted in Chapter One, for nicotine replacement therapy (NRT) to be most effective it needs to provide adequate levels of nicotine to help alleviate the physical nicotine withdrawal symptoms associated with cessation [67,69]. However, to date, the exact level of nicotine substitution required in order to optimize the effectiveness of NRT is unknown. There are few studies in pregnancy that specifically compare levels of nicotine, or its major metabolite cotinine, while smoking with those generated while using NRT and remaining abstinent; of the studies carried out there is mixed evidence as to what levels of nicotine and cotinine are generated from using NRT in pregnancy [97-99,101]. A study which attempted to provide 100% nicotine substitution to equate to ad libitum smoking still did not find NRT to be effective[88]. Yet at 2 and 8 weeks post quit date women's cotinine levels were lower when using NRT compared to smoking, which highlights that the nicotine substitution rate was not 100%. In addition, the study measured cotinine in women who reported smoking, so it remains unclear what level of nicotine was provided from using NRT and what was achieved from smoking.

In pregnancy nicotine metabolism is known to increase by the second trimester [166] and remains faster for the duration of the pregnancy[169]. Smokers are able to titrate the dose of nicotine they receive from a cigarette to meet their nicotine requirements, if nicotine metabolism was to increase during pregnancy this could potentially influence smoking topography so smokers could alter the way they smoke a cigarette to increase their nicotine intake. However, unlike a cigarette, the dose of nicotine absorbed from NRT cannot be altered. In theory, the level of nicotine substitution provided from NRT may vary depending on the

gestation at which treatment is used during pregnancy. There is limited information about how nicotine metabolism might influence the effectiveness of NRT during pregnancy. It could be that pregnant women who have a slower nicotine metabolism, have nicotine levels adequately substituted after using NRT are more likely to achieve smoking abstinence. However, this is speculation, as there are currently no studies which have explored associations between nicotine substitution and cessation during pregnancy.

Nicotine dependence has been inversely associated with smoking cessation; smokers who are highly nicotine dependant are least likely to stop smoking using smoking cessation medication[131,176]. In non-pregnant populations the heaviness of smoking index (HSI) has been shown to provide an accurate measure of nicotine dependence[199,200] defined by the number of cigarettes smoked and time to first cigarette. To our knowledge, an association between nicotine dependence and nicotine substitution has not been explored in pregnancy. By exploring the relationship between HSI and nicotine substitution we may generate a better understanding as to how nicotine dependence may influence the effectiveness of NRT during pregnancy.

We hypothesise that the blood levels of nicotine generated using standard doses of NRT are not sufficient to alleviate withdrawal symptoms and this may explain why NRT does not appear to be effective in sustaining abstinence from smoking in pregnancy. Therefore, in this study we have used paired data from a trial of NRT in pregnancy to compare the levels of cotinine in women while smoking with those from using NRT transdermal patches whilst abstinent. We have also explored the factors that may

influence the difference between these levels including NMR, HSI and gestational age.

2.2 Aims

- To compare cotinine levels in pregnant women when smoking and when using NRT and remaining abstinent.
- To examine whether cotinine substitution in pregnant women who used NRT and remained abstinent after 1 month was associated with smoking abstinence at delivery.
- To investigate whether gestational age, HSI and NMR influence the difference between cotinine levels achieved from smoking and those achieved while using NRT and remaining abstinent.
    - Gestational age can influence how quickly nicotine is metabolised and has been shown to increase with gestation[201]. However it is unknown how gestational age would influence difference in cotinine at baseline and one month.
    - Outside pregnancy, a higher HSI is related to higher relapse rates during a quit attempt[202]. An explanation for this is that smokers with a high nicotine dependency are likely to experience greater nicotine withdrawals and may need higher doses of nicotine. It was hypothesised that pregnant women who had a higher level of nicotine dependency would have lower levels of nicotine substitution from using NRT.
    - NMR was explored as it was hypothesised that women with a higher NMR would have lower substitution rates from using NRT as they would metabolise nicotine from the patches at a faster rate compared to slower metabolisers. Outside pregnancy slower metabolisers have been found to respond better to NRT compared to faster metabolisers[76] . A possible

explanation for this is NRT does not provide high enough doses of nicotine to assist faster metabolisers during a quit attempt.

2.3 Methods

2.3.1 Smoking Nicotine and Pregnancy trial (SNAP): recruitment and trial procedures

Data for this secondary analysis are from a double-blind, randomised placebo-controlled study: the SNAP trial. The trial recruited 1050 women from seven antenatal clinics within UK hospitals. The study explored the effectiveness of NRT 15mg/16hr transdermal patch use in pregnant smokers (n=521) compared with placebo patches (n=529); both groups received behavioural counselling[85]. The Medicines and Healthcare products Regulatory Agency (MHRA) accepted that the patches used in this study were bioequivalent of the 15mg/16hr nicotine patch licensed for use in the UK. A detailed description of the trial sample, interventions and methods has been published elsewhere [85]; the trial had a pragmatic design intended, as far as possible, to mimic routine clinical practice.

Participants were included in the SNAP trial if at baseline they smoked ≥5 cigarettes per day and ≥10 prior to pregnancy, were between 12-24 weeks pregnant, aged between 16-45 years and had an exhaled carbon monoxide reading (CO) of ≥8ppm. Once recruited into the trial, women received behavioural counselling and set a quit date within 2 weeks of enrolment. Women were instructed to wear the patch during daytime hours, to remove it at night and to discontinue patches if they restarted smoking. They were

advised to use the patches for a total of 8 weeks, and were initially given a 4 week supply to start on their quit date, followed by another 4 week supply if they reported smoking abstinence at the 1 month follow up, confirmed by CO reading of <8ppm. Women were followed up at delivery to establish smoking cessation outcomes and this was validated with salivary cotinine (<10ng/ml) or with CO (<8ppm) if cotinine was not available. Women who were not validated as being smoking abstinent were treated as smoking at delivery.

Data collected at baseline included socio demographic data, smoking history and behaviour, HSI and gestational age. Serum blood samples were taken for cotinine estimation and the nicotine metabolite ratio (NMR) which is a validated method of measuring nicotine metabolism[131]. The NMR was used to determine the difference between cotinine levels generated while smoking and while using NRT. Saliva samples for measuring cotinine levels while smoking were also collected at baseline. After 1 month participants who reported not smoking (5 or fewer cigarettes smoked between quit date and follow up) had their smoking abstinence validated by an exhaled CO measurement. Participants with CO-confirmed abstinence who were still using trial patches, were asked for a saliva sample to measure cotinine levels generated from using NRT.

2.3.2 Inclusion criteria for this study

Participants included in this study were those allocated to the NRT arm of the trial, who were abstinent from smoking at 1 month and were reasonably adherent. Consequently, we selected women from the NRT group, who at 1 month reported abstinence (5 or fewer cigarettes smoked between quit date and follow up), were CO-validated as such (<8ppm),

and who reported at least 80% adherence with NRT. Adherence of 80% equates to a median number of 24 days of using NRT. Adherence was calculated as the total number of days the participant reported using the trial patches as a percentage of the length of time between the participant's quit date and their follow up appointment. This ensured that participants were using NRT on a regular basis. Adherence was self-reported in this study. Evidence suggests counting pill packages may provide an accurate measure of adherence[203] yet in pragmatically designed studies this may not be practical and other studies have shown that self-report is a reliable method of obtaining information about medication adherence[204,205].

Women who were smoking abstinent and NRT adherent were also required to have a cotinine result from saliva samples taken at baseline and at one-month post quit, while using NRT. The characteristics of women who did not provide a saliva sample at both time points were compared with the women who had provided a saliva sample at each time point in order to identify if there were any observed differences between those with and without missing data.

2.3.3 Analysis strategy

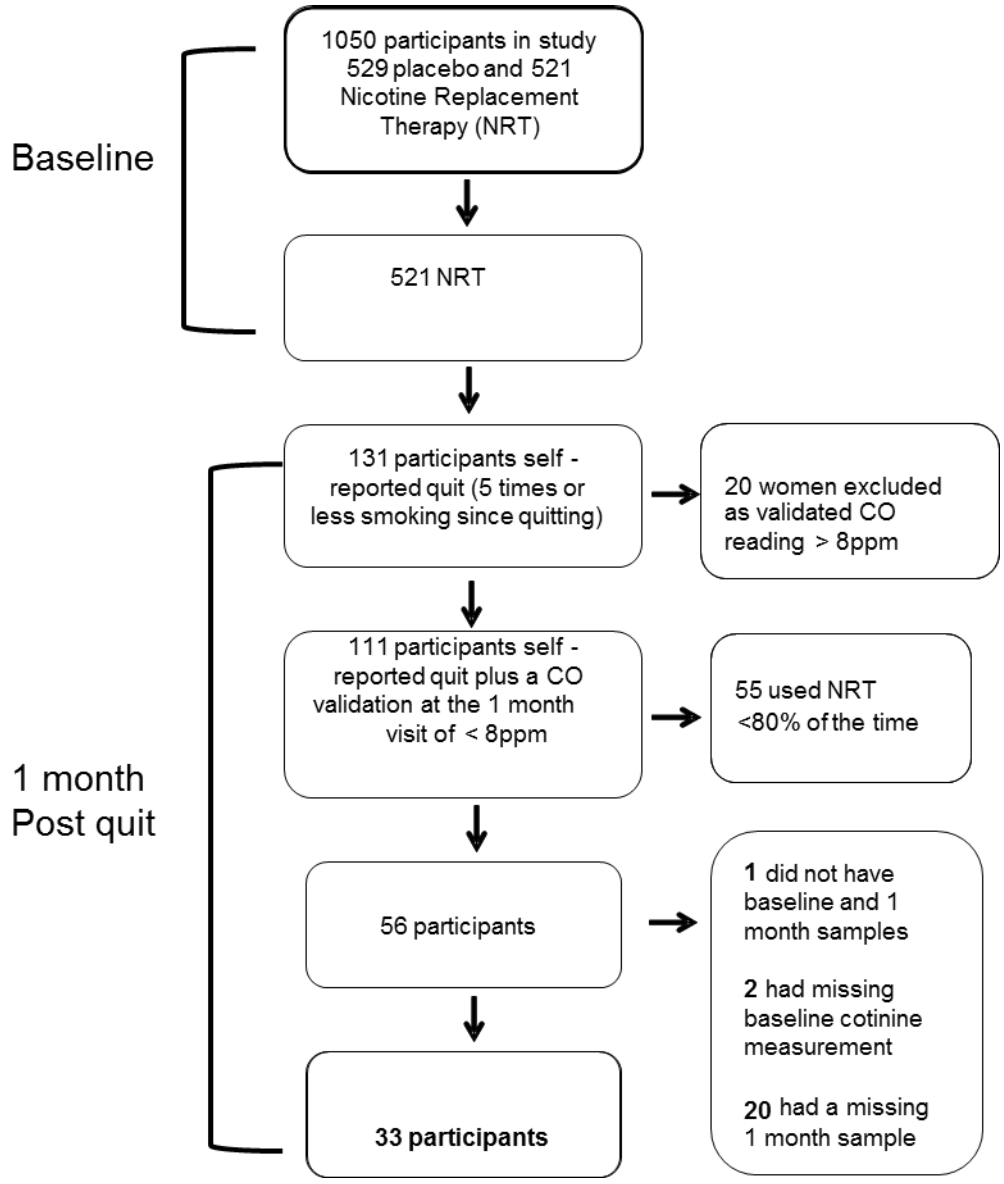
Salivary cotinine levels were not normally distributed and therefore the data was analysed using non-parametric statistics and is presented using medians and inter-quartile range (IQR). The within-subject changes in cotinine levels between baseline and 1 month were analysed using the Wilcoxon signed rank test. The difference has also been calculated to show the percentage of nicotine provided from NRT compared to smoking at baseline. Participants were then divided between those who went on to be

validated as smoking abstinent at delivery and those that were not smoking abstinent at delivery. A Wilcoxon rank sum test was conducted to compare the difference in cotinine levels whilst smoking and whilst on NRT between women who reported smoking and women who reported they were not smoking at delivery. The final part of the analysis involved investigating association between baseline and 1month cotinine levels, and between changes in cotinine levels and NMR, HSI and gestational age using scatterplots and Spearman correlation coefficients. Alternative approaches to this analysis could involve linear regression, which would quantify how these factors predicted substitution (change in cotinine levels). As these factors have never been explored before, correlations were used to quantify the strength of associations and scatter plots were used to visually display correlations. If the correlation coefficients showed a significant association then linear regression would be conducted to quantify how the two variables were associated.

2.4 Results

Of the 521 women in the NRT arm of the trial, 131 self-reported smoking abstinence at 1 month and 111 of these permitted a research midwife to visit them to validate their abstinence using an exhaled CO reading. A total of 55 women were excluded due to low self-reported adherence to taking NRT (<80% adherence), and 23 women did not have a before and after cotinine measurement to compare and subsequently were excluded (Figure 2). This left a sample size of 33 women.

Figure 2 Eligibility of SNAP trial participants for inclusion into the study



Of the 488 women in the NRT arm who were excluded from this study there was adherence data available for 403 women. Between quit date and follow up at approximately 1 month, 75 women reported using the patch for less than 10 days, of whom 23 never used the patch. A total of 328 women reported using the patch for at least 10 days. The median number

of days the patches were used was 10 days (IQR, 4-20). Women excluded from the study only used the patches for 32.4% of the time (IQR 13.33-58.97)

2.4.1 Characteristics of sample

Socio-economic, clinical and smoking factors are presented using the median and interquartile ranges for both the eligible 33 participants included in this study and for the 23 women in the NRT arm of the trial who were eligible for inclusion, but who did not provide two cotinine samples for comparison.

The 33 women included in the study were white British women, with the exception of one participant who was from an Asian/other background, and were on average 26 years old at recruitment. Most (67%) smoked between 5-10 cigarettes per day prior to beginning the trial. All were in the second trimester of pregnancy at recruitment with the median gestational age being 14.4 weeks. For 27 participants, an NMR was obtained from their baseline blood sample. Compared with the 23 women who did not provide two cotinine samples for comparison, the 33 women included in this study smoked less at baseline, had lower baseline cotinine levels, a higher NMR and more of them had adhered to NRT for 100% of the time (Table 3).

Table 3 Characteristics of eligible women

Characteristics	**Women included in study** (n =33) Median (IQR or %)	**Women who were eligible but who did not provide saliva samples at both time points** (n=23) Median (IQR or %)
Age (median, 25% & 75% percentiles)	26.12 (22.29 to 32.35)	25.06 (21.1-30.83)
Ethnicity White British Asian background	 31 (98%) 1 (2%)	 23 (100%) --
Body Mass Index (BMI)	25.6 (22.7 to 29.3)	27.96 (23.07-33.53)
Age left full time education (years)	16 (16-17)	16 (16-17)
Gestational age at baseline (weeks)	14.4 (13.3 to 17.8)	15.8 (13.3-20.4)
Cigarettes smoking per day at baseline 5-10 per day 11-15 per day 16-20 per day >20 per day	 22 (67%) 8 (24%) - 3 (9%)	 11 (48%) 5 (22%) 5 (22%) 2 (8%)
Heaviness of smoking Index (HSI)	3 (2-3)	3 (2-4)
Partner Smoking status Partner smokes Partner non smoking No partner	 23 (70%) 6 (18%) 4 (12 %)	 12 (52%) 7 (30%) 4 (17%)
Nicotine metabolite ratio (NMR)	0.4 (0.3 to 0.5)	0.3 (0.3-0.4)
Adherence with NRT trial patch + 80-99% 100%	 12 (36%) 21 (64%)	 17(74%) 6 (26%)
Smoking abstinent at delivery (validated) *Yes* *No*	 8 (24%) 25 (75%)	 10 43% 13 57%
Cotinine level Baseline (ng/mL)	98.5 (71.3 to 177.8)	105 (66.5 to 163.2)*
Cotinine level 1 month (ng/mL)	62.8 (33.3 to 82.7)	--

+% of days used trial patch from baseline to 1 month follow up
* based on 20 women in this group who provided a sample at baseline

2.4.2 Difference in cotinine levels at baseline and 1 month

The median cotinine level for participants while smoking at baseline was 98.5ng/ml (IQR 71.3-177.8) and while abstinent and using NRT at 1 month was 62.8ng/ml (IQR 33.3-82.7). There was a significant reduction in

cotinine levels between baseline when smoking and 1 month whilst using NRT (median difference -54.2ng/ml (95% CI -70.5ng/ml to -25.5.5ng/ml, p=0.045). The majority of participants had cotinine levels at 1 month that were lower than their levels at baseline (n=27). Six participants had cotinine levels at 1 month that were higher than baseline, of whom two had smoked a cigarette in the 24 hours before the 1 month cotinine sample was obtained. At baseline, for these six participants, the median gestational age was 13.92 weeks (IQR 13.2-24.1), the median number of cigarettes smoked daily was 8 (IQR 7-15), which was two fewer than the 27 participants whose 1 month cotinine level was lower than at baseline reported smoking (median 10 cigarettes/day, IQR 10-15). These six women also had lower median cotinine levels at baseline 75.2ng/ml (IQR 53.5 to 84.7) than the other 27 participants (108ng/ml, IQR 77.8 to 205.5). Only one of these six women was validated as smoking abstinent at delivery.

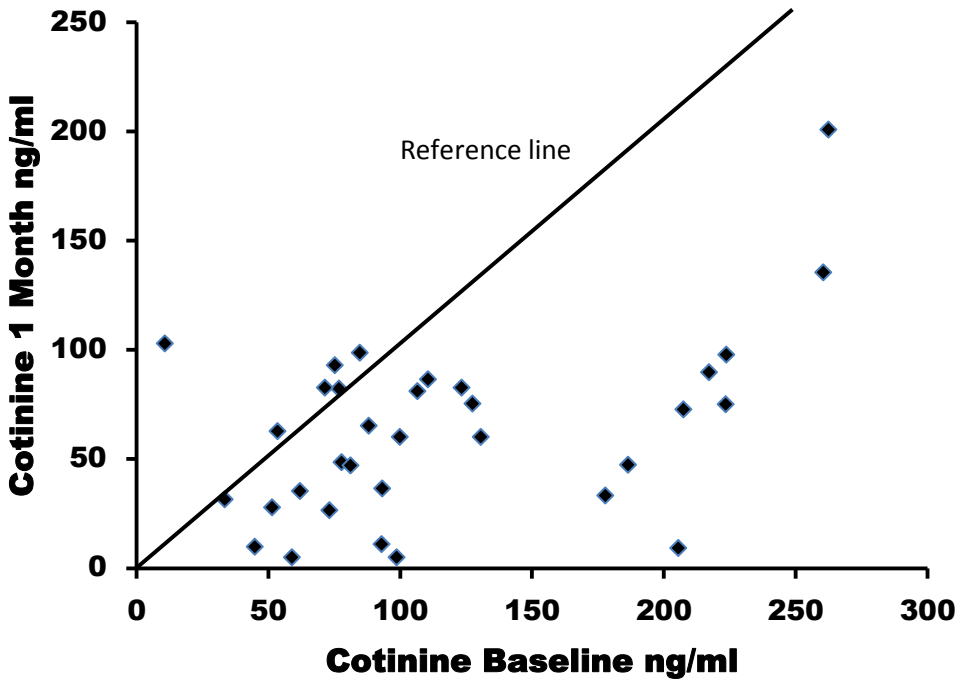
Seventeen of the 33 participants reported not smoking at all between their quit date and 1 month follow up, and the remaining 16 reported smoking five times or less. Six of the 16 participants reported smoking a cigarette in the 24 hours before their 1 month visit. We performed a sensitivity analysis on the 27 women who had not smoked in the 24 hours before the 1 month visit; the median difference in cotinine levels in this group was similar to that of the whole group (-46.6ng/ml (95% CI, -125ng/ml to -24.1ng/ml)).

A significant, positive correlation between baseline and 1 month cotinine levels was found, Spearman correlation coefficient 0.35 (p=0.04) (Figure 3). Although the association was weak, it suggests that participants with the highest baseline levels in the study also tended to have higher 1 month

levels. The scatter plot shows that for the majority of participants (n=27) cotinine levels at 1 month were lower than at baseline. The scatter plot also demonstrates that participants in the highest range of baseline cotinine levels appear to have the greatest reduction in 1 month cotinine levels. This is most noticeable among participants with baseline cotinine levels over 150ng/ml (n=9); these participants had a greater reduction in median cotinine levels (median difference -134.8ng/ml (95% CI, -144.5 to 125.9)) compared to those with a baseline cotinine level under 150ng/ml (n=24) (median difference -27.9ng/ml (95% CI, -49.35 to1.75)).

In the total sample NRT patches provided 58% (95% CI 42% to 75%) of the nicotine that women previously obtained from smoking. In the women who had the lowest cotinine baseline levels (<150ng) nicotine substitution was 64% (95% CI 56% to 98%) while using NRT. In women with the highest cotinine levels at baseline (>150ng/ml) the substitution rate was 35% (95% CI 19% to 51%) while using NRT.

Figure 3 Scatter plot of cotinine at baseline and 1 month



2.4.3 Difference in cotinine substitution in participants who were smoking and abstinent at delivery

At delivery, of the 33 women in the study, 24 were smoking and 9 were abstinent. The median cotinine level at baseline for the 24 participants who were smoking at delivery was 93.1ng/ml (IQR 74.1-182.1) while the median cotinine level at baseline for the nine participants who were abstinent at delivery was 99.1ng/ml (IQR 58.9-123.3). The difference in cotinine levels at baseline between smokers and non-smokers was not significant (p=0.8). At 1 month post quit date, the median cotinine was 62.7ng/ml (IQR 32.4-89.3) for the 25 participants who were smoking while the median cotinine level at 1 month for the nine participants who were abstinent was 62.8ng/ml (IQR 48.6-81.1). Again, there was no significant

difference (p=0.85) between non-smokers and smokers and their one month cotinine values. There was a greater difference between baseline cotinine and 1 month cotinine in the 24 participants who smoked at delivery (-49.4ng/ml, IQR -109.3 to -12.5) compared to the nine participants who were not smoking at delivery (-39.8ng/ml, IQR -53.9 to -25.5). However, this difference in change of cotinine levels from baseline and 1 month post quit between smokers and non-smokers at delivery was not found to be significant (p=0.94).

2.4.4 Factors associated with the difference in cotinine levels at baseline and 1 month

Using Spearman's correlation coefficient, we found that the individual changes in cotinine levels were not significantly related to NMR (ρ -0.13, p=0.5) (Figure 4), HSI (ρ -0.23, p=0.1) (Figure 5), or gestational age at baseline (ρ -0.06, p=0.7) (Figure 6).

Figure 4 Nicotine metabolite ratio and difference in cotinine (ng/ml)

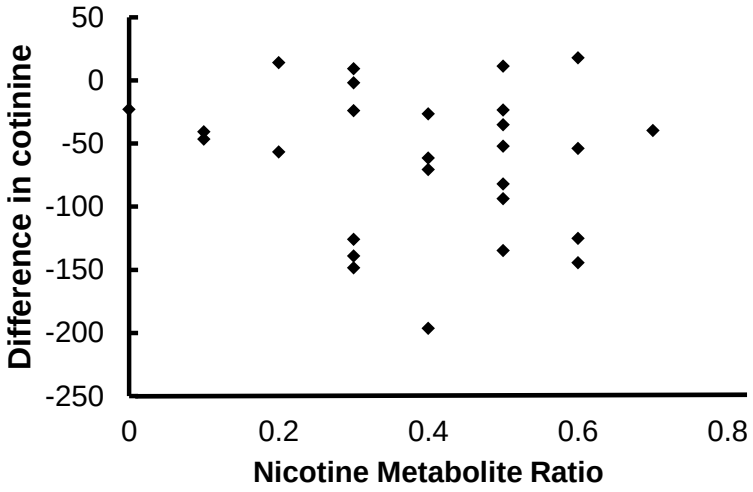


Figure 5 Heaviness of smoking and difference in cotinine (ng/ml)

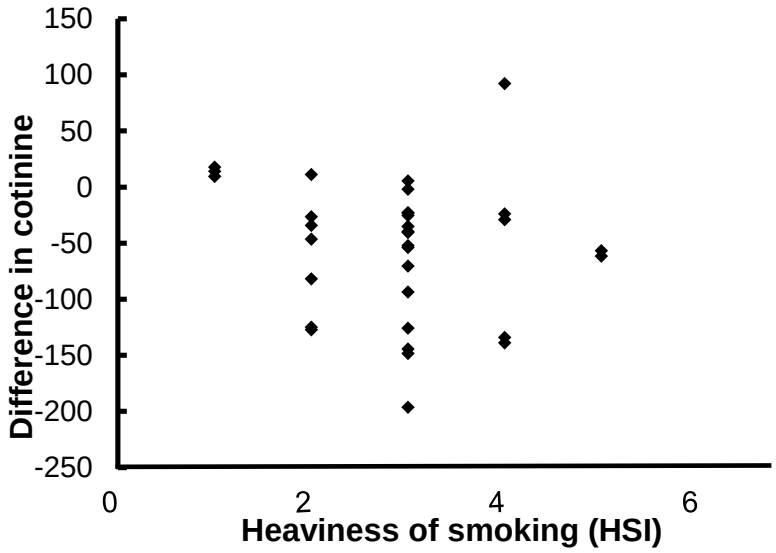
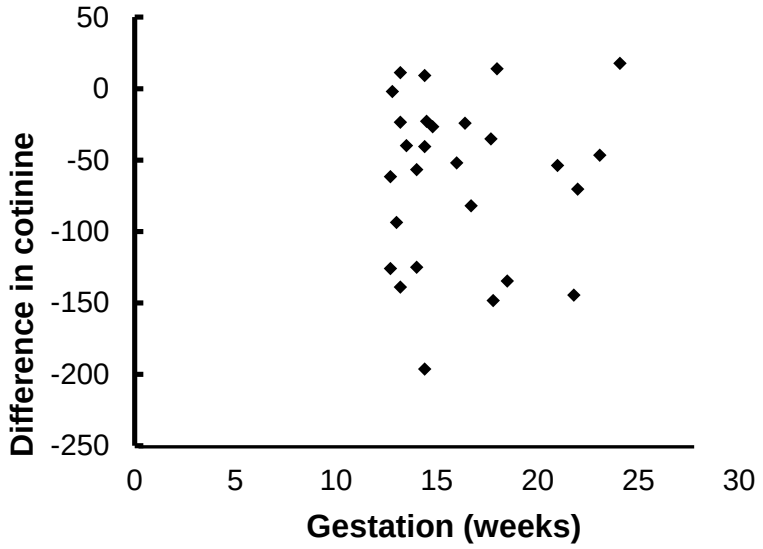


Figure 6 Gestation at baseline and difference in cotinine (ng/ml)



2.5 Discussion

2.5.1 Main findings

The findings have shown that cotinine levels generated using NRT transdermal patches in pregnancy are lower than cotinine levels generated while smoking. This indicates that nicotine substitution provided by 15mg/16hr patches may not be high enough to alleviate nicotine withdrawal symptoms effectively. Although these findings arise from a clinical trial, this had a relatively pragmatic design and was intended to replicate how NRT is used in routine clinical practice. A correlation was found between cotinine levels derived from smoking at baseline and cotinine levels whilst using NRT 1 month later. Participants with the highest cotinine measurements at baseline (>150ng/mL) tended to have the steepest reduction in cotinine levels while using NRT. Falls in cotinine levels were unrelated to the rate of nicotine metabolism, level of nicotine

dependence or women's gestational age at baseline. There was no significant difference found between the change between baseline cotinine and 1 month cotinine with smoking abstinence at delivery.

2.5.2 Strengths and Limitations

A strength of this study design was that it accounted for within-person differences and, therefore, change in cotinine levels will not have been influenced by inter-participant variation (e.g. in nicotine metabolite rate). This was observed in a setting that is similar to routine clinical practice, and so this should provide a realistic measure of cotinine levels based on regular and continuous NRT use in pregnant women who abstained from smoking. This is also, possibly, the first study to look at what factors might affect cotinine levels generated by NRT in a sample of pregnant women in this environment.

The study has some limitations. The findings reflect cotinine levels of a small sub sample of women from a large RCT who have abstained from smoking and adhered to NRT, and so it is unclear how generalizable these findings are to pregnant women who use NRT more intermittently. For example, it could be speculated that in women from the NRT arm of the study who were not included in this analysis, cotinine levels generated by NRT might have been even lower than those who were included. These lower cotinine levels may have caused these women to have stronger withdrawal symptoms, making them more likely to re-start smoking and to stop using their patches, and hence would not have been eligible for inclusion in the analyses presented in this chapter.

Although no associations were found between cotinine levels and the NMR, HSI or women's gestation (12-24 weeks) at study entry, the sample size was relatively small and a lack of power to detect these could mean that real relationships between these qualities and nicotine substitution were missed. In addition, NMR and HSI measures were only available at baseline before participants started NRT; it is possible that NMR and/or HSI could have altered in the month between baseline and follow up. If NMR increased between visits, women may have experienced more nicotine cravings and urges if the nicotine in the patches became insufficient to relieve their withdrawal symptoms. However, this is speculation, as nicotine dependence at 1 month was not measured. In addition, there was little power to look at the association between nicotine substitution and smoking cessation at delivery and this may have reduced the chance of detecting a true association.

2.5.3 Findings in context of previous research

Many large trials exploring the effects of NRT in pregnancy have measured cotinine levels in pregnant women who have been randomised to NRT[86-88,101] but in all these trials cotinine was not measured exclusively in women who were abstinent and using NRT. Overall, although these studies have shown that exposure to NRT reduces cotinine levels, they cannot accurately determine nicotine substitution from NRT as the cotinine may have been generated from NRT, or from smoking, or from a combination of NRT and smoking. The study presented in this chapter is novel in that it has been able to measure nicotine substitution from NRT by only including the cotinine levels from women using NRT patches while they were abstinent from smoking.

A study of pregnant smokers by Berlin et al, which used nicotine patches and attempted to adjust the dose of these to provide approximately 100% nicotine substitution, did not find that patches increased smoking cessation rates in women who were recruited in the second trimester of pregnancy (12-20 weeks)[88]. Cotinine levels while smoking were 119mg/ml and although they attempted to provide 100% substitution, cotinine levels while using NRT patches and remaining abstinent at 2 and 8 weeks after joining the study were still lower than levels when smoking[88]. A separate study by El-Mohandes found that in African-American pregnant smokers, who had mean baseline cotinine levels of 171ng/ml that cotinine levels did not reduce while using NRT patches[206], contrasting previous studies set in predominantly white pregnant participants. This could be due to adequate dosing as participants were given either higher or lower dose patches depending on their baseline cotinine samples or may be a result of smoking. In both these previous studies baseline cotinine levels while smoking were higher than the levels presented in this study chapter (98.5ng/ml). However in both these latter studies cotinine measurements taken while using NRT were not restricted to women that abstained from smoking. The differences in nicotine substitution levels observed in Berlin et al and El-Mohandes could also be influenced by the ethnicity of the participants; although less is known about ethnicity and nicotine metabolism in pregnant populations, in non-pregnant populations African-Americans have been found to metabolise nicotine at a slower rate [207] and so the nicotine doses provided by NRT may have provided better substitution.

Smaller, short-term laboratory based studies in pregnant women have also compared cotinine levels on NRT with pre-treatment levels generated by

smoking; they have found mixed evidence about the nicotine substitution rate. Many of the studies tried to ensure that nicotine levels generated were from NRT and not from smoking. A study by Ogburn et al administered 22mg patches for 24-hour periods over 4 days to pregnant participants (>24 weeks gestation) who were hospitalised and smoking abstinent[97]. It found that cotinine levels when using NRT were similar to baseline smoking levels (mean of 20 cigarettes per day). Morning nicotine levels while using the patches were found to be higher than morning levels recorded while smoking. However, this is likely to be due to women not smoking while sleeping, whereas nicotine from the patch would continue to be released whilst applied overnight[97]. In the UK pregnant women are usually recommended to remove nicotine patches at night to prevent continuous nicotine exposure [66,70]. A similar laboratory study by Oncken et al, administered 21 mg patches over an 8 hour period to women between 24-36 weeks gestation, who smoked a mean of 20 cigarettes per day at baseline[98]. This study also found similar cotinine levels while using patches compared to ad libitum smoking; it is possible that, in pregnancy, use of NRT for longer than 8 hours is required before stable cotinine levels are generated.

In contrast to laboratory studies which suggest that nicotine levels generated after adhering to NRT are similar to smoking, one study which administered 15mg/16hr patch over 5 days in smokers of 17 cigarettes per day found cotinine levels were 48% less on patches compared to those achieved when smoking ($p=0.029$)[99]. Participants were admitted on the first and fifth day of the study, however only 57% of this group remained abstinent between visits so cotinine levels presented may be from using a combination of NRT and smoking. Although most women reported use of

the patches daily and for recommended amount of time, the nicotine exposure was lower when compared to not using NRT and smoking. These findings are comparable to the decrease in nicotine substitution seen in the study presented in this chapter. However, this chapter has also presented data to show how levels of nicotine substitution vary based on women's cotinine values at baseline while smoking, with lower levels of substitution found in those who had the highest baseline cotinine levels.

In the study presented in this chapter, substitution levels were also compared between women who were and were not successful in smoking abstinence at delivery; a small non-significant difference was found between the change of cotinine levels from baseline and 1 month between these groups, which could imply that substitution was better in those who quit at delivery. However, the sample size was very small and so no conclusions can be drawn from this finding; further work is required to test the hypothesis that after using NRT those with nicotine substitution nearer to levels while smoking would have better quit outcomes. If such a hypothesis was proven, it could be that women from the SNAP trial who were excluded from this study due to smoking or low compliance did so because of inadequate nicotine substitution. However, it is still unknown what level of nicotine substitution is required during pregnancy to increase the chance of NRT being effective.

Outside pregnancy, literature is also limited research about the level of nicotine substitution required to ensure optimal efficacy of NRT. An RCT exploring the effectiveness of NRT found that after 1 week on nicotine patches (15ng/16hr) the nicotine substitution rate was 56% compared to baseline smoking[75]. Yet it is unknown in this study whether smokers who had higher rates of nicotine substitution had better long-term quit

outcomes. Evidence suggests that slower metabolisers of nicotine respond better to NRT compared to faster metabolisers; fast metabolisers may be more likely to quit using other smoking cessation medications such as varenicline[76]. This may be because the level of nicotine substitution supplied by NRT is sufficient for slower metabolisers, although what substitution level is required to provide optimum effectiveness of NRT is unknown. Combination NRT (i.e. patch plus short acting) has been shown to be more effective in smoking cessation compared to single form, yet it is unknown whether this is because of increased nicotine substitution or behavioural effects of having two sources of NRT[72].

The cotinine measurements presented from the analyses in this chapter were taken 1 month after the baseline cotinine samples; if, as research suggests, metabolism increases during pregnancy[166], then this may have partly contributed to the lower cotinine levels seen in our participants while using NRT. Interestingly, the 33 women included in this study were a lower gestation at baseline compared to the overall gestation of women at baseline in the SNAP trial[85]. If as research suggests metabolism does change during pregnancy it could be that women who were recruited at a later gestation were less likely to be eligible, as their metabolism may have increased and NRT may be less likely to provide adequate nicotine substitution. This could explain why the RCT used in this chapter, found that NRT was effective at 4 weeks after quit date, when the majority of women were in the second trimester, but NRT was not effective at delivery[85]. However, this is speculation and the exact time when changes in metabolism begin and how quickly they occur has not yet been determined.

Six women in the study had higher cotinine levels when using NRT compared to their baseline samples (while smoking). On further observation these six women on average smoked fewer cigarettes per day and had lower cotinine levels at baseline compared to the 27 women who had lower cotinine levels while using NRT. In non-pregnant populations smokers with slower metabolism tend to smoke less or have a lower level of nicotine intake when smoking[131]. Therefore, it could be speculated that these women were slower metabolisers during pregnancy hence why they smoked less at baseline and why cotinine levels were lower, and may explain why, when using a patch, they had higher cotinine levels compared to baseline. There were no observed differences in characteristics collected at baseline between these six women and the 27 women with lower cotinine levels, so individual variation in nicotine substitution could be due to genetic differences in the absorption of nicotine. An RCT by Berlin et al, which aimed to individualise the dose of nicotine in a patch, found that the dose of nicotine patch required to approximate a 100% substitution rate in pregnant women varied between 10-30mg/24 hr [88]; therefore the standard 15mg dose used in this study may have been too high for some women.

Our finding that, in pregnancy, cotinine levels tended to be lower from NRT than smoking is novel, as other studies on nicotine substitution in pregnancy have not specifically reported cotinine levels in abstinent and NRT adherent participants. However, our study sample was small and, in order to understand nicotine substitution more fully, larger studies where cotinine levels are obtained exclusively from women who are validated as smoking abstinent and NRT adherent would be required. By measuring anabasine and anatabine[208] it may also be possible to detect accurate nicotine substitution in pregnant women using NRT who have also been

exposed to tobacco smoke during a quit attempt. Once an increased level of understanding is achieved about nicotine substitution during pregnancy, comparisons with non-pregnant populations could be explored, by testing the hypothesis that NRT is not effective in pregnancy due to inadequate nicotine substitution. Using larger sample sizes in pregnancy could also provide the statistical power to identify whether there are any genetic and environmental factors that may influence nicotine substitution after using NRT.

2.6 Conclusions

In summary, this study found that, in pregnant women, cotinine levels generated by 15mg/16hr nicotine patches are lower than those generated by smoking. This apparently low level of nicotine substitution may be insufficient for NRT to have efficacy throughout pregnancy and may partially explain why standard dose NRT used by pregnant women has not been shown to be effective.

3 Chapter Three: Changes in the rate of nicotine metabolism across pregnancy: a longitudinal study

3.1 Introduction

As mentioned in Chapter One, nicotine replacement therapy (NRT) is often provided to assist pregnant women in their quit attempts, yet despite its effectiveness in non-pregnant populations, NRT has not been found to be effective in pregnancy [83,88]. A potential reason for this may be that nicotine metabolism is faster during pregnancy compared to outside of pregnancy [166,168,169,209] and so the dose of nicotine in NRT may not be enough to alleviate the nicotine withdrawal symptoms that pregnant women experience. Chapter Two explored nicotine substitution and the findings suggest that standard dose NRT provides insufficient levels of nicotine substitution when used during the second trimester of pregnancy.

Currently it is unknown exactly when changes in nicotine metabolism begin or the magnitude of these changes. No studies have attempted to measure nicotine metabolism from early pregnancy, throughout gestation and into the postpartum period. However, understanding the timings of changes in nicotine metabolism during pregnancy and the magnitude of these changes may be important to the efficacy of smoking cessation treatment. This could help explain why nicotine levels were lower when using NRT for one month compared to levels at baseline while smoking (Chapter Two). Therefore by using saliva samples to provide measurements of nicotine metabolism, this study will describe longitudinal variations in the metabolism of nicotine during pregnancy and postpartum.

3.2 Aims and objectives

Overall Aim

The purpose of this study was to measure pregnant smokers' nicotine metabolism using the ratio of trans-3'-hydroxycotinine to cotinine (3HC/COT) to determine how this varies across pregnancy and in the postpartum period.

Primary Objective

To determine when changes in nicotine metabolism occur during pregnancy and describe the magnitude of these changes.

Secondary Objectives

- To compare nicotine metabolism at 12 weeks postpartum with those at other time points in the antenatal period (8 to 14 weeks gestation, 18-22 weeks, 32-36 weeks) and at 4 weeks postpartum.
- To determine when nicotine metabolism stabilises in the postpartum period
- To explore cross sectional correlations between nicotine metabolism at 8 to 14 weeks gestation with the following: body mass index (BMI), nicotine dependence measures (Fagerström Test for Nicotine Dependence (FTND))[210] and maternal age.

3.3 Methods

3.3.1 Study design

This was a longitudinal cohort study measuring the nicotine metabolism of pregnant women who smoked daily during pregnancy. Saliva samples from

pregnant smokers were used to measure nicotine metabolite ratio (NMR) at three points during pregnancy and two points after pregnancy: between 8-14 weeks, 18-22 weeks and 32-36 weeks of pregnancy, and then 4 weeks and 12 weeks postpartum. The three time points measured during pregnancy would enable NMR to be observed throughout the three trimesters of pregnancy. The 12 weeks postpartum sample was the final NMR sample collected, a previous study measuring nicotine clearance during pregnancy has also used this time point as a comparison[166]. Also by 12 weeks postpartum it is possible for conception to occur again, suggesting that physiologically the body has reverted to a non-pregnant status[211].

3.3.2 Study procedure

Participants were recruited from two NHS hospital antenatal clinics in Nottingham, England, between August 2013 and October 2013, with follow-up completed by October 2014. Researchers handed screening surveys to all women who were attending either a routine scan or antenatal appointments to determine eligibility for the study. Eligible women were then approached by a researcher who discussed the study with them and took written informed consent. Participants at the QMC site were recruited by KB (PhD student) and a research midwife recruited the majority of participants at the CHN site. Participants were asked to provide a saliva sample by placing a sterile Salivette swab under their tongue until it was saturated. Participants also provided an exhaled carbon monoxide reading (CO), and had their height and weight measured.

3.3.3 Survey data

Participants were asked to complete a survey (see Appendix 8.2.3) with every saliva sample provided. Questions asked in the first survey aimed to describe the study population in terms of participant characteristics and to collect information about the factors that may influence nicotine metabolism. These included the following: age, ethnicity, body mass index (BMI), employment status, highest qualifications attained, age when they left education, and nicotine dependence (using the FTND questionnaire). The follow up surveys contained the FTND, and a question about infant feeding was added to the postpartum surveys (4 and 12 weeks postpartum). The FTND was made up of the following six questions [210].

1. *On average how many cigarettes/roll ups do you smoke each day?*
2. *How soon after waking up do you smoke your first cigarette?*
3. *Do you find it difficult to refrain from smoking in places where it is forbidden?*
4. *Which cigarette would you most hate to give up?*
5. *Do you smoke more frequently during the first few hours after waking than during the rest of the day?*
6. *Do you still smoke if you are so ill that you are in bed most of the day?*

These answers were scored and women were defined as either minimally dependent (score less than 4), moderately dependent (4 to 6) and highly dependent (7 to 10). The FTND has been shown to be a validated way of measuring nicotine dependence[212,213]. All additional details and further information on survey questions can be found in Appendix 8.2.4.

3.3.4 Inclusion criteria

Eligible women were aged 16 or over (no upper age limit), between 8-14 weeks pregnant (self-defined gestation) and were currently smoking 'regularly' (i.e. at least one cigarette per day) at recruitment. As the half-life of cotinine is approximately 16 hours, participants had to smoke at least one cigarette per day to ensure there was enough cotinine in the sample to generate a NMR measurement [1].

3.3.5 Exclusion criteria

Women were excluded from the study if they could not understand the study procedure sufficiently in order to provide informed consent e.g. due to cognitive difficulties. Women were also excluded for the following reasons:

- Multiple pregnancy; physiological changes include greater blood flow, plasma volume and basal metabolic rate compared to a singleton pregnancy [214]. Although the effects of a multiple pregnancy on NMR have not been explored before, in theory it could influence NMR.
- Consumed grapefruit within the last 24 hours at recruitment; grapefruit has been shown to decrease nicotine metabolism in non-pregnant population [155,158].
- Women with self-reported liver or kidney problems; evidence shows disease of the liver and kidney has a complex effect on drug metabolism [215].
- Women who self-reported that they used medications other than the following medications: ferritin, vitamin tablets including folic acid, medication to treat asthma. The influence of most

medications on nicotine metabolism is unknown, however to minimise the numbers of participants using other medications in the study, those using additional medication at recruitment were excluded.

3.3.6 Follow up data collection

Before each follow up the participant's hospital notes were checked to confirm pregnancy viability, and to validate gestation or to check their actual delivery date so that the dates for follow up calculations were correct. Women were asked if they were using NRT in each survey; women using NRT and not smoking would still be eligible to provide a saliva sample, as nicotine metabolism could be measured from the nicotine obtained using NRT.

Follow up saliva sample kits and surveys were posted to participants' homes when they were between 18-22 weeks and 32-36 weeks gestation, and then at 4 weeks and 12 weeks postpartum.

Follow up samples and surveys included instructions and a pre-paid leak-proof container to return both the sample and survey. Pre-paid return envelopes have been found to increase the return rate by up to 26% for postal surveys [216]. Incentives have also been shown to increase return rates [216], so for each sample received or survey completed participants received a £5 high street shopping voucher; this amount was increased to £20 for the final postpartum sample to encourage increased return rates.

Participants were notified by telephone or text to alert them that a sample kit was going to be posted to them. Women who did not return a sample kit within 2 weeks were reminded to return their sample by the researcher

either by telephone or text. In 11 cases, home visits were arranged to collect saliva samples from women who preferred not to post samples or who had difficulties receiving post. Women who had stopped smoking (self-reported) during the study and were not using NRT were instructed not to provide a sample whilst they were abstinent, but they were contacted before the next follow up to check whether they remained abstinent; if women had relapsed, they were asked to continue in the study and provide future saliva samples.

3.3.7 Ethical and research approvals

The study was given a favourable ethical opinion by East Midlands, Nottingham 2 Research Ethics Committee on 16/09/2013. Research and development approval was given by Nottingham University Hospitals NHS Trust on 9/08/2013.

3.3.8 Nicotine metabolite ratio

The outcome nicotine metabolite ratio (NMR) was measured by using the ratio of trans-3'-hydroxycotinine (3HC) to cotinine (COT) [131]. The hepatic cytochrome CYP2A6 enzyme is responsible for the metabolism of nicotine, 3HC and cotinine, therefore the NMR is a marker of CYP2A6 activity [130,131], and a higher NMR (3HC/COT) indicates faster nicotine metabolism. The ratio of 3HC/COT is often a preferred measure of nicotine metabolism in pragmatic studies compared to measuring nicotine as the cotinine half-life is longer than nicotine, so it remains in the body for longer and can be detected days after the participant has smoked [1].

3.3.9 Laboratory analysis

Samples were stored frozen at -20 degrees centigrade and stored at Nottingham Health Science Biobank before being sent by courier to ABS Laboratories Ltd, Welwyn Garden City, UK for analysis. Assays were carried out on the saliva to provide a measurement of cotinine and trans-3'-hydroxycotinine. The use of saliva to measure nicotine metabolites is a validated method of assessing exposure to nicotine [217]. The samples were quantified by liquid chromatography–tandem mass spectrometry (LC-MS/MS).

The LC-MS/MS method involved the addition of deuterated internal standards for both analytes, addition of a pH 7 buffer then liquid-liquid extraction using ethyl acetate prior to hydrophilic liquid chromatography tandem mass spectrometry using TurboIonspray in the positive ionisation mode using a CTC Agilent 1100 AB SCIEX API4000 LC-MS/MS system [218]. The assay range for both analytes was 1 to 500 ng/mL. Any over range samples were diluted and re-assayed. Precision was less than or equal to 10.3% for cotinine and less than or equal to 8.2% for 3-hydroxy cotinine; mean accuracy ranged from 88.8 to 107% for cotinine and from 98.6 to 101% for 3'hydroxycotinine. Once analysis had taken place, samples were destroyed in accordance with the Human Tissue Act, 2004.

3.3.10 Sample size and justification

This was determined based on the hypothesis that NMR will change between two points in pregnancy. The power calculation is based on a paired t-test to explore the difference between NMR at two time points. A plausible estimate of participants providing a sample at the first survey and

at another point during pregnancy was also a factor in deciding the sample size for this study. Few studies have explored NMR changes in pregnancy; only one, by Kim et al, presented NMR values at different time points in pregnancy [219]. This study included a line graph figure that showed NMR values (as 3HC/COT ratios) for 11 pregnant smokers at two time points in their second or third trimesters. By using the graphs to extract approximate NMR values for the second and third trimester for each individual, it was possible to calculate the mean difference and the standard deviation for the difference, which were 0.1 and 0.15 respectively. Thus in the study by Kim et al the change in NMR between the second and third trimester was approximately 2/3 of a standard deviation. We used this estimate of the standard deviation for change between the time points for our study, and we powered it to be able to detect an effect size that may be smaller than the one seen in the Kim et al study. Therefore, we estimated that, based on the change in NMR having a standard deviation of 0.15 between time-points, and by taking statistical significance as $p < 0.05$, a sample size of 73 participants would provide 80% power to detect a difference of 0.05 (1/3 of a standard deviation) between points in pregnancy. It was planned that 100 pregnant smokers would be recruited into study and anticipated that up to 30% may drop out after providing the first sample, with drop out defined as participants who do not return any follow up samples after the initial sample.

There were approximately 10,051 infants born at Queens Medical Centre and Nottingham City Hospital annually between 2011 and 2012. A national survey has shown that 12% of women will smoke throughout their pregnancy [35], so based on the number of women delivering at these hospitals there would potentially be 1080 eligible participants within 1 year.

Nottingham was known to have particularly high rates of smoking in pregnancy compared to the national average, therefore the number of women eligible for the study may have been even higher [220]. Baseline data taken from a recent longitudinal cohort study in Nottingham had found that 19% of pregnant women between 8-26 weeks currently smoked in pregnancy [53]. Therefore it was expected that the sample size estimated was achievable within the time frame and the resources available to recruit women into the study.

3.3.11 Statistical analysis

NMR was not normally distributed, and so was transformed to normality using a natural logarithm. NMR was compared descriptively in all available samples at each of the five time points: 8-14, 18-22 and 32-36 weeks gestation, and 4 and 12 weeks postpartum. It was expected the impact of pregnancy on NMR would have disappeared at 12 weeks postpartum. Previous research has used samples taken near to 12 weeks postpartum as a point of comparison with nicotine samples taken in pregnancy[166]. The overall significance of the change in NMR between time points was assessed using a multi-level model also known as a random effects model, which allows the significance of a change over time to be assessed, whilst allowing for the correlation between repeated measurements made on individuals [221,222]. Results of the multi-level model are presented in appendix 8.2.16.

A priori determined paired t-tests were then performed to determine the difference in NMR between 12 weeks postpartum and all other time points during pregnancy and 4 weeks postpartum. The Bonferroni correction was

used for multiple testing in the 4 pairwise comparisons of each time point with the 12 week postpartum value [223]. So the p values for each t-test were divided by the overall number of tests conducted (n=4) meaning that p values of 0.0125 or smaller were considered significant at the 5% level. Effect sizes are presented as percent change from the 12 week postpartum, result by anti-logging the logged mean difference. In a final comparison, all measurements made on an individual during the antenatal period were averaged (i.e. 8-14 weeks, 18-22 weeks and 32-36 weeks) and the postpartum period (i.e. 4 and 12 weeks postpartum). After which they were compared using a paired t-test to determine the magnitude and statistical significance of change in NMR between the antenatal and postpartum period.

3.3.12 Sensitivity methods

Two sensitivity analyses were carried out. In the first of these, data from the 26 women who returned all five saliva samples were looked at to see if their NMR followed a similar pattern over time to that described using all available samples.

The second sensitivity analysis excluded those who reported using hormonal contraceptive medication at either 4 or 12 weeks postpartum as there is evidence that suggests the contraceptive pill increases nicotine metabolism[146,148]. Self-reported data on current medication use was collected at each sample, however, aside from contraceptive medication there is limited research on how drugs may influence nicotine metabolism[1]. Therefore it was not deemed necessary to perform any further analysis on

women who, after recruitment, went on to use medication other than contraceptives.

3.3.13 Exploratory analyses

Exploratory analyses were carried out to investigate associations between logged NMR and age, nicotine dependence (FTND) and BMI at the 8 to 14 week time point. Associations between age[224], nicotine dependence[131], BMI[143] and NMR have not been explored before in pregnancy. Although there is some data outside pregnancy to suggest these factors may be weakly associated with NMR in non-pregnant populations there is currently no evidence about this during pregnancy. Cross sectional analysis at 8-14 weeks gestation was investigated to explore associations with NMR. This time point was chosen as it had the largest number of samples returned and so had the most power to detect a result. These variables were treated as continuous data for this analysis. Pearson correlation was used to measure associations, as NMR at 8 to 14 weeks was normally distributed and scatter plots were created to visually display associations. Associations were only explored with NMR at 8 to 14 weeks, as this time point had the highest number of samples analysed, enabling enough power to detect statistical associations. The association between ethnicity and NMR at 8 to 14 weeks was not investigated as the majority of the participants were from a white background (96 white verses 5 non-white) and therefore it was unlikely a statistical association would be detected. The computer statistical package Stata SE (version 13) was used to perform all statistical analyses.

3.4 Results

3.4.1 Recruitment rates

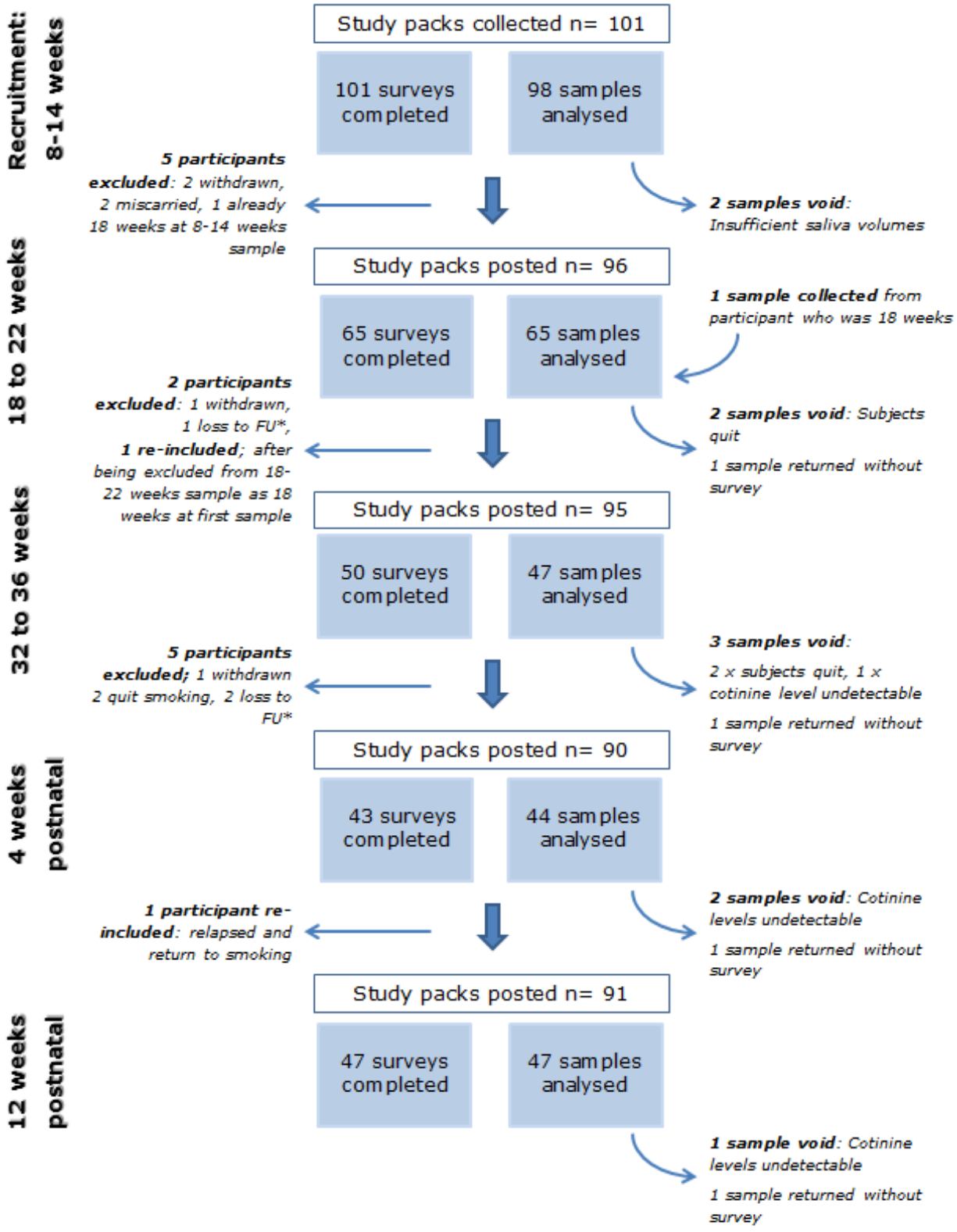
Participants were recruited from Nottingham University Hospitals NHS Trust between 1st of August 2013 and 25th October 2013, and follow-up was completed by October 2014. At City Hospital Nottingham (CHN) 52 (51%) participants were recruited and at Queens Medical Centre (QMC) 49 (49%) participants. At the QMC site, data was recorded on the number of screening surveys distributed, but this data was not recorded at CHN. At the QMC site 429 screening surveys were handed out, of which 271 (63%) women self-completed the screening questions. The remaining 158 (37%) women declined to complete the survey after it had been explained that the study was about smoking; most of these women stated they did not smoke and therefore were not asked to complete a screening survey. Six women who completed the screening survey were eligible but declined to take part in the study. Compared to eligible women who did take part in the study, women who declined were likely to be of earlier gestation (mean 9 weeks, SD 0.70, compared to 12 weeks, SD 1.78) and were older (median 26 years IQR 25-29, compared to 24 years IQR 24-28).

3.4.2 Response rate

Figure 7 shows the response rates for the saliva samples and surveys at each time point. A total of 98 out of 101 saliva samples provided at recruitment (8 to 14 weeks) were able to be analysed; two contained insufficient saliva and one woman self-reported she was 14 weeks pregnant but later her ultrasound scan showed that she was 18 weeks and the sample obtained at recruitment was therefore included in the 18-22 week analysis. The first follow up at 18-22 weeks gestation had the highest

return rate (n=65, 64%) and the lowest response rate was 4 weeks postpartum (sample n=44, 43%). Samples provided by two participants at both 18-22 and 32-36 weeks were classed as 'void' as they self-reported to having stopped smoking. These two participants were contacted again prior to the 4 week postpartum sample being posted to check they remained abstinent; both remained abstinent at this point so were not eligible. One of these participants had returned to smoking by 12 weeks postpartum and was sent a final sample kit to complete. Four samples had cotinine levels that were undetectable, as the levels of cotinine were too low to be analysed. The response for the final samples (12 weeks postpartum) was 47 (46%). A total of 79 (78%) women provided more than one sample for comparison during this study, and 26 (26%) women provided a sample at all five time points.

Figure 7 Recruitment and follow up rates for samples and surveys



* FU= Follow up, *loss to FU= moved address/not contactable

3.4.3 Characteristics of the sample

The baseline characteristics of the 101 participants recruited into the study can be seen in Table 4; these were compared with the baseline characteristics of participants who returned the 12 week postpartum sample (n=47) to make sure there was no bias between the entire population and this sub-sample used for the paired t-tests. There was a slight difference in the median number of cigarettes smoked; at recruitment the median smoked was 10 per day for the entire sample whereas women who returned the 12 week postpartum sample smoked 8 cigarettes per day at recruitment. Also only one of the five non-white background participants returned a sample at 12 weeks postpartum. The remaining characteristics appeared similar between the two groups. We recorded breastfeeding rates in the postpartum surveys; of the 47 women who returned surveys at 12 weeks postpartum, four were breastfeeding.

The median number of cigarettes participants smoked per day at each time point was: 8-14 weeks, 10 (IQR, 5-10); 18-22 weeks, 10 (IQR, 4-11); 32-36 weeks, 10 (IQR, 5-10); 4 weeks postpartum, 10 (IQR, 7.5-15); and 12 weeks postpartum, 10 (IQR, 7-15). Two participants reported using NRT during the study; one participant used nicotine lozenges and was also smoking at 18 to 22 weeks gestation. A second participant, who reported not smoking, was using nicotine gum at 4 weeks postpartum, and nicotine patches and e-cigarettes at 12 weeks postpartum.

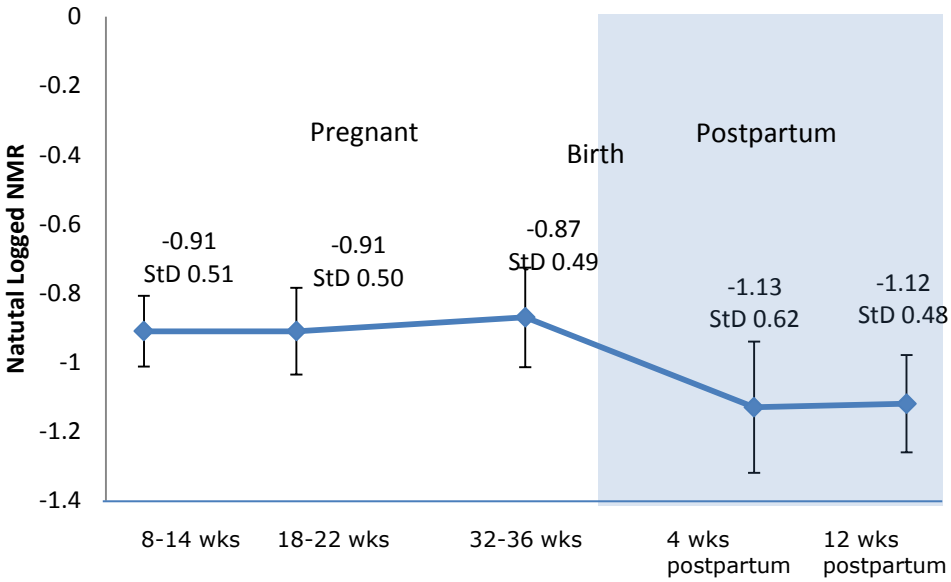
Table 4 Characteristics of all participants at 8 to 14 week gestation (recruitment) and those participants who returned a sample at 12 weeks postpartum

Variables at recruitment	Recruitment: 8-14 weeks gestation (n=101)		Returned sample at 12 weeks postpartum (n=47)	
Maternal age (Median, IQR)	24 (20-28)		25 (20-29)	
Gestation at recruitment (Mean and SD)	12.3 (1.78)		12.3 (1.77)	
Cigarettes smoked at recruitment (Median IQR)	10 (5-10)		8 (5-12)	
Carbon monoxide reading (CO)	11 (7-16)		11 (8-16)	
Employment (N & %)				
Employed	32	32%	18	41%
Unemployed	34	34%	12	25%
Full Time Student	3	3%	1	2%
Homemaker/Parent	30	30%	14	29%
Other	2	2%	2	4%
Qualifications (N & %)				
None	20	20%	9	18%
GCSE/Or Equivalent	57	57%	24	53%
A Levels/Equivalent	19	19%	11	22%
Degree/Equivalent	2	2%	1	2%
Other	3	3%	2	4%
Partner smokes (N & %)				
Yes	71	70%	36	77%
No	19	19%	11	23%
No Partner	11	11%	--	--
Partner/Family smoke in home (N & %)				
Yes	36	*35%*	18	*50%*
No	35	*36%*	18	*50%*
No Partner/partner not a smoker	30	*29%*	--	--
Ethnicity (N & %)				
White-British/Irish/Other White	96	95%	46	98%
Other Background	5	5%	1	2%
Fagerstrom (N & %)				
Mildly Addicted <4	56	55%	25	53%
Moderately Addicted 4-6	37	36%	19	41%
Highly Addicted 7-10	8	7%	3	6%
BMI (8-14 weeks gestation) (N & %)				
Underweight Less Than 18.5	9	8%	3	7%
Healthy Weight 18.5-24.9	50	50%	21	46%
Overweight 25-29.9	20	20%	10	22%
Obese Above 30	21	21%	12	26%
Missing	1	1%	1	2%

3.4.4 Pattern of change over time

The mean of the log-transformed values of NMR and StD at different time points are shown in Figure 8. Nicotine metabolism appears to be higher in pregnancy than outside of pregnancy; after pregnancy, the ratio had decreased by 4 weeks postpartum.

Figure 8 Nicotine metabolite ratio (NMR) over time



The bars are the 95% confidence intervals (CI) for the precision of the estimate at each time point.

Table 5 shows the pairwise comparison of NMR at 12 weeks postpartum with NMR at other time points. After accounting for multiple comparisons NMR was significantly higher at 18-22 weeks (p=0.001) and 32-36 weeks (p=0.003) compared to NMR at 12 weeks postpartum. There was no statistical difference between NMR at 8-14 weeks gestation or at 4 weeks postpartum compared to 12 weeks postpartum. The comparison of mean log transformed NMR from samples collected during pregnancy with those collected after delivery (4 and 12 weeks postpartum combined) showed

that NMR was 17% higher in the antenatal period (95% CI: 6% to 26% (p=0.023)).

Table 5 Pairwise comparisons of NMR at 12 weeks postpartum with each time point antenatally and at 4 weeks postpartum

Comparison to 12 weeks postpartum	*Percentage difference*	*95% confidence intervals*	*P value*
8-12 weeks gestation (46 paired samples)	+15%	+1% to +26%	0.034
18-22 weeks gestation (37 paired samples)	+26%	+12% to +38%	0.001*
32-36 weeks gestation (31 paired samples)	+23%	+ 9% to +35%	0.003*
4 weeks postpartum (35 paired samples)	+9%	-12% to +26%	0.405

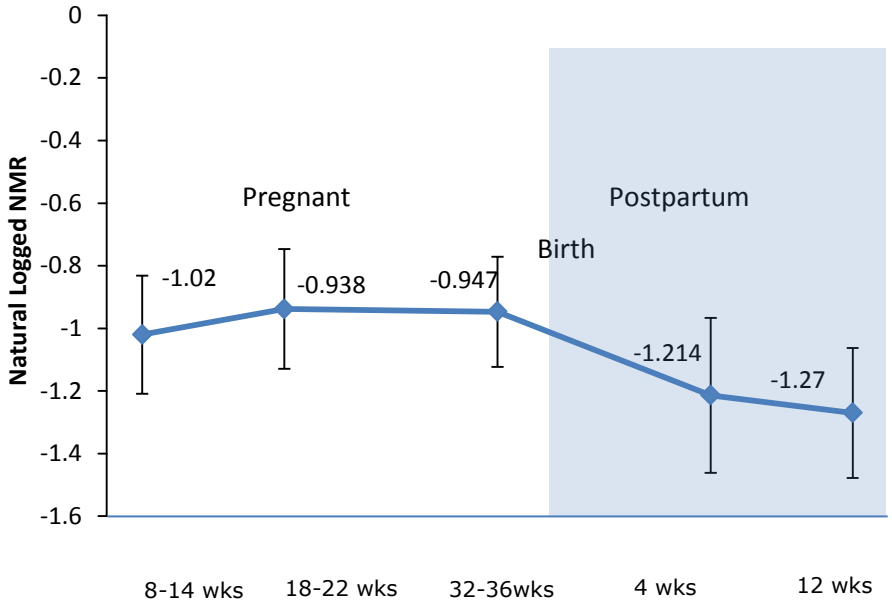
**significant after accounting for multiple testing using the Bonferroni correction*

3.4.5 Sensitivity analysis

3.4.5.1 Women who returned all 5 samples

The mean of the log-transformed values of NMR at different time points in the 26 women who returned a sample at every time point is shown in Figure 9. Nicotine metabolism appears to be higher in pregnancy than outside of pregnancy; after pregnancy NMR has decreased by 4 weeks postpartum.

Figure 9 Nicotine metabolite ratio over time in women with complete data (26 women)



The bars are the 95% confidence intervals (CI) for the precision of the estimate at each time point

3.4.5.2 Contraception

A list of medications women reported to have taken during the follow up can be seen in Appendix 8.2.15. Overall, seven women reported using hormonal contraception at either 4 or 12 weeks postpartum; one of the seven women provided a postpartum sample at 4 and 12 weeks and was taking hormonal contraceptives at both time points. After excluding women who reported using hormonal contraception postpartum (n=7) the observed pattern of NMR over time appeared similar.

3.4.6 Associations with NMR at 8 to 14 weeks gestation

Using log transformed NMR, Pearson's correlation coefficients showed a negative correlation between NMR and nicotine dependency, measured by the FTND (ρ -0.2794, p=0.0053) (Figure 10). Although the associations are

weak, they suggest women with higher nicotine dependence scores have a lower NMR. Age was weakly associated with NMR, and was of borderline significance (ρ -0.139, p=0.052) (Figure 11). There was no association between BMI and NMR at 8 to 14 weeks gestation (ρ -0.1490, p=0.1452) (Figure 12).

Figure 10 Scatterplot of Fagerstrom Score and NMR at 8-14 weeks gestation

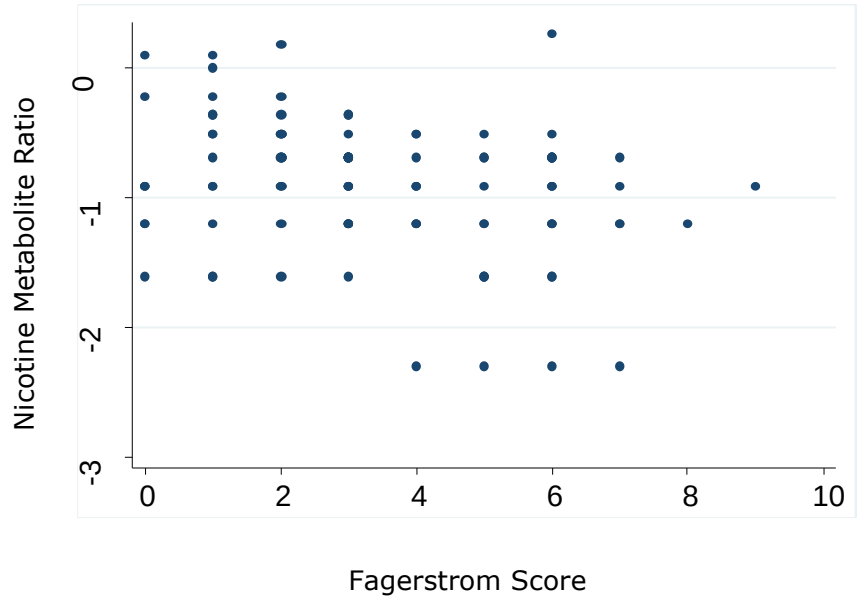


Figure 11 Scatterplot of maternal age and NMR at 8-14 weeks gestation

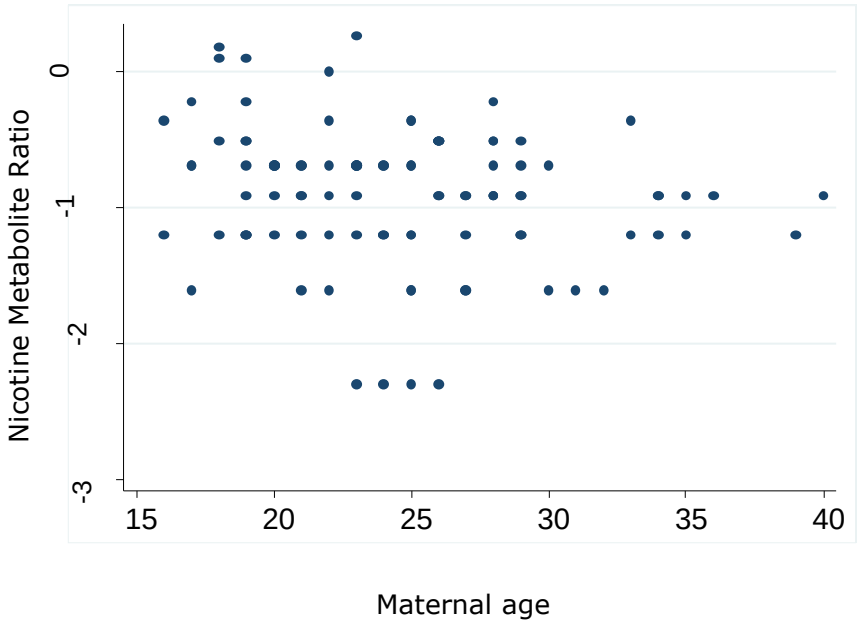
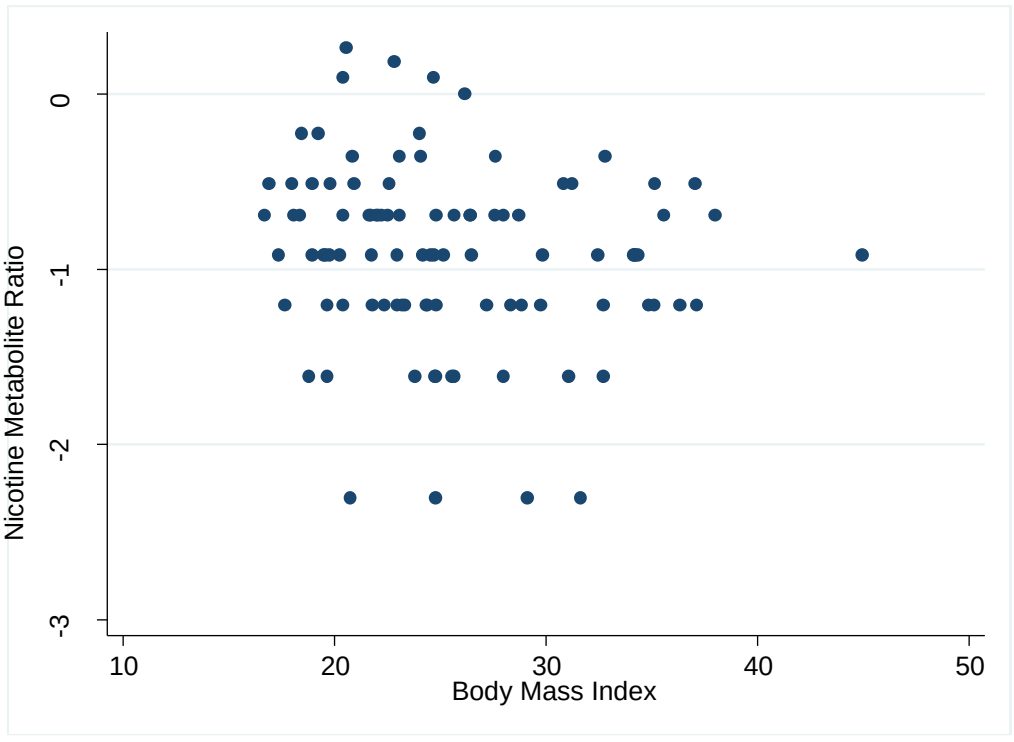


Figure 12 Scatterplot of BMI and NMR at 8-14 weeks gestation



3.5 Discussion

3.5.1 Main findings

This is the first study to describe the longitudinal pattern of nicotine metabolism from early pregnancy until 12 weeks following birth. The findings show that nicotine metabolism appears to be faster in pregnancy; this faster metabolism is apparent from 18 to 22 weeks of pregnancy and appears to fall to an 'outside pregnancy' rate by 4 weeks after childbirth.

3.5.2 Strengths and limitations

This study is one of the largest pharmacokinetic studies of any drug throughout pregnancy[161,163]. Using a large prospective cohort of pregnant women in early pregnancy, repeated measures of NMR were taken from the same women, three antenatally and two postpartum. By measuring NMR from as early as 8 weeks gestation this is also the first study to measure NMR at such an early time point in pregnancy. This has enabled the exploration of within-subject changes over time. This study was a pragmatic observational study and although the sample size was larger than any other previous similar study, after accounting for multiple testing more participants would be required in order to provide enough power in the study to ensure results observed were not due to chance. Yet given the period allotted for recruitment, study analysis and reporting, a larger sample size was not feasible. Future studies need to consider larger sample sizes to account for multiple testing as post-hoc calculation suggests there should be a minimum of 104 participants at each time point.

This is the only study to accurately record nicotine metabolism soon after pregnancy (4 weeks postpartum) and again at a much later point following

birth (12 weeks postpartum). Therefore, unlike any other study, this study has been able to report when nicotine metabolism may change after birth. This is the first study of nicotine metabolism that recruited a cohort from a routine antenatal care setting, and so it is likely this sample will be more representative of pregnant women than studies that recruited to laboratory studies by advertisements. By using saliva-based assays, an NMR measurement was obtained at precise time points during pregnancy, unlike hair analysis that will only provide a measure of metabolism over a defined period. Therefore, using saliva it has been possible to show longitudinal changes in the rate of nicotine metabolism between specific time points.

The response rate fell to below 50% by the time of the 4 week postpartum sample; this could have given rise to response bias, as the participants who returned samples could have been different to those that did not. However, no major differences between women who returned the final 12 week postpartum sample and all women recruited to the cohort was found, so this does not seem to have been influential. Sensitivity analysis was conducted on participants who provided a sample at every time point; this showed a similar pattern of nicotine metabolism change to that observed in the primary analysis, again suggesting that response bias did not impact unduly on study results.

Two participants had quit smoking during the study, and it is possible other women had quit smoking, which may have lowered the response rate. Quit attempts have been shown to fluctuate throughout pregnancy, yet most occur in the first trimester and many are unsuccessful [47] so it is unlikely this would account for many of the women who didn't respond in this study. Apart from the samples collected at recruitment, most of the follow-

up samples were returned by post; therefore there is a small possibility that the saliva provided was not from the participant. However, as the sample was not for validation of smoking cessation, it is unlikely that participants would do this as there were no benefits to gain from this.

The 12 weeks postpartum sample was used as a comparison time point and was measured against all the time points during pregnancy and shortly after birth. By 12 weeks postpartum, it is possible for a woman to become pregnant again, suggesting that sex hormones which have been shown to influence nicotine metabolism have stabilised to levels of a non-pregnant woman[146,148]. Ideally, NMR would have been collected before conception and participants would have been followed into pregnancy. However, recruiting women prior to conception would be very difficult to achieve given the time frame of this study, particularly as most women do not access healthcare until they know they are pregnant and those that access healthcare before becoming pregnant are likely to be a different population of women to those that do not plan a pregnancy. There were no significant differences in the rates of metabolism between 4 and 12 weeks postpartum, suggesting that by 12 weeks following birth the rate of NMR had not decreased further. However, ascertaining a NMR sample at a much later time point, for example one year later could determine if nicotine metabolism alters further after 12 weeks postpartum. No previous studies have measured nicotine metabolism postpartum at sequential time points, so the finding that there was no further fall in nicotine metabolism between 4 and 12 weeks postpartum is a novel finding.

3.5.3 Findings in context of previous research

Consistent with a previous study by Dempsey et al, we have shown that nicotine metabolism is faster during pregnancy compared to the postpartum period [166]. Our study also found that at 18-22 weeks and 32-36 weeks gestation NMR was significantly different from 12 weeks postpartum. The difference between 8-14 weeks and 12 weeks postpartum was in the same direction but was not significant after allowing for multiple testing. Those that provided a sample at 8-14 weeks and 12 weeks postpartum had similar mean logged NMR values compared to those that provided samples at 18-22 weeks and 12 weeks postpartum, yet the 8-14 weeks samples was not significantly different from 12 weeks postpartum. A possible explanation for this could be that there were differences in the characteristics of women who provided samples at these time points..

The study found that, from 8-14 weeks onwards, NMR remained fairly constant throughout gestation. Studies that have analysed hair segments from each gestation of pregnancy have also shown that nicotine metabolism appears to increase during pregnancy, peaking in the third trimester of pregnancy[168,169,209]. A laboratory based study by Dempsey et al that used serum and urine samples from pregnant smokers did not find a pharmacokinetic trend with increasing gestation[166]. However, change in nicotine metabolism during pregnancy was not the primary focus of this study, so only five of the 10 women in the study provided two samples antenatally and most participants were in their late second and third trimester of pregnancy when measurements were obtained[166]. Most studies which have been able to derive longitudinal trends during pregnancy have relied on the analysis of hair samples which cannot provide measurement

at precise time points, and may be problematic due to contamination from hair products, hair pigmentation and hair washing [168,209].

Studies outside pregnancy have found that weight, gender, ethnicity and other environmental factors account for 7.6% in the variation in NMR [159] but most differences in nicotine metabolism are likely to be explained by genetic factors [159,225]. However, it is important to understand what factors may influence metabolism, as nicotine metabolism can influence how smokers respond to smoking cessation treatments [76]. This study involved cross sectional analysis which explored the influences of NMR at one time point, however in future repeated measures studies containing larger sample sizes these factors could be explored with change in NMR overtime.

In pregnancy there has been no research on factors that may influence nicotine metabolism. By using the largest sample size available in this study, which were the samples obtained between 8 to 14 weeks gestation, we were able to investigate factors that may be associated with nicotine metabolism during pregnancy. In contrast to evidence from outside pregnancy, BMI was not associated with NMR during this study [143,144]. However, no firm conclusions can be drawn from this one study, and future studies investigating body weight should be conducted using a larger sample size and investigate the effects of BMI on NMR over time. Also comparisons with non-pregnant populations may be difficult as weight in pregnancy is physiologically different when taking into account the weight of the growing uterus and changing blood volumes[211].

This study is the first to explore the relationship between maternal age and nicotine metabolism during pregnancy, and the finding of a negative

correlation, albeit weak, is novel. The weak association found may be due to chance. A theoretical explanation could be that with increased age factors such as reduced liver blood flow [130] However, the relationship between age and nicotine metabolism in non-pregnant populations is limited and the findings inconclusive [130,141,224]. Findings are difficult to compare with pregnant populations as the age ranges in the study of non-pregnant populations involve wider age ranges than the biologically restricted age ranges due to pregnancy, seen in this study.

An inverse relationship between increased nicotine dependence, measured by FTND, was found with nicotine metabolism in early pregnancy. It is unclear why this occurred, as in non-pregnant populations a systematic review did not find any study which showed a significant relationship between the FTND and nicotine metabolism [131]. However, some studies have found associations with nicotine metabolism when looking at individual questions on the FTND such as which cigarette they would hate to give up [131]. The number of cigarettes smoked per day has been associated with increased nicotine metabolism [131] and as the FTND incorporate the number of cigarettes smoked, theoretically it would be expected that they would be positively associated with NMR. It is unclear why an inverse relationship between the FTND during pregnancy was found; it may be that women in pregnancy respond differently to measures of nicotine dependence compared to non-pregnant smokers, due to social stigma [43,44] and increased motivation to change smoking habits in early pregnancy [45].One large study did, however, find that the validity of the nicotine dependency measured by the heaviness of smoking index during pregnancy was comparable to non-pregnant populations [92]. However these pregnant women were participants in a RCT exploring the effectiveness of

NRT, and knew they were being biologically validated, so may have been more open about their nicotine dependence.

Outside pregnancy, women using oral contraceptives have a higher rate of nicotine metabolism compared to women not using contraceptives [146,148]. Contraceptives contain progesterone or a combination of oestrogen and progesterone hormones. During pregnancy progesterone levels rise markedly, in early pregnancy progesterone is initially produced by the corpus lutetium of the ovaries before being taken over by the placenta at around two months. At this point, progesterone levels rise further and stay constant until birth, when the placenta is removed. Oestrogen is made and released by the corpus luteum, it is regulated by the pituitary gland. The levels of oestrogen increase steadily throughout pregnancy until birth[226]. It is speculated that these hormones are responsible for the increased CYP2A6 activity [130]. This could explain why NMR was observed to be faster during pregnancy, and why there was a decline in NMR at 4 weeks postpartum, as following birth there would be a rapid reduction in these hormones. However, the exact causes of increased nicotine metabolism require investigation.

It was originally hypothesized that if nicotine metabolism increased during pregnancy then the dose of nicotine in NRT may not be enough to alleviate nicotine withdrawal symptoms. As an increase in nicotine metabolism during pregnancy has been shown in this study, it suggests that higher doses of NRT may be necessary. Observational evidence has shown that, during pregnancy, using a combination of long and short term acting NRT (patch and a faster acting form) is associated with higher short term quit rates compared to those attributable to using either no medication or single

form NRT [89]. However, to date no randomised control trials have investigated this, so further research is required on the effectiveness of higher dose NRT.

3.6 Conclusion

This is the largest, longitudinal study to have measured nicotine metabolism across pregnancy and the first to show that metabolism is faster from 18 to 22 weeks week of pregnancy onwards. This study has shown that in pregnancy nicotine metabolism is increased compared to the postpartum period; it falls between birth and 1 month and does not seem to fall further after this. Nicotine metabolism influences the effectiveness of pharmacotherapies used by non-pregnant smokers [76] and study findings could help to explain why NRT has, to date, been found ineffective, in pregnancy. Increased nicotine metabolism could explain why in Chapter Two it was found that, in pregnant women, cotinine levels generated by nicotine patches one month into a quit attempt were lower than those generated by smoking at baseline.

4 Chapter Four: Understanding pregnant smokers' adherence to nicotine replacement therapy during a quit attempt: a qualitative study

4.1 Introduction

Pregnant smokers may be offered NRT alongside behavioural support to assist with a quit attempt [66], yet trials investigating NRT during pregnancy have found that pregnant smokers typically have low adherence to NRT [83]; this may have contributed to its apparent lack of effectiveness in pregnancy[83,88]. Data from routine practice indicates that adherence with NRT is also low in routine care settings with many women only being prescribed NRT for a 2 week period [82]. Chapter Two has shown that NRT given at standard dose may not provide adequate nicotine substitution and Chapter Three has shown that nicotine metabolism is increased during pregnancy compared to outside of pregnancy. These latter findings could help explain why NRT adherence is low, as the dose of nicotine in NRT may not be high enough to alleviate nicotine withdrawal during pregnancy. However, aside from changes to nicotine metabolism there could be other explanations as to why pregnant women do not adhere to NRT, and the study presented in this chapter will seek to explore these.

There is little uniformity in the definitions or reporting of adherence to NRT that are used in trials, both in and outside of pregnancy, which can make it difficult to compare between studies [85,172]. Adherence can be defined by the degree to which the individual conforms to using the medication as it is recommended, such as the timing, dosage and frequency of using treatment [173]; low adherence also includes early discontinuation of treatment[154]. Poor medication adherence in general may be either intentional - where the person weighs up their perceived risk and benefits of treatment and makes an informed choice - or unintentional - where the person forgets to adhere to treatment and is not sufficiently careful to administer medication as recommended [174].

There is limited knowledge about how women use NRT in pregnancy. Survey data suggests medication use during pregnancy can be influenced by unpleasant side effects, by individuals wanting to test their ability to quit without using NRT and by a belief that NRT is not working[185]. There is also evidence that both non-pregnant [227] and pregnant smokers[195,228] believe using NRT is harmful to health and so reduce their use of NRT accordingly. One study interviewed women about their experience of using NRT in pregnancy and reported that while women perceived NRT to be useful in alleviating stress from nicotine cravings, the most helpful factor for assisting a quit attempt was midwifery support [189]. Yet women in this study were interviewed at time points after their quit attempt with some women being postpartum at interview, so responses may be subject to recall bias. Therefore, current attitudes and beliefs of women who experience using NRT at a point close to their current quit attempt are unknown.

4.2 Aims

The aim of this study was to understand the experience of pregnant women using NRT who discontinue this early or who do not use this medication as recommended by smoking cessation advisors.

4.3 Methods

Qualitative semi-structured telephone interviews were conducted with pregnant women who had used NRT as part of their most recent quit attempt, but had not used NRT as it was recommended. Favourable ethical opinion was given by the West of Scotland Research Ethics Committee (Appendix 8.3.6). All interviews were conducted by researcher KB (PhD

student) between May and December 2014. The qualitative research Consolidated Criteria for Reporting Qualitative Research (COREQ) tool [229] was used to promote a comprehensive report of the methods and findings of this research.

4.3.1 Recruitment

Two NHS stop smoking services (SSS) in Nottinghamshire were used as participant identification centres; pregnant women attending these who received NRT as part of their quit attempt were informed about the study. Smoking cessation advisors first approached these women, either face to face or by telephone during a smoking cessation consultation, and those who expressed an interest to take part were asked for verbal consent to pass their contact details onto a researcher. A participant information sheet (PIS) (Appendix 8.3.1) was posted to all interested women, after which they were telephoned by the researcher to seek consent to take part in the study.

4.3.2 Screening Procedures

One week after being posted a PIS women were telephoned by a researcher (KB) to seek consent to take part in the study. They were then asked to complete a short telephone 'screening' survey lasting 5-10 minutes in order to determine whether they had used NRT as recommended by the SSS (Appendix 8.3.2). The researcher sought information from SSS as to how pregnant women were prescribed NRT, in order to determine whether women had adhered to NRT as recommended. Currently there are no national guidelines as to how NRT should be

prescribed during pregnancy. Table 6 shows how each NRT product was recommended for use by the SSS included in this study.

Treatment adherence can be defined as the degree to which the smokers' behaviour follows health recommendations[230]. After liaising with the SSS involved in this study a description of how NRT is recommended was established and this was used to determine whether women had adhered to these recommendations. After completing the survey, women who, from their responses, were considered to have not used NRT as recommended, were asked to participate in a qualitative telephone interview. Women were excluded if they reported abstinence from smoking and were using NRT as recommended.

Table 6 Recommendations given by stop smoking services for NRT use during pregnancy

NRT	***Wear daily, remove at night***	***Single use NRT, on hour every hour****	***Dual use NRT per day~***	***Smoke while using***	***Length of NRT use***
Patches 5mg/10mg/15mg in 16hrs	Yes	NA	NA	No	6-12 weeks
Gum 2mg or 4mg	NA	Yes	Take if craving; Max 6-8 uses	No	6-12 weeks
Inhalator 15mg	NA	Yes	Take if craving Max 3 cartridges	No	6-12 weeks
Mouth Spray 1mg per spray	NA	Yes	Take if craving Max 6-8 uses	No	6-12 weeks
Microtab 2mg	NA	Yes	Take if craving Max 6-8 uses	No	6-12 weeks
Women are encouraged to contact the services should they require support in-between appointments.					

* *single use, using only a short acting product, advice for waking hours*

~ dual use, using a patch and a short acting product

4.3.3 Interviews

The interviewer in this study (KB) was female, had a background in midwifery and had some previous experience of conducting qualitative research. Separate recorded verbal consent was obtained for interviews. Women were not eligible for the study if they could not understand the study procedure sufficiently to provide informed consent or were under the age of 16 years. KB introduced herself as a researcher from the University of Nottingham who worked outside the SSS organisation; this was clearly stated so that participants might feel able to talk more openly about their experiences and use of NRT without concern that it could influence their care. Participants were made aware that the study was being conducted in order to understand how pregnant women experience using NRT as part of their quit attempt. Interviews lasted between 20-60 minutes, and were audio recorded so that the interviewer could fully concentrate on listening and engaging with the respondent during the interview. Data was transcribed verbatim by Clayton Research support. All participants who completed a qualitative interview were sent a £20 high street shopping voucher in exchange for their time.

Interviews covered the following areas: expectations about using NRT, experience of using NRT, perceived barriers of using NRT, perceived usefulness of NRT, and general opinions about using NRT as part of a quit attempt during pregnancy (see 8.3.3 for the interview topic guide). A member of the public who was on the UK Centre for Tobacco and Alcohol studies, research smokers' panel, who had smoked during her own pregnancy, reviewed the interview schedule; the input from this person

ensured the wording and language was appropriate to pregnant women. Background questions included gestation, age, ethnic group, partner status and partner smoking status. Data was analysed throughout the data collection phase which enabled reflexion about the interviews as they were being completed. Data collection continued until little or no new insight was gained on the experience of using NRT from the final few interviews, indicating that data saturation had been reached. Each participant was interviewed once and interviews took place between 4 and 49 days since the first day NRT had been used. No repeat interviews were carried out and it was not felt necessary to return the transcripts to participants to comment after the interviews had took place, as clarification of views and experiences had often taken place during the interview.

4.3.4 Analysis

Inductive thematic analysis was used to identify and report patterns and themes within the text, and which captured the data in relation to the research questions or other relevant topics mentioned by the women during their interviews [231]. Thematic analysis enables the researcher to describe patterns and themes across data sets. Data can be easily linked to the themes and validated to ensure they reflect the data [232]. This technique enables the participants experience to be reflected in the research findings, without the interviewer placing their own interpretation and version of events on to the data[231]. The inductive nature of this analysis also meant that themes identified would be explored even if they did not relate necessarily to the original research question. Data was read, re-read and coded; codes were then reordered by importance, combined where appropriate, and the codes grouped into emerging themes. The emerging

themes were explored in depth and mapped back onto the interview transcripts and coded data and consequently refined. Attention was paid to cases which deviated from the norm, as a way of increasing credibility of the findings [233]. A second researcher (KC) independently coded 20% of the interview transcripts to establish the coherence of the coding framework; the coding consistency was high. NVivo 10 software was used to assist with the coding of the data.

4.4 Results

4.4.1 Response and characteristics of sample

Twenty-three women expressed an interest in participation; six were non-contactable, three declined participation, and 14 (61%) women were surveyed for eligibility, all of whom were eligible for the qualitative interviews and took part in the study. Table 7 displays socio-demographic data and information about participants' self-reported smoking and NRT use during their most recent quit attempt. The most frequently prescribed and used NRT product was the inhalator. Most women reported using their NRT product 'every day or almost every day'. However, of the eight women who only used short-acting NRT products, four (50%) reported they used their NRT product four times or fewer per day, which is far less than the 'on the hour, every hour' recommended advice (see Table 6). All women had smoked during their quit attempt, and at the time of the interview 10 were smoking; seven of these 10 women were also still using NRT. Two women were smoking, using NRT and an e-cigarette at the time of the interview. Apart from one woman who was interviewed one month after discontinuing NRT, all other women were either using NRT at the time of the interview or had discontinued using NRT within the previous 7 days.

Table 7 Participants' socio-demographic characteristics, self-reported NRT use and smoking behaviour during quit attempt

Self-reported NRT use and smoking behavior	**Mean & StD/Number& % (N=14)**
Maternal age (years) (mean, SD)	28 (4.6)
Gestation (weeks) (mean, SD)	14 (3.6)
Cigarettes smoked before quit attempt (n) (mean, SD)	14 (3.6)
Ethnicity	
White	13 (93%)
Mixed British and Caribbean	1 (7%)
Partner smoking status (n, %)	
Smoker	9 (64%)
Non-smoker	4 (27%)
No Partner	1 (7%)
NRT product(s) prescribed and used (n, %)	
Patches	5 (36%)
Gum	1 (7%)
Microtab	1 (7%)
Inhalator	6 (43%)
Patches, mouth spray and inhalator	1 (7%)
Used E-cigarettes during current pregnancy (n, %)	
Yes	5 (36%)
No	9 (64 %)
Frequency NRT used on average in period leading up to interview (n, %)	
Every day or almost everyday	10 (72%)
Every other day	2 (14%)
Less than every other day	2 (14%)
If patches worn, length of time (n=6)	
16 hours	4 (67%)
<16 hours	1 (7%)
>16 hours	1 (7%)
***Short acting NRT only (n=8)**	
more than 12 times per day	1(12%)
5 to 12 times per day	3 (38%)
4 or fewer times per day	4 (50%)
Smoked at all during quit attempt	
Yes	14 (100%)
No	0 (0%)
Continuing to smoke at time of interview	
Yes	10 (72%)
No	4 (28%)
Continuing to use NRT at time of interview	
Yes	9 (64%)
No	5 (36%)
Days after commencing NRT interview took place	
1 to 7 days	6 (43%)
8-14 days	2 (14%)
15-21 days	5 (36%)
>22 days	1 (7%)

** Those that used only short acting NRT (i.e. gum, microtab, lozenges, spray, inhalator) and did not use patches alongside.*

4.4.2 Themes

The following themes were identified within the data: expectations of NRT, experience of using NRT (perceived effects of NRT use, concomitant smoking and side effects), safety concerns and experience of using e-cigarettes.

Expectations of NRT

All women expressed an increased desire to stop smoking once they became aware they were pregnant; protecting the health of their baby was a priority. Some women were initially surprised that they were offered NRT in pregnancy as they had expected that they would have to quit smoking with willpower alone. Women who were aware about the possibility of using NRT to help them quit tended to have made a decision about the type of NRT product they wanted to use before meeting with their smoking cessation advisor. Some women who chose the inhalator often did so as they felt it would imitate the behavioural actions they associated with smoking (e.g. being held, like a cigarette and having the hand to mouth action associated with smoking):

I don't know what to do with my hands when I'm not smoking – so that's why I used the inhaler [inhalator] *because it's something in my hands and it can help – feels like I'm smoking a fag* [cigarettes] *kind of thing*
Respondent 2 (Inhalator)

That [inhalator] *was my first choice because obviously when you're putting a cigarette to your mouth or you know, it's kind of a similar kind of effect*
Respondent 3 (Inhalator)

However, other women who wanted to avoid the behavioural habits they associated with smoking chose products they felt would not remind them of smoking. Patches were viewed as a discreet method of using NRT that were easy to use, easily forgotten about and did not interfere with daily activities:

I didn't want anything really in my mouth because of the taste of it and I thought with patches, you don't see them, you don't really know you've got them on do you? Respondent 13 (Patches)

All women had been eager to stop smoking as soon as possible, and often reported starting NRT at the earliest opportunity; many commenced on the first day it was prescribed. Women had felt confident that it would help them with their quit attempt. However, one woman recollected how anxious she felt about the prospect of incorporating NRT into her life and stopping smoking:

Oh I was looking and thinking crikey, how am I going to get used to this, you know cos it's totally different to cigarettes… But once you get used to it I think you're all right. I think it's like anything else, change is a bit unwary isn't it cos you don't know what's going to happen with a change but once you get used to it it's not that bad. Respondent 3 (Inhalator)

Experience of using NRT

Perceived effects of NRT use

Women had mixed feelings about the effectiveness of NRT to manage cravings and urges to smoke. Some women thought NRT had helped to

reduce their cravings. One woman described the relief she sometimes felt after using NRT:

I mean if I'm stressed or anything, if I get a squirt, once I take my squirt [description of inhalator] *it's like a relief, I feel calm, I feel relaxed and that's all it is pretty much. …..But that's how you basically feel, you feel calmer, more relaxed.* Respondent 6 (Inhalator).

However, this same woman also described situations where she needed to smoke to relieve her nicotine cravings. Another woman described how she struggled to manage her cravings and urges to smoke, and felt NRT was not working:

I was really struggling. It [the patch] *was stuck but it felt like I was getting no nicotine. And it didn't matter how much I puffed on the inhalator I was just suffering like really bad. So I started smoking over that weekend* Respondent 7 (Patches, mouth spray and inhalator)

A couple of women who used patches reported feeling the benefits of using them almost instantly after applying the patch, and some felt that after removing the patch they became anxious and began to experience cravings. In such instances some women took measures to manage cravings by keeping the patches on for longer. These decisions were made without consulting a smoking cessation advisor or midwife. One woman decided to wear her patch for 24 hours against the advice of her advisor in order to manage her urges to smoke:

She did say to put them on and take them off before bedtime to give you and baby a rest. I was finding I was waking up and wanting a cigarette so I took it upon myself to leave them on for 24 hours.
Respondent 9 (Patches)

I thought you couldn't have them [NRT patches] *on in the bath, so that's why I were taking them off and then I was like oh it's a bit early, you know, I've got another three or four hours before I go to bed yet and then I think it was my friend, she said 'well why don't you just leave it on', so I have been doing and it's been fine.* Respondent 10 (Patches)

Most felt that short-acting NRT was helpful because it enabled them to keep to the routines they had before their quit attempt by using NRT at times when otherwise they might have smoked. Some women who had used the inhalator expressed disappointment that it did not replicate a cigarette in design and shape, finding it aesthetically off-putting:

I thought it'd be more like a cigarette, like a sort of fag taste but it's not really like a fag taste, maybe because it don't look like a cigarette. I think it's still you know it's not a fag so you still need a fag if you get what I mean where I think if it looked more like one and acted more as a cigarette, a fake cigarette, I think it'd be more effective definitely than just a little white stick thing Respondent 3 (Inhalator)

Concomitant smoking

As highlighted previously, all women interviewed had smoked during their quit attempt; for some these were sporadic relapses, but for the majority smoking was a regular occurrence. Some women reported using NRT to cut down their smoking; a few women had planned to do this before quitting, while for others it was something they decided to do after quitting as they reported that avoiding smoking was harder than they had anticipated. Many women felt smoking a cigarette occasionally in order to satisfy their nicotine cravings was necessary as they weren't always alleviated by NRT.

Yeah, I planned to cut out the cigarettes, like halve my cigarette intake and start using the nicotine replacement for the other half that I took out. So I was gradually quitting smoking rather than just trying to go cold turkey and just have the nicotine replacement altogether because I don't think I would have been able to do it otherwise. Respondent 1 (Microtab)

I *decided not to punish myself and I'd have a few* (cigarettes) *in the morning, a few in the evening and then none in the day* [alongside NRT] Respondent 7 (Patches, mouth spray and inhalator).

Most women had not consulted with their smoking cessation advisor about smoking while using NRT. One woman who had previously tried to quit smoking believed that combining smoking and NRT was the only method that would enable her to eventually quit:

I've not really spoke to her [smoking cessation advisor] *….it wasn't a case of just stopping completely for me and then just going on to nicotine replacement, because I've tried quitting smoking quite a lot of times and*

it's been unsuccessful……So I set myself on a realistic target in terms of saying right on this day I'll stop completely and so ….so if I needed it [NRT] as and when, having a cigarette at those times, then I would lower the amount of cigarettes I am actually smoking in a day. Respondent 5 (Gum)

Side effects

Many women experienced physical side effects when using NRT. Those who had used short acting products reported disliking the taste, experienced swollen lips, sore throats, and nausea. In some cases women prolonged the time between doses of NRT to avoid the side effects:

Yeah, totally delayed cos I keep saying oh I don't want to taste it yet, I'll give it another ten minutes you know or I'll give it a bit longer. It is delaying it cos you think I'm not looking forward to the taste of it so I'll just wait a bit longer. Respondent 3 (Inhalator)

Some women experienced episodes of nausea when they used NRT; many were already feeling such symptoms as a result of being pregnant, but using NRT could make symptoms worse. Some women felt they would have liked some warning about the potential side effects, as it wasn't something they considered when they were deciding on their NRT product of choice:

'I was just more worried about the side effects obviously because I'm quite early on in pregnancy, and especially with morning sickness anyway, I didn't know that it [NRT] would cause - obviously [it] made me feel more nauseous and [I] vomited quite a few times when I had the gum. So just would have been nice to have a heads up about it that it makes you feel sick' Respondent 5 (Gum)

A few women developed coping mechanisms to manage the side effects such as using mints or sipping water after using NRT. Most women using patches reported skin irritation, but were less able to self-manage these side effects compared to those using short acting NRT products. Often the patches would not adhere to the skin, and some women who experienced skin irritation discontinued NRT early.

Safety concerns

Often women reported reservations about using NRT and some queried whether it would harm their baby. A few women were anxious that NRT was addictive and therefore would not end their addiction to nicotine but would simply change the mode of delivery. These women felt NRT had the potential to increase nicotine dependence, and believed that the levels of nicotine delivered by NRT products could be higher than that acquired by smoking:

Well, all they keep saying is you know it gets rid of the toxins, you still get the nicotine but it gets rid of the toxins, this, that and the other and it's just in that the nicotine you take it in. The nicotine itself is what makes it addictive, so to me the more nicotine that you're taking in anyway, the more you're going to want to smoke or you know you're going to need that nicotine. Respondent 6 (Inhalator)

A few women could recall querying the safety of NRT with smoking cessation advisors or with their midwife when they attended an antenatal appointment, and some felt reassured when informed about the likelihood of NRT being safer than smoking. However, some women felt information

about the likely safety of nicotine compared to smoking could be made more explicit during consultations.

My main concern was obviously 'smoking passes on horrible chemicals to the child, does this [NRT] *still do that' and* [the smoking cessation advisor] *was like 'no, it's just one main one, it's just the nicotine' so I'd have liked to have known a bit more on how that affects the baby*
Respondent 11 (Patches)

One woman was not convinced about the safety of using her nicotine patch even after being advised about this; she decided to revert back to smoking as she felt that the harms of using her patch on her lower back would be too dangerous for her baby:

I can remember the conversation we had about it and [the smoking cessation advisor] *was letting me know where I can put* [the patches] *and what not, but to myself I just thought no, that's just a bit too – you know you sit there thinking about it. I don't know, it's weird, I just think it's too close to the baby to be having all that nicotine going in.*
Respondent 10 (Patches).

Experience of e-cigarettes

Some women talked about e-cigarettes when discussing NRT and smoking cessation but a few were reluctant to use these due to uncertainty surrounding their safety. Five women had used e-cigarettes during their pregnancy; two continued to use them alongside their NRT products despite discussing the unknown safety risks of their use during pregnancy with a health professional. Three women decided to discontinue e-

cigarettes after becoming aware they were not recommended for use in pregnancy, from either their friends or their advisor:

I was, yeah I was doing really well, I mean cos with vaping you do get the sensation like you're still smoking so yeah I felt like I was vaping and you know I didn't feel that bad I mean obviously, but then when I found out through friends that midwives doesn't recommend them I thought I'd better stop them just in case.
Respondent 7 (Patches, mouth spray, inhalator)

Yet this same woman, after swapping to NRT felt strong urges and cravings to smoke, and began to smoke again as she felt unable to manage using NRT alone. However, women who continued to use e-cigarettes felt that overall these were not helpful to their quit attempt; one complained it made her cough and the second woman described how it continued the behavioural aspect of smoking:

So, kept on with the vapour stick, but I still found that I still wanted – because I was doing the same technique as a smoker, as in I'm putting something – you know, and actually smoking something, it still made me want to have a cigarette even more, which I thought, 'Well, that's not a good thing". Respondent 14 (Gum and patches)

4.5 Discussion

4.5.1 Main findings

This study provides novel insight into why some pregnant women do not adhere to NRT advised by health professionals. Most women smoked

regularly while using NRT and many used NRT to cut down their cigarette intake rather than to quit abruptly, such decisions were made both before and during the quit attempt. A few women were concerned that using NRT instead of smoking could increase their nicotine intake and dependence or cause greater harm to the fetus, and so often underutilised their NRT to avoid perceived harm.

4.5.2 Strengths and limitations

There are some limitations to this study. Firstly, the researcher did not directly observe the advisors' advice and so there may be some disparity between the actual advice given by SSS advisors and that intended by the SSS that employed them. If women were given advice which was different from that intended then this could have influenced the way in which women used NRT. Also the majority of participants were from a White British background and were recruited from a specific geographic location, so the results may not be transferable to other ethnic groups or to SSS that may be organised differently. Telephone, rather than face-to-face, interviews were used in this study; while it is acknowledged that this removes the presence of visual cues[234], pregnancy is often a busy time with various antenatal appointments to attend, and so we felt this method of interviewing would be most convenient for women. Telephone interviews may also enable participants to talk more freely about sensitive information, as they may be more relaxed by having distance from the researcher[234,235]. Data from general practice shows nicotine patches to be the most commonly prescribed product during pregnancy [82] yet in the study presented in this chapter the majority of women interviewed had

used an inhalator, so some of the experiences described in this study may not be as relevant to women using other NRT products.

A strength of this qualitative study was that women were interviewed whilst they were still pregnant and during or close to their quit attempt. This should have reduced recall bias as the interview took place so close to the event and was likely to have captured current beliefs and attitudes. The majority of women were still using NRT at the time of the interview and so it is more likely that they could accurately recall how they felt about using NRT, and their attitudes and views towards its use during pregnancy. Another strength of this study is its sampling strategy; the screening survey was carefully constructed to ensure a very specific group of participants, who did not adhere to NRT as intended by their SSS, were selected and invited to be interviewed. Also, interviews were analysed using an inductive approach, the advantage of this was we were able to report new information that emerged from the data, and so have been able to discover novel information about the attitudes of pregnant women towards the use of e-cigarettes during pregnancy.

4.5.3 Findings in context of previous research

This is the first study to describe in detail the way in which pregnant smokers integrate NRT into their quit attempts, and this has highlighted reasons as to why pregnant women may not adhere to NRT. Most simultaneously used NRT whilst smoking and some women clearly intended to use NRT to cut down rather than make an abrupt quit. NRT may be used to assist non-pregnant smokers to gradually cut down the number of cigarettes they smoke and can assist smokers who feel unable to quit

abruptly[236]. The cut down method has previously been found to appeal to both new parents[237] and pregnant smokers, who view cutting down their cigarette intake as a form of harm reduction and a method to quit smoking[238,239]. Evidence suggests that the effectiveness of using NRT in this manner is comparable to quitting smoking abruptly[236]. This study has highlighted that using NRT to reduce cigarette intake appeals to pregnant women, and could be an alternative approach to assist pregnant smokers who may feel unable to quit abruptly. The cut down method should be approached cautiously in pregnancy as one would not like pregnant women to receive more nicotine levels than achieved from ad libitum smoking and quitting abruptly would be safest for the health of the baby.

Women who used the cut down method or relapsed during their quit attempt often justified their smoking by explaining it helped to control nicotine withdrawals. In non-pregnant populations relapse is often associated with reduced motivation to quit[178]. During pregnancy relapse may also be due to changing pharmacokinetics and smoking could be a response to increased nicotine metabolism and low nicotine substitution from NRT, which was identified in Chapter Two and Chapter Three.

Many of the women in this study modified how they used NRT to meet their individual perceived needs; some tried to manage their nicotine withdrawal symptoms by using more NRT than recommended, for example by using patches overnight. However, others who reported not using NRT as regularly as advised, did so because; they were alternating between cigarettes and NRT; had safety concerns; or experienced side effects. By not using NRT as regularly as recommended, it is likely that the nicotine levels provided from NRT would be low as even among adherent smokers

in the Chapter Two study, nicotine levels while using NRT were lower than continued smoking. A fall in nicotine could explain why women in this qualitative study smoked as smoking enabled them to self-titrate their nicotine levels to help ease withdrawal symptoms.

A high level of confidence in the role of NRT prior to a quit attempt is evident in non-pregnant smokers. Yet unrealistic expectations about the role of NRT can disrupt a quit attempt[178]. Women reported feeling optimistic and eager to commence NRT before commencing their quit attempt. However, expectations did not always match their experience, and some women became anxious about the safety of NRT or complained about the side effects of using NRT shortly after commencing their quit attempt. Few women discussed their concerns with a smoking advisor and consequently made decisions as to how they used NRT independently. A lack of dialogue between participant and advisor could be due to the timing of the interview, as most participants would have had little opportunity to meet with their advisor between quitting and the interview taking place. However, a previous qualitative study of pregnant smokers conducted at a later time point after quitting found women also used NRT independently from the advice provided by health professionals[189], suggesting the timing of the interview may not have impacted the findings.

Women in this study raised similar concerns about the safety of using NRT to women in a previous study[189]; concerns about the effects of NRT on the fetus or concern that using NRT would be substituting one addiction for another were expressed. The similarity between responses in these studies suggests these are common concerns among pregnant women using NRT, irrespective of the length of time between using NRT and being asked to

reflect on their experience. Evidence outside pregnancy suggests a strong correlation between unaddressed concerns about the safety of using NRT and underutilizing NRT[240]. Women in this study feared that NRT could increase their nicotine addiction, which meant they delayed or avoided using NRT in order to maintain nicotine levels they perceived were safe. Such fears are most likely unfounded as NRT does not appear to be associated with increasing nicotine dependence [241,242].

Despite concerns some women had about using NRT they continued to smoke during their quit attempt. A study by Naughton et al found pregnant smokers often downplay the dangers of smoking to justify their continued smoking [228], and it could be that women in our study were accentuating the dangers associated with NRT to justify their continued smoking. Also during pregnancy, knowledge about the risks of smoking is related to women's intention to quit smoking and pregnant women are often misinformed about the efficacy of smoking cessation treatments[243]. Evidence outside pregnancy has shown that misconceptions about the health risks associated with NRT influence adherence[227]. Therefore, by addressing any underlying concerns about NRT prior to a quit attempt it may result in less women being able to justify smoking during their quit attempt.

While the study was not designed to explore women's views of e-cigarettes they often talked about them as a smoking cessation method which was being used by smokers in their communities. Some women were concerned about the health and safety effects of using e-cigarettes during pregnancy. However survey data suggests pregnant women view e-cigarettes as less

harmful than smoking [244], which is similar to the views of some young adults [245] and obstetrician-gynaecologists[246] Further research about attitudes and experiences of women who have used e-cigarettes is required, as well as robust research into their safety. Although as with NRT, evidence suggests that they are no more harmful than smoking[124].

4.6 Conclusion

This study has shown how women experience using NRT and the influence this has on adherence. Women modified how they used NRT, often by underutilizing or discontinuing NRT due to safety concerns or having experienced negative side effects. However, all women smoked during their quit attempt, suggesting they felt smoking was less harmful than using NRT. Concomitant smoking may be a consequence of NRT providing inadequate nicotine substitution, as women described situations where they felt NRT was not alleviating their nicotine withdrawal symptoms. However, some women never intended to adhere to NRT during a quit attempt and used NRT to cut down smoking rather than to quit smoking abruptly. Overall, this study has highlighted the need for; interventions to help reduce the causes of underutilization of NRT; measures to ensure women understand the dangers of smoking compared to continued use of NRT; and professional support to help reduce and manage negative side effects. This study has also highlighted that for some women quitting smoking abruptly may not be feasible and alternative approaches to quitting, such as using NRT to cut down cigarettes may need to be considered.

5 Chapter Five: Summary of each study

Overall Aim

The overall aim of this thesis was to investigate the use of nicotine replacement therapy (NRT) in pregnancy, including individuals' reasons for not adhering to a treatment regime, and to increase understanding of the changes in women's nicotine metabolism across pregnancy and what implications this has for NRT treatment. The thesis is composed of three studies, which are summarised below.

5.1 Summary of Findings

Study One; Comparison of cotinine levels in pregnant women whilst smoking and when using nicotine replacement therapy

This study aimed to measure the level of nicotine substitution provided by standard dose nicotine patches during pregnancy. It was the first study in pregnancy to accurately measure the nicotine levels provided by standard dose NRT patches (15mg/16hr) and compare these with nicotine levels while smoking. An estimation of nicotine substitution was obtained from cotinine serum samples collected as part of an RCT. The study found evidence that, in pregnant women, the level of nicotine substitution provided by standard dose NRT patches was lower than that generated by smoking. This disparity may partially explain why standard dose NRT used by pregnant women has not been shown to be effective. The lower cotinine levels may be indicative of insufficient levels of nicotine from NRT, so it may not provide high enough levels of nicotine to alleviate nicotine withdrawal symptoms. This could be one explanation as to why pregnant women often do not adhere to NRT and return to smoking.

This study included only a small select sub-sample of women from the Smoking Nicotine and Pregnancy (SNAP) trial, who had quit smoking at 1

month post their quit date[85]. The majority of the sub-sample was smoking again at the time of delivery, so it could be that the nicotine substitution provided by standard dose NRT was sufficient short term at one month but levels of nicotine were not sufficient long term during pregnancy. The majority of participants in the SNAP trial did not achieve cessation even at 1 month post quit date, so women who were not in the sub-sample and hence in the study described in this thesis, who were smoking, may have had lower levels of substitution.

Study Two; Changes in the rate of nicotine metabolism across pregnancy: a longitudinal study

This study explored the longitudinal pattern of nicotine metabolism during pregnancy. It showed that the rate of nicotine metabolism is faster in pregnancy compared to postpartum, falling between birth and 1 month after delivery, at which point it appears to stabilise, presumably returning to non-pregnant levels by this point. It is also the first study to show that metabolism is increased from 18 to 22 weeks of pregnancy onwards. This finding could help explain why NRT has, to date, been found ineffective in pregnancy; as nicotine metabolism increases during pregnancy, so the dose of nicotine in NRT may become insufficient at providing therapeutic levels of nicotine.

As mentioned previously, the SNAP trial data that was used for Study One, found that NRT was effective 1 month after quit date when most participants would have been in the second trimester of pregnancy, but this was not sustained to delivery[85]. The findings from this longitudinal study, which showed that nicotine metabolism increased by 18 to 22 weeks, could help to explain why in Study One nicotine substitution levels were less than

when smoking for the majority of participants, as metabolism may have increased by the 1 month post quit date. Also, Study One found that by delivery the majority of participants were smoking, so it may be the dose of nicotine in the patches may have become insufficient with increased gestation. Although it remains unknown exactly how metabolism relates to nicotine substitution throughout pregnancy, this study provides further support for the hypothesis that the apparent lack of effectiveness of NRT during pregnancy may relate to inadequate dosing.

Study Three; Understanding pregnant smokers' adherence to nicotine replacement therapy during a quit attempt: a qualitative study

This study aimed to understand the experiences of women not adhering to NRT, in order to determine the barriers to adherence. It was designed to identify whether or not there were other reasons aside from increased nicotine metabolism and inadequate nicotine substitution that may explain why women often do not adhere to NRT during pregnancy. Many women in the study continued to smoke regularly while using NRT; some smoked as they felt NRT did not alleviate their cravings, whereas others intentionally used NRT to substitute a proportion of their cigarette intake (i.e. to cut down rather than quit). This study also found that women underutilised their NRT, often as a result of side effects or safety concerns. Yet despite these concerns women continued to smoke during their quit attempt, highlighting that some women may have had greater concerns about the safety of using NRT compared to smoking.

Although not a prior aim of the work, this study highlighted the views of pregnant women towards using e-cigarettes when pregnant. The majority of women had heard about e-cigarettes and reported smokers in their

communities were using them as a measure to cut down or quit smoking. Women were curious about the potential benefits of using e-cigarettes in pregnancy and some women in the study had used e-cigarettes during their current quit attempt in order to help them stop smoking. Women who were not using e-cigarettes during their quit attempt reported feeling wary and uncertain about their safety.

6 Thesis Conclusions

The overall aim of this thesis was to understand why NRT might not be effective during pregnancy. When considering the findings of all three studies within this thesis, the ineffectiveness of NRT during pregnancy is likely to be due to a complex mix of reasons. The first study showed that in adherent pregnant smoker's nicotine substitution after using standard dose NRT was lower compared to levels achieved from continued smoking. The ideal nicotine substitution rate required to help smokers achieve abstinence is unknown, however NRT works by alleviating nicotine withdrawal symptoms, and therefore if the level of nicotine in NRT is too low then it is unlikely to effectively alleviate nicotine withdrawal symptoms. The thesis findings have also shown that nicotine metabolism increases by the second trimester of pregnancy and this increase persists until after birth. An increase in nicotine metabolism during pregnancy could potentially result in NRT providing even lower levels of nicotine substitution.

The qualitative work conducted as part of this thesis has provided some insight into the quit attempt of women who do not adhere to NRT as recommended by stop smoking services. Women often individualise how they use NRT to manage their nicotine levels and quit attempt, for some this involved using NRT to cut down smoking rather than quit. Others relapsed and smoked because they did not feel NRT was assisting adequately with their nicotine cravings. In addition, women had concerns about the safety of NRT and some had experienced negative side effects, both of which led women to either discontinue or underutilize NRT. Overall,

the thesis has shown that inadequate nicotine substitution, increased nicotine metabolism and underutilization of NRT during pregnancy may contribute to why NRT has not been found to be effective during pregnancy.

6.1 Implications for research or clinical practice

Study One, research implications:

This study found lower nicotine substitution levels after using NRT compared to smoking. However, this study measured nicotine substitution only once, at one-month following the quit date, so there could be variation in substitution over time that was unexplored. This study also explored whether nicotine substitution was associated with smoking cessation, however the sample size was very small and is likely to have been underpowered. Further research is required to understand nicotine substitution during pregnancy as it may affect the efficacy of NRT:

- Using a large prospective cohort of pregnant women who use NRT, frequent measurements of cotinine levels generated in women who are adherent and smoking abstinent may provide more insight into whether substitution levels change with gestation. If nicotine substitution levels decrease with gestation, this would support the hypothesis that there is a relationship between nicotine substitution and increased nicotine metabolism. Within such a study, associations between nicotine substitution and smoking cessation at differing time points within pregnancy, delivery and the postpartum could be explored. This would increase understanding as to whether there is a relationship between nicotine substitution and smoking cessation during pregnancy and help to distinguish what nicotine

substitution levels are most efficacious. However, as adherence to NRT is often low during pregnancy sample sizes would need to be sufficiently large in order to ensure adequate power to detect associations.

Study Two, research implications:

This study established that nicotine metabolism increases during pregnancy, so it may mean that the dose of nicotine in standard dose NRT is too low to alleviate nicotine cravings. In order to test this hypothesis future work trialling higher dose NRT is required; if proven effective this could explain why standard dose NRT is not effective during pregnancy. It could be that low nicotine substitution and increased nicotine metabolism may affect the efficacy of NRT by adversely affecting adherence. A study comprising of the following may be required:

- An RCT using higher dose NRT with a placebo or standard dose NRT could determine if higher dose nicotine is more effective for smoking cessation during pregnancy. Higher dose NRT may consist of either higher dose nicotine patches or providing women with dual therapy NRT; each would provide higher levels of nicotine. By obtaining frequent cotinine measurements, the level of nicotine substitution after using higher dose NRT could be determined and associations with smoking cessation could be explored. In order to determine whether nicotine metabolism is associated with adherence this would require measuring adherence rates among women allocated to the higher dose group and comparing with the control group.

Study Three, research implications and clinical implications:

This study described the experience of using NRT amongst pregnant smokers that were non-adherent during a quit attempt. Although low adherence may be the result of increased nicotine metabolism and inadequate nicotine substitution, this qualitative study has highlighted the ways in which women use and modify NRT use during a quit attempt. The findings have highlighted some potential implications for clinical practice and future research:

- This qualitative study has provided an insight into the ways in which women deviate from the recommendations provided by SSS about using NRT. Quantitative data on how pregnant women administer NRT after receiving a prescription from SSS is unknown. Future work could involve collecting survey data about how women use NRT during a quit attempt that would provide SSS with a greater understanding of how pregnant women deviate from their recommendations that may enable services to tailor support and advice to suit the needs of their smokers.
- The finding that some women use NRT to cut down cigarettes rather than to stop smoking abruptly suggests that current recommendations on NRT use may not be acceptable or feasible for some women. Women often individualised how they used NRT to suit their needs, and this involved going against the advice of health professionals. Health professionals should continue to advise women to quit smoking abruptly as a first option, but the cut down method should be considered if an abrupt quit attempt continuously fails or pregnant women feel they are unable to stop smoking completely. Offering some flexibility in how smoking cessation is achieved may encourage more women to work in conjunction with SSS and could potentially lessen situations where women act independently from the advice given be SSS.

- Women who plan an abrupt quit attempt may relapse and SSS should work with women to establish what caused the relapse. Assessing the cause could enable advisors to determine whether women are using their NRT product correctly. The qualitative study found that women who relapsed were often underusing their NRT, which is likely to explain why they smoked. Frequent investigation by advisors about the daily use of NRT could help to determine if women are maximising the benefit of NRT.
- Some women had specific safety concerns about using NRT, including potential harm to their baby and fears that using NRT could increase their addiction to nicotine. These concerns often led women to discontinue NRT or reduce the amount of NRT they used. However, despite having concerns about the safety of using NRT they all smoked during their quit attempt. Health professionals may need to offer pregnant women more reassurance about the safety of using NRT compared to continued smoking. This could include providing women with scientific evidence about the safety of using NRT and presenting the risks of smoking. This would enable women to weigh up the evidence and make informed choices. This information could be delivered by stop smoking advisors at the beginning of a quit attempt in order to help combat any negative preconceptions women may have about using NRT. It would be equally as important to provide this information in leaflet form or via a website in order to help support women who develop safety concerns during a quit attempt, between appointments with their advisor.

7 References

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8 Appendices

8.1 Publications

BRIEF REPORT

Comparison of Cotinine Levels in Pregnant Women Whilst Smoking and When Using Nicotine Replacement Therapy

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ABSTRACT

Background: Nicotine replacement therapy (NRT) helps smokers quit smoking, but trials indicate that there is no evidence that it is effective in pregnancy. As metabolism increases during pregnancy, NRT may deliver insufficient nicotine to alleviate withdrawal symptoms. There is mixed evidence as to what levels of cotinine are reached from nicotine exposure in pregnancy while using NRT, compared with smoking.

Methods: We analyzed data on 33 pregnant participants from the NRT arm of a randomized control trial who had stopped smoking and were still using 15 mg/16 hr nicotine patches 1 month after quitting. Salivary cotinine levels when smoking at baseline were compared with levels on NRT at 1 month using the Wilcoxon test.

Results: Cotinine levels were a median of 98.5 ng/ml while smoking and 62.8 ng/ml while using NRT and remaining abstinent ($p = .045$). Participants with the highest cotinine measurements when smoking also tended to have the steepest reduction in cotinine levels while using NRT. This was most noticeable among participants with baseline cotinine levels more than 150 ng/ml ($n = 9$) who had a greater reduction in median cotinine levels (median difference −134.8 ng /ml [95% CI = −144.5 to −125.9]) than those with a baseline cotinine level under 150 ng/ml ($n = 24$; median difference −27.9 ng/ml [95% CI = −49.35 to −1.75]).

Conclusions: In a pragmatic trial that replicated clinical practice, cotinine levels generated using NRT in pregnancy were lower than levels achieved from smoking. Although the sample size of this study was small, our findings are significant and are consistent with the hypothesis that NRT patches deliver an inadequate dose of nicotine to aid smoking cessation in pregnancy.

INTRODUCTION

Smoking in pregnancy is strongly associated with adverse pregnancy and birth outcomes (Cnattingius, 2004; Dechanet et al., 2011; Jaddoe et al., 2008; Li & Daling, 1991). In the United Kingdom, 26% of women smoke at some point during pregnancy and 12% of women continue to smoke throughout (The NHS Information Centre, 2011). Nicotine replacement therapy (NRT) in combination with behavioral support is often used to help pregnant smokers quit smoking (National Institute for Clinical Excellence, 2010). NRT is thought to be safer than smoking, as it does not contain all of the toxins present in tobacco smoke, and delivers only nicotine in doses aimed at relieving withdrawal symptoms (Benowitz et al., 2000).

NRT is effective in nonpregnant smokers (Stead, Bergson, & Lancaster, 2008), but its efficacy is unproven in pregnancy (Coleman, Chamberlain, Davey, Cooper, & Leonardi-Bee, 2012). Cotinine is the major metabolite of nicotine and is a specific marker for assessing nicotine exposure (Benowitz, Hukkanen, & Jacob, 2009). Nicotine is metabolized more rapidly in pregnancy, with a reported 60% increase in nicotine clearance and 140% increase in cotinine clearance, obtained by both plasma and urinary measurement (Dempsey, Jacob, & Benowitz, 2002). This faster metabolism could mean the nicotine supplied from a standard NRT patch is insufficient to alleviate smoking withdrawal symptoms in pregnancy.

This study has used data from a trial of NRT in pregnancy, which attempted to replicate routine clinical practice, to compare the cotinine levels in women generated by smoking with those from using NRT transdermal patches while abstinent.

METHODS

Data for this secondary analysis are from a double-blind, randomized placebo-controlled study: the Smoking, Nicotine, and Pregnancy (SNAP) trial. The trial recruited 1050 women

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from antenatal clinics within U.K. hospitals and investigated NRT 15 mg/16 hr transdermal patch use in pregnant smokers ($n = 521$) compared with placebo patches ($n = 529$; Coleman et al., 2012). The trial had a pragmatic design and intended, as far as possible, to mimic routine clinical practice; a description is available elsewhere (Coleman, Cooper, et al., 2012).

Participants were included in SNAP if they smoked ≥5 cigarettes/day and ≥10 prior to pregnancy, were 12–24-weeks pregnant, were aged 16–45 years, and had an exhaled carbon monoxide reading (CO) of ≥8 p.p.m. After enrolment, data on sociodemographics and smoking behavior were asked, and saliva cotinine was measured. Women were initially given a 4-week NRT supply to start on their quit date, followed by another 4-weeks' supply if they were abstinent, confirmed by an exhaled CO reading of <8 p.p.m. Women were instructed to remove patches at night and discontinue them if they restarted smoking.

At 1 month, women who reported not smoking (≤5 cigarettes smoked since quit date) had their abstinence validated by exhaled CO readings; those with validated abstinence, and who were also still using patches, were asked for a saliva sample to measure cotinine levels. Adherence with NRT trial patches between quit date and follow up was calculated as the total number of days the participant reported using the trial patches as a percentage of the length of time between the participant's quit date and their follow-up appointment.

We wanted to perform analyses on participants who were allocated to NRT and who were reasonably adherent. Consequently, we included in analyses, women from the NRT group with validated abstinence, who reported at least 80% adherence with NRT (defined previously). This final criterion was used to ensure that participants were pregnant women using NRT on a regular basis.

Salivary cotinine levels were not normally distributed and were analyzed using nonparametric statistics; within-subject changes in cotinine levels between baseline and 1 month were analyzed using the Wilcoxon rank sum test.

RESULTS

Of the 521 women in the NRT arm of the trial, 131 self-reported smoking abstinence at 1 month and 111 permitted a research midwife to visit them to validate their abstinence using an exhaled CO reading. A total of 55 women were excluded due to low self-reported adherence to taking NRT (<80% adherence) and 23 women did not have a before and after cotinine measurement to compare and subsequently were excluded. This left a sample size of 33 women.

Participant characteristics are presented using the median and interquartile ranges (IQRs) for the eligible 33 participants (Table 1). The participants were White British women, with the exception of one participant who was from an Asian/other background. They were on average 26 years old at recruitment. Most (67%) smoked 5–10 cigarettes/day prior to beginning the trial. All participants were in the second trimester of pregnancy at recruitment with the median gestational age being 14.4 weeks. We looked at how many of our sample stopped smoking in the SNAP trial, and nine participants (27%) were validated as abstinent from smoking at delivery.

The median cotinine level for participants while smoking at baseline was 98.5 ng/ml (IQR 71.3–177.8) and while abstinent and using NRT at 1 month was 62.8 ng/ml (IQR 33.3–82.7). There was a significant reduction in cotinine levels between baseline when smoking and 1 month while using NRT (median difference −54.2 ng/ml, [95% CI = −70.5 to −25.5 ng/ml], $p = .045$). The majority of participants had cotinine levels at 1 month, which were lower than their levels at baseline ($n = 27$); six participants had cotinine levels at 1 month, which were higher than baseline.

Of the 33 participants, 17 participants reported no smoking at all between their quit date and 1-month follow up, and the remaining 16 participants reported smoking five times or less. Six of the 16 participants reported smoking a cigarette in the 24 hr before their 1-month visit. We performed sensitivity analysis on the 27 women who had not smoked in the previous 24 hr before the 1-month visit; the median difference in cotinine levels in this group was similar to that of the whole group −46.6 ng/ml (95% CI = −125 to −24.1 ng/ml).

We found a significant, positive correlation between baseline and 1-month cotinine levels, Spearman correlation coefficient ρ 0.35 ($p = .04$). Although the association was weak, it suggests that participants with the highest baseline levels in the study also tended to have higher 1 month levels. However, the scatter plot (Figure 1) shows that for the majority of participants ($n = 27$), cotinine levels at 1 month were lower than at baseline. The scatter plot also demonstrates that participants in the highest range of baseline cotinine levels appear to have the greatest reduction in 1-month cotinine levels. This is most noticeable among participants with baseline cotinine levels more than 150 ng/ml ($n = 9$); these participants had a greater reduction in median cotinine levels (median difference −134.8 ng/ml [95% CI = −144.5 to −125.9]) compared with

Table 1. Participant Characteristics

Characteristics	Women included in study ($n = 33$), median (interquartile range or %)
Age (median, 25% and 75%)	26.12 (22.29–32.35)
Ethnicity	
White British	31 (98%)
Asian background	1 (2%)
Body mass index	25.6 (22.7–29.3)
Age left full time education (years)	16 (16–17)
Gestational age at baseline (weeks)	14.4 (13.3–17.8)
Current cigarettes smoking per day	
5–10	22 (67%)
11–15	8 (24%)
16–20	–
>20	3 (9%)
Heaviness of Smoking Index	3 (2–3)
Partner smoking status	
Partner smokes	23 (70%)
Partner non smoking	6 (18%)
No partner	4 (12 %)
Nicotine metabolite ratio	0.4 (0.3–0.5)
Adherence with NRT trial patch[a]	
80%–99%	12 (37%)
100%	20 (63%)
Cotinine level baseline (ng/ml)	98.5 (71.3–177.8)
Cotinine level 1 month (ng/ml)	62.8 (33.3–82.7)
Validated smoking abstinent at delivery	9 (27%)

Note. NRT = nicotine replacement therapy. [a]% of days used trial patch from baseline to 1-month follow up.

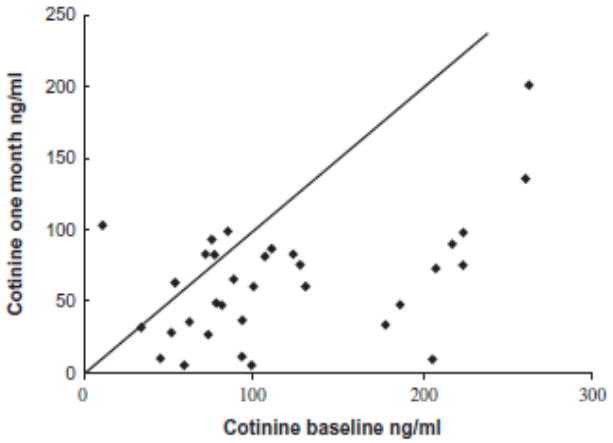


Figure 1. Cotinine levels at baseline while smoking and at 1 month while abstinent and using nicotine replacement therapy.

those with a baseline cotinine level under 150 ng/ml (n = 24; median difference –27.9 ng/ml [95% CI = –49.35 to–1.75]).

DISCUSSION

Main Findings

Our findings have shown that cotinine levels generated using NRT transdermal patches in pregnancy are lower than cotinine levels generated while smoking. Although these findings arise from a clinical trial, this had a relatively pragmatic design and was intended to replicate how NRT is used in routine clinical practice. We found a correlation between baseline and 1-month cotinine levels and observed that participants in the highest range of cotinine measurements at baseline (>150 ng/ml) tended to have the steepest reduction in cotinine levels while using NRT.

A strength of our study design was that it accounted for within-person differences and, therefore, change in cotinine levels will not have been influenced by inter-participant variation (e.g., in nicotine metabolic rate). We were also able to observe cotinine levels generated in a setting, which is similar to routine clinical practice, while also only including abstinent women who adhered to patches, so cotinine levels are very likely based on regular and continuous NRT use.

For unknown reasons, as in other randomized controlled trials, compliance with NRT in SNAP was low (Pollak et al., 2007; Wisborg, Henriksen, Jespersen, & Secher, 2000) and, as our analyses included only women who used patches regularly, cotinine levels measured are unlikely to reflect those generated in women using NRT intermittently. Additionally, it could be speculated that women who were excluded from the analysis might have lower cotinine levels than those included. In order for NRT to effective, it is expected that cotinine levels achieved from using NRT would need to be similar to those achieved by smoking (Benowitz, Zevin, & Jacob, 1997). Such lower cotinine levels could have caused these women to suffer from more withdrawal symptoms, making them more likely to re-start smoking and stop using patches, resulting in their exclusion. A final weakness of our study is that cotinine measurements on NRT and when smoking were taken 1 month apart; if, as research suggests, nicotine metabolism increases during pregnancy (Dempsey et al., 2002), then this may partially explain lower cotinine levels seen when using NRT at the 1-month follow up. It could be speculated that NRT may have become increasingly insufficient as gestation increased, which is why only nine of the 33 women had validated smoking abstinence at delivery.

Smaller laboratory-based studies have been able to report on the paired difference while using NRT. In one study, researchers administered 15 mg/16 hr patch over 5 days and found cotinine levels were 48% less than those achieved when smoking (p = .029) (Oncken, Campbell, Chan, Hatsukami, & Kranzler, 2009); our findings are consistent with this. However, another study that used patches over 5 days (Oncken et al., 1997) and a study that applied patches over an 8-hr period (Ogburn et al., 1999) did not. It is possible that, in pregnancy, use of NRT for longer than 8 hr is required before stable cotinine levels are generated. Although informative, these studies were laboratory based and may not accurately represent cotinine levels achieved from using NRT in routine clinical practice.

Trials exploring the effects of NRT have reported lower cotinine levels in pregnant women randomized to NRT than those randomized to placebo; they have also reported lower levels after randomization to NRT than prior to study enrolment (Oncken et al., 2008; Wisborg et al., 2000). However, these studies have not restricted comparisons to those women who achieved abstinence from smoking; consequently, these comparisons may reflect cotinine levels generated by smoking, NRT use, or both.

CONCLUSIONS

In summary, in pregnancy, cotinine levels generated by 15 mg/16 hr nicotine patches are lower than those generated by smoking. Although the sample size of this study was small, our results are significant. They do indicate that an apparently low level of nicotine substitution may be insufficient for NRT to have efficacy in pregnancy and may, at least partially, explain why standard dose NRT used by pregnant women has not been shown to be effective.

FUNDING

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DECLARATION OF INTERESTS

On two occasions since 2008, TC has been paid to attend and present at symposia arranged by Pierre Fabre Laboratories (PFL); PFL are a manufacturer of NRT.

ACKNOWLEDGMENTS

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RESEARCH REPORT
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Changes in the rate of nicotine metabolism across pregnancy: a longitudinal study

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ABSTRACT

Aims Increased nicotine metabolism during pregnancy could explain why nicotine replacement therapy (NRT) appears to be less effective on smoking cessation in pregnancy than in non-pregnant smokers, but little is known about nicotine metabolism across pregnancy. This study was conducted to determine when changes in nicotine metabolism occur during pregnancy and to describe the magnitude of these changes. **Design** Longitudinal cohort study of pregnant smokers' nicotine metabolite ratio (NMR). **Setting and Participants** 101 pregnant smokers recruited from hospital antenatal clinics in Nottingham, UK were asked to provide saliva samples at 8–14 weeks (n = 98), 18–22 weeks (n = 65), 32–36 weeks gestation (n = 47), 4 weeks postpartum (n = 44) and 12 weeks postpartum (n = 47). **Measurements** Nicotine metabolite ratio (NMR) was measured using the ratio of cotinine to its primary metabolite trans-3'-hydroxycotinine. Multi-level modelling was used to detect any overall difference in NMR between time points. The 12 week postpartum NMR was compared with the NMRs collected antenatally and 4 weeks postpartum. **Findings** NMR changed over time (p = 0.0006). Compared with NMR at 12 weeks postpartum, NMR was significantly higher at 18–22 weeks (26% higher, 95% CI 12% to 38%) and 32–36 weeks (23% higher, 95% CI 9% to 35%). There was no significant difference between the 8-14 weeks gestation or 4 weeks postpartum NMR and 12 weeks postpartum. **Conclusions** Nicotine metabolism appears to be faster during pregnancy; this faster metabolism is apparent from 18 to 22 weeks of pregnancy and appears to fall by 4 weeks after childbirth.

Keywords Antenatal, metabolism, nicotine, pregnancy, postpartum, smoking.

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INTRODUCTION

Smoking in pregnancy is associated strongly with adverse pregnancy and birth outcomes [1]. Nicotine replacement therapy (NRT) is often provided to assist pregnant women to stop smoking, yet despite its effectiveness in non-pregnant populations, NRT has not been found in randomized controlled trials to be effective in pregnancy, except possibly in the short term [2,3]. A potential reason for this may be that nicotine metabolism is increased during pregnancy [4–7]. It is possible that the dose of nicotine in NRT may not be enough to reduce cigarette cravings adequately.

Currently, we do not know exactly when changes in nicotine metabolism begin. Most studies have suggested that change in nicotine metabolism occurs by the second trimester of pregnancy, but little is known about nicotine metabolism in the first trimester and postpartum. No studies have attempted to measure the rate of nicotine metabolism from early pregnancy, throughout gestation and into the postpartum. Using saliva samples to provide current measurements of nicotine metabolism, this study aims to address the following: to (1) determine at what gestation changes in nicotine metabolism occur; and (2) describe the magnitude of changes in nicotine metabolism across pregnancy.

METHODS

This is a longitudinal cohort study measuring the nicotine metabolism of pregnant and postpartum women who smoke daily. Saliva samples were analysed to obtain their nicotine metabolite ratio at five points; at recruitment between 8 and 14 weeks' gestation with follow-up at 18–22, 32–36 weeks' gestation, and at 4 and 12 weeks postpartum.

Participants were recruited from hospital antenatal clinics in Nottingham, UK, between August 2013 and October 2013, with follow-up completed by October 2014.

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Participants were included if they smoked one cigarette or more per day, were aged 16 years or over, had a singleton pregnancy and self-reported being between 8 and 14 weeks' gestation. Participants were excluded if they self-reported that they had consumed grapefruit within the last 24 hours [8], had liver or renal health problems or used medication other than the following: iron supplementation, vitamin supplements, folic acid and medications to treat asthma.

The researcher took written informed consent and asked participants to provide a saliva sample using a sterile Salivette swab, an exhaled carbon monoxide reading (CO) and had their height and weight measured. They were also asked to complete a short survey containing questions about their age, education, employment status and nicotine dependence, measured by the Fagerström Test for Nicotine Dependence (FTND) [9].

Participants were notified by telephone or text to alert them that follow-up saliva kits were being posted to their home, along with surveys containing questions about nicotine dependence. Anyone who had stopped smoking during the study was instructed to not provide a sample while they were abstinent. In order to encourage response rates, participants received a £5 high street shopping voucher; this amount was increased to £20 for the final postpartum sample in order to improve return rates. The study was given a favourable ethical opinion by East Midlands, Nottingham 2 Research Ethics Committee.

Sample analysis

Samples were stored frozen at −20 degrees centigrade and stored at Nottingham Health Science Biobank before being sent by courier to ABS Laboratories Ltd, Welwyn Garden City, UK for analysis. Assays were carried out on the saliva to provide a measurement of cotinine and trans-3'-hydroxycotinine. The use of saliva to measure nicotine metabolites is a validated method of assessing exposure to nicotine [10]. The samples were quantified by liquid chromatography–tandem mass spectrometry (LC-MS/MS).

The LC-MS/MS method involved the addition of deuterated internal standards for both analytes, addition of a pH 7 buffer then liquid–liquid extraction using ethyl acetate prior to hydrophilic liquid chromatography tandem mass spectrometry using TurboIonspray in the positive ionization mode using a CTC Agilent 1100 AB SCIEX API4000 LC-MS/MS system [11]. The assay range for both analytes was 1–500 ng/mL. Any over-range samples were diluted and re-assayed. Precision was less than or equal to 10.3% for cotinine and less than or equal to 8.2% for 3-hydroxy cotinine; mean accuracy ranged from 88.8 to 107% for cotinine and from 98.6 to 101% for 3'-hydroxycotinine. Once analysis had taken place, samples were destroyed in accordance with the Human Tissue Act, 2004.

Outcome measures

Nicotine metabolite ratio (NMR) was measured by using the ratio of cotinine (COT) to its primary metabolite trans-3'-hydroxycotinine (3HC) in saliva [12]. This method is a validated measure of nicotine metabolism [13,14]. The hepatic cytochrome CYP2A6 enzyme is responsible for the metabolism of nicotine, cotinine and 3HC, therefore NMR is a marker of CYP2A6 activity [12,15], and a higher NMR (3HC/COT) indicates faster nicotine metabolism.

Statistical analysis

Based on previous research we assumed a standard deviation of 0.15 between time-points [16], and taking statistical significance as $P < 0.05$, a sample size of 73 participants would provide 80% power to detect a difference of 0.05 (1/3 of a standard deviation). NMR was not distributed normally, and was log-transformed to normality. Levene's test was used to confirm that there was no difference in variance of NMR between time-points. The overall significance of the change in NMR between time-points was assessed using a multi-level model, which allows us to assess the significance of a change over time while allowing for the correlation between repeated measurements made on individuals.

NMR was compared descriptively between each of the five time-points: 8–14, 18–22 and 32–36 weeks' gestation and 4 and 12 weeks postpartum. Similar to previous research, we used the 12-week postpartum NMR as the comparison value in this study [4] and a priori-determined paired *t*-tests were performed to determine differences in NMR at 12 weeks postpartum and all other time-points. The Bonferroni correction was used for multiple testing in the four pairwise comparisons of each time-point with the 12-week postpartum value [17], so *P*-values of 0.01 or smaller were significant at the 5% level. We also averaged all measurements made on an individual during the antenatal period (8–14, 18–22 and 32–36 weeks) and the postpartum period (4 and 12 weeks postpartum) and compared these using a paired *t*-test to determine the magnitude and statistical significance of change in NMR between the antenatal and postpartum periods. Effect sizes are presented as percent change from the 12-week postpartum result by anti-logging the mean difference.

We observed the 26 women who returned all five saliva samples and looked to see if the NMR followed a similar pattern of NMR over time to that described using all available samples, and then conducted multi-level analysis to test for change over time within this subsample. Evidence suggests the contraceptive pill increases nicotine metabolism [18,19], so we excluded those who reported using contraception at either 4 or 12 weeks postpartum.

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RESULTS

Of 101 saliva samples provided at recruitment (8–14 weeks), 98 were able to be analysed (Fig. 1). The first follow-up at 18–22 weeks' gestation had the highest return rate ($n = 65$, 64%) and the lowest response rate was at 4 weeks postpartum (sample $n = 44$, 43%). The response for the final sample at 12 weeks postpartum was 47 (46%). A total of 79 (78%) women provided more than one sample for comparison and 26 (26%) women provided a sample at all five time-points. The median (IQR) number of cigarettes participants smoked at each time-point was: 8–14 weeks, 10 (5–10); 18–22 weeks, 10 (4–11); 32–36 weeks, 10 (5–10); 4 weeks postpartum, 10 (7.5–15); and 12 weeks postpartum, 10 (7–15). Two participants who provided follow-up samples reported to have used NRT.

The characteristics of the 101 participants recruited can be seen in Table 1. We compared the characteristics of the entire sample at recruitment with those of participants who returned the 12-week postpartum sample ($n = 47$). There was a slight difference in the median number of

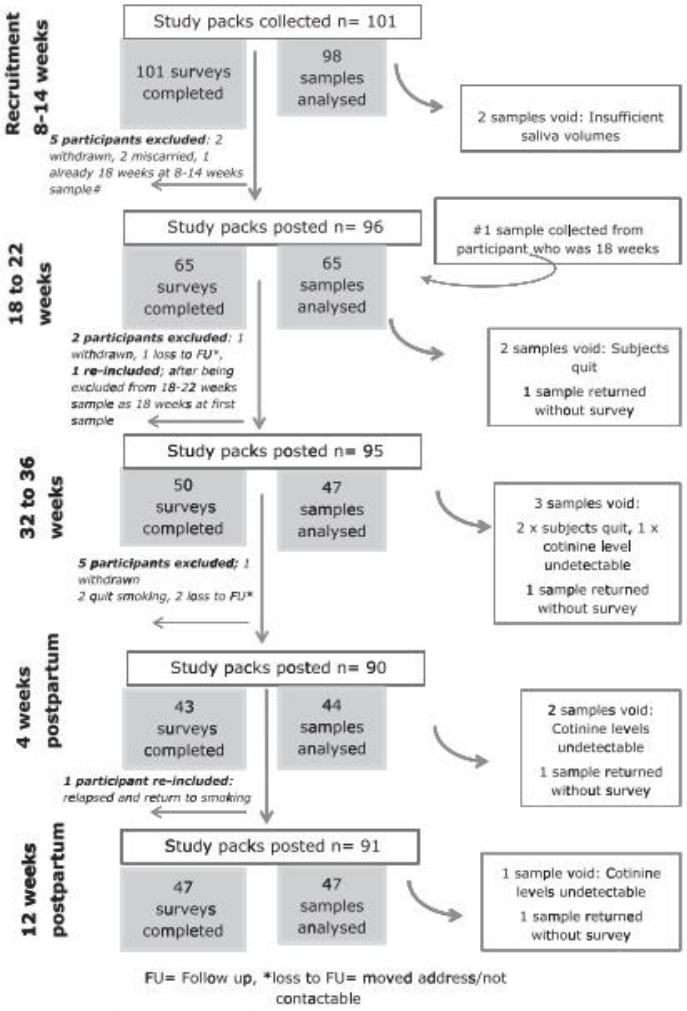


Figure 1 Recruitment and follow-up rates for samples and surveys

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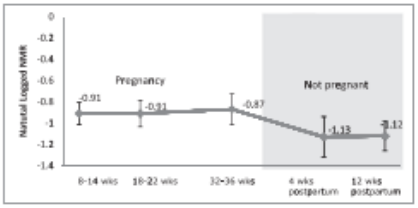
Table 1 Characteristics of sample at recruitment and 12 weeks postpartum.

Variables at recruitment	*Recruitment: 8–14 weeks' gestation (n = 101)*		*Returned sample/survey at 12 weeks postpartum (n = 47)*	
Maternal age (median, IQR)	24 (20–28)		25 (20–29)	
Gestation at recruitment (mean and SD)	12.3 (1.78)		12.3 (1.77)	
Cigarettes smoked at recruitment (median IQR)	10 (5–10)		8 (5–12)	
Carbon monoxide reading (CO)	11 (7–16)		11 (8–16)	
Employment (*n*, %)				
Employed	32	32%	18	41%
Unemployed	34	34%	12	25%
Full-time student	3	3%	1	2%
Homemaker/parent	30	30%	14	29%
Other	2	2%	2	4%
Qualifications (*n*, %)				
None	20	20%	9	18%
GCSE or equivalent	57	57%	24	53%
A levels/equivalent	19	19%	11	22%
Degree/equivalent	2	2%	1	2%
Other	3	3%	2	4%
Partner smokes (*n*, %)				
Yes	71	70%	36	77%
No	19	19%	11	23%
No partner	11	11%	–	–
Partner/family smoke in home (*n*, %)				
Yes	36	35%	18	50%
No	35	36%	18	50%
Ethnicity (*n*, %)				
White British/Irish/other white	96	95%	46	98%
Other background	5	5%	1	2%
Fagerström categorical (*n*, %)				
Mildly addicted < 4	56	55%	25	53%
Moderately addicted 4–6	37	36%	19	41%
Highly addicted 7–10	8	7%	3	6%
BMI categorical (8–14 weeks' gestation) (*n*, %)				
Underweight, less than 18.5	9	8%	3	7%
Healthy weight, 18.5–24.9	50	50%	21	46%
Overweight, 25–29.9	20	20%	10	22%
Obese, above 30	21	21%	12	26%
Missing	1	1%	1	2%

BMI = body mass index; GCSE = General Certificate of Secondary Education; IQR = interquartile range; SD = standard deviation.

cigarettes smoked. Also, only one of the five non-white ethnic participants returned a sample at 12 weeks postpartum. The remaining characteristics appeared similar between the two groups. The mean of the log-transformed values of NMR at different time-points is shown in Fig. 2. There was a significant difference in NMR over time (multi-level model, $P = 0.0006$). Nicotine metabolism appears higher in pregnancy than outside pregnancy, and after pregnancy the ratio appears to fall by 4 weeks postpartum.

Table 2 shows the pairwise comparison of NMR at 12 weeks postpartum with NMR at other time-points. After accounting for multiple comparisons, NMR was significantly higher at 18–22 weeks ($P = 0.001$) and 32–36 weeks ($P = 0.003$) compared to NMR 12 weeks postpartum. There was no statistical difference between the samples



Mean log transformed NMR with 95% confidence intervals giving the precision of the estimate at each time point; the multilevel model showed a significant change over time (p=0.0006)

Figure 2 Nicotine metabolite ratio (NMR) over time

obtained at 8–14 weeks' gestation or 4 weeks postpartum and 12 weeks postpartum. The 'within-participant'

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Table 2 Pairwise comparisons of nicotine metabolism ratio (NMR) at 12 weeks postpartum with each time-point antenatally and postpartum

Comparison to 12 weeks postpartum	*Percentage difference*	*95% confidence intervals*	*P-value*
8–14 weeks' gestation (46 paired samples)	+15%	+1% to +26%	0.034
18–22 weeks' gestation (37 paired samples)	+26%	+12% to +38%	0.001*
32–36 weeks' gestation (31 paired samples)	+23%	+9% to +35%	0.003*
4 weeks postpartum (35 paired samples)	+9%	−12% to +26%	0.405

* *P* values < 0.125 are significant.

comparison of mean log-transformed NMR from samples collected during pregnancy with those collected after delivery (4 and 12 weeks postpartum combined) showed that NMR was 17% higher in the antenatal period (95% CI = 6–26%, $P = 0.023$).

The analysis involving the 26 women who returned a sample at every time-point showed a similar pattern of NMR, and there remained a significant change in NMR over time ($P = 0.003$). After excluding women who reported using contraception at either 4 or 12 weeks postpartum ($n = 7$) the pattern of NMR over time appeared similar, and there was still a significant difference in NMR over time ($P = 0.002$).

DISCUSSION

To our knowledge, this is the first study to describe the longitudinal pattern of nicotine metabolism from as early as 8 weeks' gestation until 12 weeks following birth. Our findings show that nicotine metabolism appears to be faster in pregnancy; this faster metabolism is apparent from 18 to 22 weeks of pregnancy and appears to fall by 4 weeks after childbirth

This study is one of the largest pharmacokinetic studies of any drug throughout pregnancy [20,21]. From the same women, we have been able to take repeated measures of NMR which enabled us to explore the within-subject changes over time. To our knowledge, this is also the only study to record nicotine metabolism accurately soon after pregnancy (4 weeks postpartum) and also at a much later point following birth (12 weeks postpartum), so our finding that there was no further fall in nicotine metabolism between 4 and 12 weeks postpartum is a novel finding. We used the 12 weeks postpartum sample as our comparison sample. Ideally, the comparison NMR would have been collected before conception and participants would have been followed into pregnancy. However, recruiting prior to conception would be difficult, as most women access health-care after finding out they are pregnant.

Our response rate fell to below 50% by the time of the 4-week postpartum sample; this could have given rise to response bias. However, we found no major differences between women who returned the 12-week postpartum sample and all women recruited to the cohort. We used a multi-level model which enabled us to include all available data, but also conducted sensitivity analysis on participants who provided a sample at every time-point; this showed a similar pattern of nicotine metabolism changes to that observed in the primary analysis, again suggesting that response bias did not impact unduly upon study results.

Consistent with a previous study, we have shown that nicotine metabolism is faster during pregnancy compared to the post-partum period [4]. Our study also found that at 18–22 and 32–36 weeks' gestation NMR was significantly different from 12 weeks postpartum. The difference between 8–14 weeks' gestation and 12 weeks postpartum was in the same direction, but was not significant after allowing for multiple testing; low statistical power may have contributed to this finding. Studies which have been able to derive longitudinal trends during pregnancy have relied upon the analysis of hair samples which cannot provide measurement at precise time-points, and may be problematic due to contamination from hair products or passive smoking [6,7].

Non-pregnant women who use contraceptives containing either progesterone or a combination of oestrogen and progesterone hormones metabolize nicotine faster compared to women not using contraception [18,19]. During pregnancy these hormones begin to rise after conception, and are increased markedly throughout pregnancy. It is speculated that these hormones are responsible for the increased CYP2A6 activity during pregnancy [15]. This could explain why at 4 weeks postpartum there was a decline in NMR, as following birth there would be a rapid reduction in these hormones. However, the exact causes of increased nicotine metabolism require investigation.

We hypothesized originally that if nicotine metabolism increased during pregnancy then the dose of nicotine in NRT may not be enough to alleviate nicotine withdrawal symptoms. As we have shown an increase in nicotine metabolism during pregnancy, this suggests that higher doses of NRT may be necessary. Observational evidence has shown that, during pregnancy, using a combination of long- and short-term-acting NRT (patch and a

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faster-acting form) is associated with higher short-term quit rates compared with those attributable to using either no medication or single form NRT [22]. However, to date no randomized control trials have investigated this, so further research is required on the effectiveness of higher-dose NRT and combinations of patch plus a faster-acting form.

In conclusion, we have shown that in pregnancy nicotine metabolism is increased compared to the postpartum period; it falls between birth and 1 month and does not seem to fall further after this.

Declaration of interests

This research is part funded by the National Institute for Health Research School for Primary Care Research (NIHR SPCR) and under the NIHR Programme Grants for Applied Research scheme (RP-PG-0109-10020). The views expressed are those of the author(s) and not necessarily those of the NIHR, the NHS or the Department of Health. K.B., T.C., S.C. and S.L. are members of the NIHR School for Primary Care Research and are members of the UK Centre for Tobacco and Alcohol Studies (http://www.ukctas.ac.uk). T.C. acknowledges the support of the East Midlands Collaboration for Leadership in Applied Health Research and Care (CLARHC).

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8.2 Changes in the rate of nicotine metabolism across pregnancy: a longitudinal study

8.2.1 Participant information sheet

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Nottingham University Hospitals NHS
NHS Trust

INFORMATION ABOUT THE RESEARCH
(Final version 1.1: 03.07.13)

Study title: Nicotine breakdown during pregnancy

Exploring how quickly nicotine is broken down in the body during pregnancy and following birth

Name of Researchers: Katharine Bowker, Sue Cooper, Tim Coleman, Sarah Lewis

We would like to invite you to take part in our research study. This research will form part of a research degree (PhD) being undertaken by researcher Katharine Bowker. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. Talk to others about the study if you wish. Ask us if there is anything that is not clear.

What is the purpose of the study?
People who smoke sometimes use Nicotine Replacement Therapy (NRT), such as nicotine patches or gum, to help them to stop smoking. In pregnancy, NRT is not always helpful for stopping smoking, and we are carrying out this research to try to find out why.

Cigarettes contain a drug called nicotine that is inhaled (breathed) into the body when someone smokes. The body breaks down nicotine and begins to metabolise it (i.e. process it) almost immediately after it is inhaled. In this study we want to find out how quickly pregnant smokers get rid of the nicotine they receive from smoking. How quickly the body does this varies from one person to another and being pregnant may make the body get rid of nicotine more quickly. This is one of the possible reasons why NRT does not seem to work as well in woman who are pregnant. Therefore, we are asking pregnant smokers to provide saliva (spit) samples in pregnancy and afterwards, so that we can get a measurement of how quickly you process nicotine.

Why have I been invited?
You are being invited to take part because you are a smoker. We are inviting 100 participants like you to take part

Do I have to take part?
It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This would not affect your legal rights.

What will happen to me if I take part?
We will ask you to give up to five saliva samples and complete five short questionnaires altogether.

Sample one will be collected during your first antenatal appointment/dating scan. You will be also asked to complete a very short questionnaire about smoking. A researcher will also measure your height and weight, and will ask you to blow into a carbon monoxide monitor to measure the amount of carbon monoxide in your body.

Sample two will be collected when you are between 18-22 weeks pregnant. You will be sent a collection kit in the post, which will include instructions on how to provide a saliva sample, plus a short questionnaire, which will ask about your current smoking behaviour. You will be asked to return the questionnaire, and if you are still smoking or are using NRT then you will also be asked to provide a saliva sample by post. You will be provided with a free postage envelope to do this.

Sample three will take place when you are between 32-36 weeks pregnant, and will follow the same method as sample two.

Sample four will take place about 4 weeks after you have had your baby.

Sample five this will take place about 12 weeks after having your baby.

Expenses and payments
Participants will be paid (an inconvenience allowance) to participate in the study by being given a £5 shopping voucher for each questionnaire and (if required) sample completed.

What are the possible disadvantages and risks of taking part?
It will take between 10 or 15 minutes to complete both the questionnaire and to provide a saliva sample.

What are the possible benefits of taking part?
We cannot promise the study will help you but the information from this study may help future pregnant women. It will increase the researcher's understanding of how pregnant women process nicotine, it may help improve how NRT is given to help pregnant women who wish to stop smoking.

What happens when the research study stops?
When you have returned the final questionnaire and if (if required) saliva sample you will receive a thank you letter and voucher from the research team, contact details will be provided about how you can get in touch to obtain any publications from this research.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. The researchers contact details are given at the end of this information sheet. If you remain unhappy and wish to complain formally, you can do this by contacting NHS Complaints. The Patient Advice and Liaison Service (PALS) on 0800 183 0204.

Will my taking part in this study be kept confidential?
If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised persons from the University of Nottingham who are organising the research. They may also be looked at by authorised people to check that the study is being carried out correctly. All will have a duty of confidentiality to you as a research participant and we will do our best to meet this duty.

Your medical records will be checked to make sure you and your baby are well during pregnancy and to find out the date when you deliver your baby, this will enable us to establish dates of when to request the following samples and questionnaires. We will follow ethical and legal practice and all information about you will be handled in confidence.
All information which is collected about you during the course of the research will be kept strictly confidential, stored in a secure and locked office, and on a password protected database. Any information about you which leaves the hospital will have your name and address removed (anonymised) and a unique code will be used so that you cannot be recognised from it.

Your personal data (address, telephone number) will be kept for 3 years after the end of the study. All other data (research data) will be kept securely for 7 years. After this time your data will be disposed of securely. During this time all precautions will be taken by all those involved to maintain your confidentiality, only members of the research team will have access to your personal data.

What will happen if I don't want to carry on with the study?
Although we would like you to complete all five questionnaires and if requested saliva samples, whether or not you do so is up to you. If you change your mind and do not want to provide samples, you can let us know by phone, text or email, or you can return the Freepost card we will give you. Your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. If you withdraw then the information collected so far cannot be erased and this information may still be used in the project analysis.

What happens to the saliva samples I give?
The saliva samples you give will be stored in a secure freezer within Nottingham University Hospital campus, samples will be sent outside the university to a university approved laboratory to be analysed. Your samples will be anonymous, and only the research team will be able to link your sample to you. Once the laboratory have analysed your sample and

we have checked your results, your samples will be destroyed in accordance with the Human Tissue Act 2004.

Will any genetic tests be done?
No, we will only use your saliva sample to measure how quickly your body metabolises nicotine.

What will happen to the results of the research study?
We will publish the study results but you will not be identified in any report. You will be able to find information about any work published from this study by contacting the researcher whose contact details are available on this form.

Who is organising and funding the research
This research is being organised by the University of Nottingham and is being funded by the school of Community Health Sciences and the NIHR National School of Primary Care.

Who has reviewed the research?
All research in the NHS is looked at by independent group of people, called a Research Ethics Committee, to protect your interests. This study has been reviewed and given favourable opinion by East Midlands Nottingham 2 Research Ethics Committee.

Katharine Bowker Tel 0115 7484040 Email katharine.bowker@nottingham.ac.uk Smoking and Pregnancy Research Office Division of Primary Care, Room 1406, Tower Building University of Nottingham NG7 2RD	**Dr Sue Cooper** Chief Investigator Tel 0115 823 1898 Email sue.cooper@nottingham.ac.uk Smoking and Pregnancy Research Office Division of Primary Care, Room 1406, Tower Building University of Nottingham NG7 2RD

This information sheet is for you to keep.
Thank you very much for your time and help – we are very grateful for this.

8.2.2 Consent form



CONSENT FORM
(Final version 1.1: 03/07/2013)

Title of Study: **Nicotine breakdown during pregnancy**

REC ref: 13/EM/0236

Name of Researcher:

Name of Participant: **Please initial box (e.g.*AC*)**

1. I confirm that I have read and understood the information sheet version number ……dated…..... for the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. I understand that should I withdraw then the information collected so far cannot be erased and that this information may still be used in the project analysis. ☐

3. I understand that relevant sections of my medical notes and data collected in the study may be looked at by authorised individuals from the University of Nottingham, the research group and regulatory authorities where it is relevant to my taking part in this study. I give permission for these individuals to have access to these records and to collect, store, analyse and publish information obtained from my participation in this study. I understand that my personal details will be kept confidential. ☐

4. I understand and agree that I am being asked to provide saliva samples in this research and that these samples will be stored until the end of the project. ☐

5. I understand that my saliva will not be analysed for any purpose other than to measure how quickly nicotine is processed. ☐

6. I understand at the end of the study, or if I withdraw from the study, the samples will be destroyed. ☐

7. I agree to take part in the above study. ☐

Name of Participant	Date	Signature
Name of Person taking consent	Date	Signature

3 copies: 1 for participant, 1 for the project notes and 1 for the medical notes

8.2.3 Baseline survey

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Participant ID number								
Date returned								
Researcher's initials								

Nicotine breakdown during pregnancy

Exploring how quickly nicotine is broken down in the body during pregnancy and following birth

Baseline questionnaire

Final version number;1.1

Version date 03/07/2013

Thank you for reading the participant information sheet and agreeing to take part in this study. We would now like you to answer some questions about your health, lifestyle and smoking and to provide a sample of your saliva.

Once you have completed this questionnaire and provided a sample of your saliva we will give you a £5 gift voucher to thank you for your time and effort.

Smoking behaviour

1. Do you smoke cigarettes or roll ups?

Yes ☐	No ☐

2. On average how many cigarettes/roll ups do you smoke each day?

3. How many cigarettes or roll ups did you smoke per day before you found out you were pregnant?

4. Do you live with a partner or family member who smokes?

Yes ☐	No ☐

5. If yes, does this person smoke in the house?

Yes ☐	No ☐

6. How soon after you wake up do you smoke your first cigarette or roll up?

Within 5 minutes ☐	31-60 minutes ☐
6-30 minutes ☐	After 60 minutes ☐

7. Do you find it difficult to refrain from smoking in places where it is forbidden (e.g., in a church, at the library, in cinema, etc)?

Yes ☐	No ☐

8. Which cigarette would you most hate to give up?

The first one in the morning ☐	Any other one ☐

9. Do you smoke more frequently during the first few hours after waking than during the rest of the day?

Yes ☐	No ☐

10. Do you still smoke if you are so ill that you are in bed most of the day?

Yes ☐	No ☐

About you

11. How would you describe your ethnic group?

White – British/Irish/other white background ☐	
Mixed - white and black/Caribbean ☐	Mixed - white and Asian ☐
Other mixed background ☐	Asian/Asian British/other Asian background ☐
Black or black British ☐	Chinese ☐
Other. Please specify:_____	

12. At what age did you leave/finish full time education?

☐ Years of age	☐ I am still in education

13.Which of the following qualifications do you have?

None ☐
GCSEs or similar (e.g. level 1 diploma or NVQ level1/2) ☐
A-levels/AS-levels or similar (e.g. Level 2 diploma or NVQ level 3) ☐
Degree or similar (e.g. diploma in higher education or NVQ level 4) ☐
Other ☐

14.Which of the following applies to you?

I am in paid work at the moment ☐	I'm unemployed ☐
I'm a full time student ☐	I'm a homemaker/full time parent ☐
Other ☐	

Thank you for completing the questionnaire. We are very grateful for your help. Please hand this back to the researcher so that they can complete the rest of the visit with you.

Your health measurements (to be completed with the researcher after you have completed the survey)

15.What is your current height in metres?	
16. What is your current weight in kg?	
17. Carbon monoxide reading	

8.2.4 Survey follow up one

The University of Nottingham
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Participant ID number								
Date returned								
Researcher's initials								

Follow up 1 questionnaire (18-22 weeks gestation)

Final version number;1.1

Version date 03/07/2013

Nicotine breakdown during pregnancy

Exploring how quickly nicotine is broken down in the body during pregnancy and following birth

Dear Madam,

Thank you for your participation in this study. We are grateful for your time and input. We would like you to answer the following questions about your health and smoking habits. Depending on your answers, you may also be asked to send us a saliva (spit) sample.

If you are asked to send a saliva sample, please follow the instructions enclosed and return it with this questionnaire in the free post, secure package provided.

Once we have received the questionnaire, and if requested a sample, we will send you a £5 gift voucher to thank you for your effort.

Smoking behaviour

1. Have you smoked any cigarettes or roll ups in the past 3 days?

Yes ☐	No ☐

If you answered YES go to Question 3

2. Have you used Nicotine Replacement therapy (NRT) i.e nicotine patches/gum etc. in the last 3 days?

Yes ☐	No ☐

If you answered YES to using NRT go to Question 9

If you have not smoked in the past 3 days and are not using NRT you have now finished the questionnaire- we do NOT need a saliva sample from you. Please return this form using the free postage packaging.

Otherwise please continue, and we will require a saliva sample

3. On average how many cigarettes/roll ups do you smoke approximately each day?

☐

4. How soon after you wake up do you smoke your first cigarette or roll up?

Within 5 minutes ☐	31-60 minutes ☐
6-30 minutes ☐	After 60 minutes ☐

5. Do you find it difficult to refrain from smoking in places where it is forbidden (e.g., in a church, at the library, in cinema, etc)?

Yes ☐	No ☐

6. Which cigarette would you most hate to give up?

The first one in the morning ☐	Any other one ☐

7. Do you smoke more frequently during the first few hours after waking than during the rest of the day?

Yes ☐	No ☐

8. Do you still smoke if you are so ill that you are in bed most of the day?

Yes ☐	No ☐

Please go to Question 11

Nicotine replacement therapy (NRT)- *please complete this section if you are using NRT*

9. What type of NRT product are you using? Please select all that apply to you

Patches ☐	Spray ☐	Gum ☐
Lozenges ☐	E Cigarettes ☐	None of these ☐

10. When did you last use NRT?

Last 24 hours ☐	Between 1 and 2 days ago ☐
Between 2 and 3 days ago ☐	Over 3 days ago ☐

Please continue

Your health and environment

11. Do you live with a partner or family member who smokes?

Yes ☐	No ☐

12. If yes, does this person smoke in the house?

Yes ☐	No ☐

13. Are you currently taking any medication except the ones stated below:
Iron medication, vitamin tablets (including folic acid), or an inhaler for asthma

Yes ☐	No ☐	Don't know ☐

If yes, Please state: _____

If you answered 'YES' to either question 1 or question 2 and you smoke or use NRT, we would like you to give a saliva sample

Please follow the instructions which are in the envelope.

If you are unsure, please send a sample with the questionnaire anyway

Thank you for your participation in this study

8.2.5 Survey follow up two

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Participant ID number								
Date returned								
Researcher's initials								

Nicotine breakdown during pregnancy

Exploring how quickly nicotine is broken down in the body during pregnancy and following birth

Follow up 2 questionnaire (32-36 weeks gestation)

Final version number;1.1

Version date 03/07/2013

Dear Madam,

Thank you for your participation in this study. We are grateful for your time and input. We would like you to answer the following questions about your health and smoking habits. Depending on your answers, you may also be asked to send us a saliva (spit) sample.

If you are asked to send a saliva sample, please follow the instructions enclosed and return it with this questionnaire in the free post, secure package provided.

Once we have received the questionnaire, and if requested a sample, we will send you a £5 gift voucher to thank you for your effort.

Smoking behaviour

1. Have you smoked any cigarettes or roll ups in the past 3 days?

Yes ☐	No ☐

If you answered YES go to Question 3

2. Have you used Nicotine Replacement therapy (NRT) i.e nicotine patches/gum etc. in the last 3 days?

Yes ☐	No ☐

If you answered YES to using NRT go to Question 9

If you have not smoked in the past 3 days and are not using NRT you have now finished the questionnaire- we do NOT need a saliva sample from you. Please return this form using the free postage packaging.

Otherwise please continue, and we will require a saliva sample

3. On average how many cigarettes/roll ups do you smoke approximately each day?

☐

4. How soon after you wake up do you smoke your first cigarette or roll up?

Within 5 minutes ☐	31-60 minutes ☐
6-30 minutes ☐	After 60 minutes ☐

5. Do you find it difficult to refrain from smoking in places where it is forbidden (e.g., in a church, at the library, in cinema, etc)?

Yes ☐	No ☐

6. Which cigarette would you most hate to give up?

The first one in the morning ☐	Any other one ☐

7. Do you smoke more frequently during the first few hours after waking than during the rest of the day?

Yes ☐	No ☐

8. Do you still smoke if you are so ill that you are in bed most of the day?

Yes ☐	No ☐

Please go to Question 11

Nicotine replacement therapy (NRT)- *please complete this section if you are using NRT*

9. What type of NRT product are you using? Please select all that apply to you

Patches ☐	Spray ☐	Gum ☐
Lozenges ☐	E Cigarettes ☐	None of these ☐

10. When did you last use NRT?

Last 24 hours ☐	Between 1 and 2 days ago ☐
Between 2 and 3 days ago ☐	Over 3 days ago ☐

Please continue

Your health and environment

11. Do you live with a partner or family member who smokes?

Yes ☐	No ☐

12. If yes, does this person smoke in the house?

Yes ☐	No ☐

13. Are you currently taking any medication except the ones stated below:
Iron medication, vitamin tablets (including folic acid), or an inhaler for asthma

Yes ☐	No ☐	Don't know ☐

If yes, Please state: _____

If you answered 'YES' to either question 1 or question 2 and you smoke or use NRT, we would like you to give a saliva sample

Please follow the instructions which are in the envelope.

If you are unsure, please send a sample with the questionnaire anyway

Thank you for your participation in this study

8.2.6 Survey follow up three

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Participant ID number								
Date returned								
Researcher's initials								

Nicotine breakdown during pregnancy

Exploring how quickly nicotine is broken down in the body during pregnancy and following birth

Follow up 3 questionnaire (4 weeks postnatal)

Final version number;1.1

Version date 03/07/2013

Dear Madam,

Thank you for your participation in this study. We are grateful for your time and input. We would like you to answer the following questions about your health and smoking habits. Depending on your answers, you may also be asked to send us a saliva (spit) sample.

If you are asked to send a saliva sample, please follow the instructions enclosed and return it with this questionnaire in the free post, secure package provided.

Once we have received the questionnaire, and if requested a sample, we will send you a £5 gift voucher to thank you for your effort.

Smoking behaviour

1. Have you smoked any cigarettes or roll ups in the past 3 days?

Yes ☐	No ☐

If you answered YES go to Question 3

2. Have you used Nicotine Replacement therapy (NRT) i.e nicotine patches/gum etc. in the last 3 days?

Yes ☐	No ☐

If you answered YES to using NRT go to Question 9

If you have not smoked in the past 3 days and are not using NRT you have now finished the questionnaire- we do NOT need a saliva sample from you. Please return this form using the free postage packaging.

Otherwise please continue, and we will require a saliva sample

3. On average how many cigarettes/roll ups do you smoke approximately each day?

☐

4. How soon after you wake up do you smoke your first cigarette or roll up?

Within 5 minutes ☐	31-60 minutes ☐
6-30 minutes ☐	After 60 minutes ☐

5. Do you find it difficult to refrain from smoking in places where it is forbidden (e.g., in a church, at the library, in cinema, etc)?

Yes ☐	No ☐

6. Which cigarette would you most hate to give up?

The first one in the morning ☐	Any other one ☐

7. Do you smoke more frequently during the first few hours after waking than during the rest of the day?

Yes ☐	No ☐

8. Do you still smoke if you are so ill that you are in bed most of the day?

Yes ☐	No ☐

Please go to Question 11

Nicotine replacement therapy (NRT)- *please complete this section if you are using NRT*

9. What type of NRT product are you using? Please select all that apply to you

Patches ☐	Spray ☐	Gum ☐
Lozenges ☐	E Cigarettes ☐	None of these ☐

10. When did you last use NRT?

Last 24 hours ☐	Between 1 and 2 days ago ☐
Between 2 and 3 days ago ☐	Over 3 days ago ☐

Please continue

Your health and environment

11. Do you live with a partner or family member who smokes?

Yes ☐	No ☐

12. If yes, does this person smoke in the house?

Yes ☐	No ☐

13. Are you currently taking any medication except the ones stated below:
Iron medication, vitamin tablets (including folic acid), or an inhaler for asthma

Yes ☐	No ☐	Don't know ☐

If yes, Please state: _____

14. How are you feeding your baby?

Breastfeeding ☐	Bottle-feeding ☐	Mixed Feeding ☐

If you answered 'YES' to either question 1 or question 2 and you smoke or use NRT, we would like you to give a saliva sample

Please follow the instructions which are in the envelope.

If you are unsure, please send a sample with the questionnaire anyway

Thank you for your participation in this study

8.2.7 Survey follow up four

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Participant ID number								
Date returned								
Researcher's initials								

Nicotine breakdown during pregnancy

Exploring how quickly nicotine is broken down in the body during pregnancy and following birth

Follow up 4 questionnaire (12 weeks postnatal)

Final version number;1.1

Version date 03/07/2013

Dear Madam,

Thank you for your participation in this study. We are grateful for your time and input. We would like you to answer the following questions about your health and smoking habits. Depending on your answers, you may also be asked to send us a saliva (spit) sample.

If you are asked to send a saliva sample, please follow the instructions enclosed and return it with this questionnaire in the free post, secure package provided.

Once we have received the questionnaire, and if requested a sample, we will send you a £5 gift voucher to thank you for your effort.

Smoking behaviour

1. Have you smoked any cigarettes or roll ups in the past 3 days?

Yes ☐	No ☐

If you answered YES go to Question 3

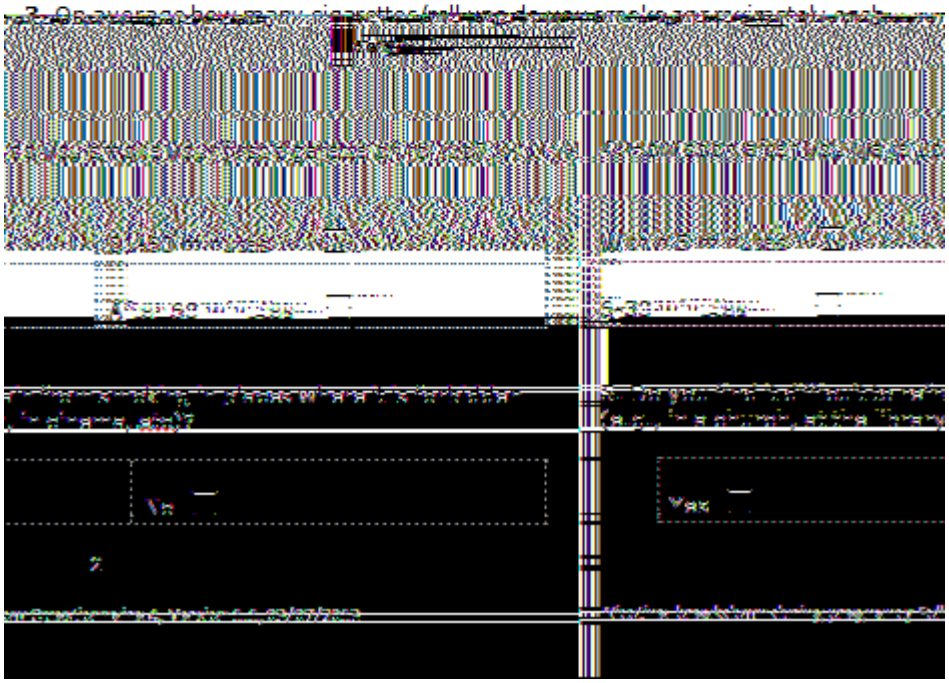
2. Have you used Nicotine Replacement therapy (NRT) i.e nicotine patches/gum etc. in the last 3 days?

Yes ☐	No ☐

If you answered YES to using NRT go to Question 9

If you have not smoked in the past 3 days and are not using NRT you have now finished the questionnaire- we do NOT need a saliva sample from you. Please return this form using the free postage packaging.

Otherwise please continue, and we will require a saliva sample



6. Which cigarette would you most hate to give up?

The first one in the morning ☐	Any other one ☐

7. Do you smoke more frequently during the first few hours after waking than during the rest of the day?

Yes ☐	No ☐

8. Do you still smoke if you are so ill that you are in bed most of the day?

Yes ☐	No ☐

Please go to Question 11

Nicotine replacement therapy (NRT)- *please complete this section if you are using NRT*

9. What type of NRT product are you using? Please select all that apply to you

Patches ☐	Spray ☐	Gum ☐
Lozenges ☐	E Cigarettes ☐	None of these ☐

10. When did you last use NRT?

Last 24 hours ☐	Between 1 and 2 days ago ☐
Between 2 and 3 days ago ☐	Over 3 days ago ☐

Please continue

Your health and environment

11. Do you live with a partner or family member who smokes?

Yes ☐	No ☐

12. If yes, does this person smoke in the house?

Yes ☐	No ☐

13. Are you currently taking any medication except the ones stated below:
Iron medication, vitamin tablets (including folic acid), or an inhaler for asthma

Yes ☐	No ☐	Don't know ☐

If yes, Please state: _____

14. How are you feeding your baby?

Breastfeeding ☐	Bottle-feeding ☐	Mixed Feeding ☐

If you answered 'YES' to either question 1 or question 2 and you smoke or use NRT, we would like you to give a saliva sample

Please follow the instructions which are in the envelope.

If you are unsure, please send a sample with the questionnaire anyway

Thank you for your participation in this study

8.2.8 Letter of follow up

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Smoking and Pregnancy Research Office
University of Nottingham
Tower Building, Rm 1406
University Park Campus
Nottingham
NG7 2RD
Tel: 0115 7484040
Email:
Katharine.bowker@nottingham.ac.uk

Date

Nicotine breakdown during pregnancy

Dear

I hope that you remember providing a sample of your saliva earlier in your pregnancy. We are extremely grateful to have had your help with this.

We would therefore be very grateful if you could now fill in another questionnaire and, if the questionnaire asks for one, to provide a sample of your saliva. The questionnaire and / or saliva sample can then be returned in the pre-paid package. We would be very pleased if you could do this **within the next 5 days**. Please follow the instruction sheets for guidance on taking and returning the questionnaire and sample.

Once we have received the questionnaire and if requested the saliva sample, we will send you a £5 shopping voucher to thank you for your support. Your participation in this study is extremely valuable for the research. The research team will treat your samples and answers confidentially and will not share these with anybody else.

Thank you again for participating in this study. We look forward to receiving your reply. If you have any questions about the study please contact the researcher Katharine Bowker using the contact details below.

With best wishes,

Yours sincerely

Katharine Bowker,
PhD student
Telephone 0115 7484040 or email katharine.bowker@nottingham.ac.uk.

8.2.9 Thank you letter

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Smoking and Pregnancy Research Office
University of Nottingham
Tower Building, Rm 1406
University Park Campus
Nottingham
NG7 2RD
Tel: 0115 7484040
Email: Katharine.bowker@nottingham.ac.uk

Date

Name

Congratulations you have now finished the study. Thank you for returning the questionnaire and if requested a sample of your saliva as part of the study. Please find enclosed a £20 shopping voucher to thank you for this. We really do appreciate your help.

Once again, thank you for your support. When all particpants have completed the study, a summary of the findings will be written up and you can request to see any publications from this study by contacting the researcher Katharine Bowker (details above).

With best wishes,
Yours sincerely

Katharine Bowker

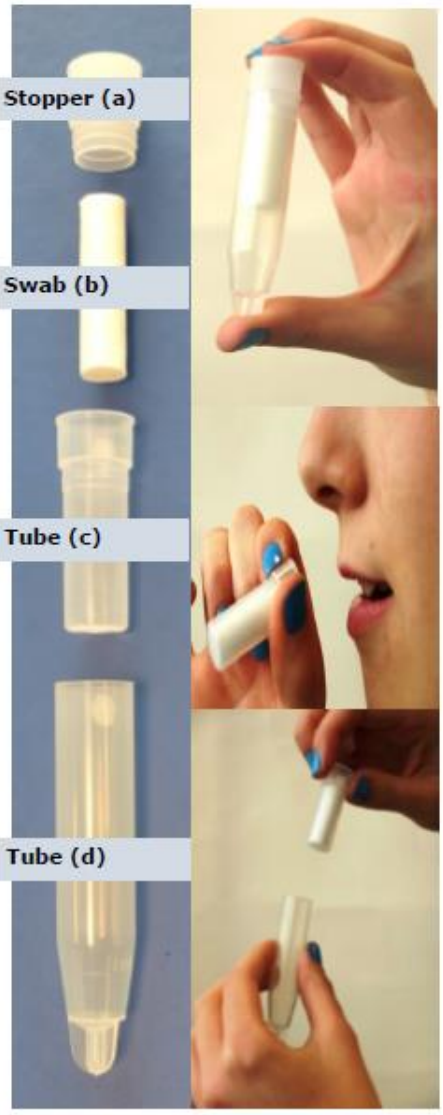
8.2.10 Sample instructions letter

The University of Nottingham

How to take a saliva sample

Before giving a sample

- Wash your mouth out with water
- Wait at least 24 hours before giving your sample if you have eaten or drank grapefruit in the past 24 hours.



Now follow these 5 simple steps!

1. Remove tube c which is located in tube d and then remove the Stopper (a).

2. Inside tube c you will find a swab (b).

Try not to touch the swab with your fingers!

Put the swab under your tongue by tipping up the tube. When the swab is under your tongue close your mouth.

3. Leave the swab underneath your tongue until it is soggy, this may take up to **5 minutes**.

4. Without using your fingers, use your mouth to place the swab back into tube (c) and put the stopper firmly on the tube.

5. Finally put the tube (c) which contains the swab into the larger tube (d).

Please write the date you have taken the saliva on the sample tube!

*Please do not send your sample on a **Friday**.*

Thank you for providing this saliva sample

Nicotine breakdown during pregnancy Instruction sheet picture, Version1.1, 03/07/2013

8.2.11 Sample posting instructions

The University of Nottingham

Instructions for collection of saliva samples

Storing sample and posting

1. Wrap the sealed sample tube in the silver foil and place inside the clear self-seal bag.
2. Seal the clear bag and place the sample into the clear plastic compartment of the postal box.
3. Ensure the questionnaire is completed, and fold and place in the compartment next to the sample.
4. Once the package has been closed, it cannot be reopened without destroying it so please ensure that all contents are in the safebox (see picture below).
5. Remove the cardboard separator in the lid of the safebox and place the lid over the base and press to close the safebox.
6. Peel the outer backing from the address label and wrap around the blue box.
7. Please post at any pillar box or post office (address label already completed) by 5.00pm on day of collection. No postage needed.



Important Information

1. Please ensure that you post the sample on the day of collection. If the sample cannot be mailed immediately, it should be wrapped in the silver foil, placed in the clear self-seal bag and frozen in your freezer until it can be posted.
2. Saliva samples will be disposed of following completion of the study.

Contact Information

If you need any advice regarding these instructions please telephone;
Katharine Bowker on 0115 748 4040

Nicotine breakdown during pregnancy Instruction Sheet, Version 1.1 03/07/2013

8.2.12 NHS R&D approval

research & innovation

Nottingham University Hospitals NHS
NHS Trust

Research & Innovation
Nottingham Integrated Clinical Research Centre

C Floor, South Block
Queen's Medical Centre Campus
Derby Road, Nottingham NG7 2UH

Tel: 0115 9249924 ext 70659
www.nuhrise.org

Dr Sue Cooper

19 JUL 2013

Smoking and Pregnancy Research Office

Room 1406, 14th Floor, Tower Building

University Park

Nottingham

NG7 2RD

Dear **Dr Sue Cooper**

Re: **13OB009**

CSP **125756**

REC **13/EM/0236**

Investigating Nicotine Metabolism in pregnant smokers

The R&I Department have reviewed the following documents and NHS permission for the above research has been granted on the basis described in the application form, protocol, and supporting documentation. The documents reviewed were:

REC favourable opinion dated 5 July 2013
Protocol, v1.1, 03 July 2013
Participant information sheet, v1. 1, 03 July 2013

Participant consent form, v1.1, 03 July 2013
Advertisement: Poster, v1.1, 03 July 2013
Questionnaire: Screening, v1.1, 03 July 2013
Questionnaire: Baseline, v1.1, 03 July 2013
Questionnaire: Follow up 1, v1.1, 03 July 2013
Questionnaire: Follow up 2, v1.1, 03 July 2013
Questionnaire: Follow up 3, v1.1, 03 July 2013
Questionnaire: Follow up 4, v1.1, 03 July 2013
Study instruction sheet picture, v1. 1, 03 July 2013
Study instruction sheet, v1. 1, 03 July 2013
Letter of invitation to participant (not consented in clinic) v1.1, 03 July 2013
Letter of follow up 1-3, (in REC letter as 'Other: Request Letter Follow Up') v1. 1, 03 July 2013
Letter of follow up 4, v1.1, 03 July 2013
Thank you letter follow up 1-3, (in REC letter as 'Other: Thank you Letter') v1. 1, 03 July 2013
Thank you letter follow up 4, v1. 1, 03 July 2013
Contact information sheet, v1.1, 03 July 2013
Postcard information, v1.1, 03 July 2013

NB. The REC letter lists 2 documents which have been superseded and the current versions also approved within the letter. These documents are labelled in the REC letter as: -

Letter of invitation to Participant: Participants not consented in clinic V1 28 May 2013
Letter of invitation to Participant: Follow up four invite V1 28 May 2013

Your study now has NHS permission, on the understanding and provision that you will follow the conditions set out below.

Conditions of Approval

The Principal Investigator is responsible for

1. Compliance with all relevant laws, regulations and codes of practice applicable to the trial including but not limited to, the UK Clinical Trials Regulations, Medicines for Human Use (Clinical Trial) Regulations 2004, principles of Good Clinical Practice, the World Medical Association Declaration of Helsinki entitled 'Ethical Principles for Medical Research Involving Human Subjects' (1996 version), the Human Rights Act 1998, the Data Protection Act 1998 the Medicines Act 1968, the NHS Research Governance Framework for Health and Social Care (version 2 April 2005). Should any of these be revised and reissued this will apply. Copies of the up-to-date regulations are available from the R&I Office or via the R&I website http://nuhrise.org

2. Submission of study amendments to the Ethics committee and MHRA in accordance with the IRAS guidelines. Amendments and information with regards to changes in study status must be sent to R&I, (this includes changes to the local study team). Within 35 days from the receipt of a valid amendment submission, NUH will inform you if may not locally implement the amendment. If no objections are raised NHS

permission is valid and the amendment may be implemented.

When submitting documents for studies adopted into the NIHR portfolio please send the information to NUHNT.TRENTCLRN@nhs.net
When submitting documents for all other studies please use the email address rdamend@nuh.nhs.uk

3. Ensuring all study personnel, not employed by the Nottingham University Hospitals NHS Trust hold either honorary contracts/letters of access with this Trust, before they have access to any patients or staff, their data, tissue or organs or any NUH facilities.

4. In accordance with the Department of Health's Plan for Growth, for initialising and delivering research within the NHS the 'first patient, first visit should occur 70 days from receipt of a valid submission in R&I. Therefore for all research where:

    - The sponsor is a commercial partner
    - NUH holds a funding contract with the National Institute for Health Research (NIHR)
    - The research is classed as a "clinical trial "on the IRAS filter page.

The research team is expected to collaborate with the department of R&I in reporting recruitment data to rdmon@nuh.nhs.uk.

1. For GTAC-approved studies, the NHS permission should be forwarded to GTAC via the sponsor. GTAC should then issue a site authorisation letter which must be received by each site prior to recruitment commencing. A copy of this letter must be forwarded to R&I.

2. Comply with requests from NUH R&I to allow monitoring of research to comply with the Research Governance framework.

3. Record *all* types of adverse events (including Suspected Unexpected Serious Adverse Drug Reaction - SUSARS) in the patient medical records and study documentation and report to the sponsor as required by the protocol. Further guidance can be found in R&I SOP 11 - "Adverse Event Monitoring, Recording and Reporting for investigators".

4. Report any Serious Breach of the UK Clinical Trial regulations in connection with the trial or Serious Breach of the protocol, immediately after becoming aware of the breach to the study sponsor.

For NUH sponsored studies only, the Chief Investigator is responsible for:

i. All duties as detailed in the "Clinical Trial Delegation of Sponsorship responsibilities to Chief Investigator" agreement.
ii. Contacting the sponsor for review of all amendment documentation prior to submission to NRES and MHRA. Please note that according to NRES and MHRA regulations, all submissions of amendments need to be signed by the authorised sponsor's representative. All relevant documentation should be emailed

to rdappl@nuh.nhs.

iii. Send copies of the completed Annual Safety, Progress reports and End of Study reports required by the Ethics Committee and the MHRA (if appropriate) to the Quality Assurance manager at NUH R&I.

iv. Notify NUH R&I of all SAEs by completing and sending the *"Serious Adverse Event reporting form"* to R&I **(only via fax, e-mail or by hand),** within 24hrs of becoming aware of the event. If the event is defined as a SUSAR then a follow up report must also be submitted to R&I, via the above channels-**no longer than 7 days after the original report was submitted**

v. Reporting any Serious Breach of the UK Clinical Trial regulations in connection with the trial or Serious Breach of the protocol, immediately after becoming aware of the breach to NUH R&I as sponsor. Further guidance can be found in R&I SOP 12 "Protocol Violation and Serious Breach Reporting"

This approval letter constitutes a favourable Site Specific Assessment (SSA) for this site.

Please note that the R&I department maintains a database containing study related information, and personal information about individual investigators e.g. name, address, contact details etc. This information will be managed according to the principles established in the Data Protection Act.

Yours sincerely,

Dr Brian Thomson / Dr Maria Koufali
Director of Research and Innovation / Deputy Director Research and Innovation

cc Nottingham Research Ethics Committee

8.2.13 Ethics approval

NHS
Health Research Authority
NRES Committee East Midlands - Nottingham 2
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Telephone: 0115 8839697

05 July 2013

Ms Katherine Bowker
Room 1406 Tower Building
University of Nottingham
NG7 2RD

Dear Ms Bowker,

Study title:	**Investigating Nicotine Metabolism in pregnant smokers during pregnancy and postpartum**
REC reference:	**13/EM/0236**
Protocol number:	**13060**
IRAS project ID:	**125756**

Thank you for your letter of 04 July 2013. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 02 July 2013.

Documents received

The documents received were as follows:

Document	*Version*	*Date*
Advertisement	1.1	03 July 2013
Covering Letter	(Email)	04 July 2013
Letter of invitation to participant	1.1	03 July 2013
Other: Follow up letter 4	1.1	03 July 2013
Other: Request Letter Follow Up	1.1	03 July 2013
Other: Contact Information Sheet	1.1	03 July 2013
Other: Postcard Information	1.1	03 July 2013
Other: Thank you Letter	1.1	03 July 2013
Other: Instruction Sheet Picture	1.1	03 July 2013
Other: Thank you Letter 4	1.1	03 July 2013
Participant Consent Form	1.1	03 July 2013
Participant Information Sheet: Instruction Sheet	1.1	03 July 2013
Participant Information Sheet	1.1	

Protocol	1.1	03 July 2013
Questionnaire: Follow up Questionnaire 1	1.1	03 July 2013
Questionnaire: Follow up Questionnaire 2	1.1	03 July 2013
Questionnaire: Follow up Questionnaire 4	1.1	03 July 2013
Questionnaire: Baseline Questionnaire	1.1	03 July 2013
Questionnaire: Follow Up Questionnaire 3	1.1	03 July 2013
Questionnaire: Screening Questionnaire	1.1	03 July 2013

Approved documents

The final list of approved documentation for the study is therefore as follows:

Document	*Version*	*Date*
Advertisement	1.1	03 July 2013
Covering Letter		01 June 2013
Covering Letter	(Email)	04 July 2013
Evidence of insurance or indemnity		25 July 2012
Evidence of insurance or indemnity		06 August 2012
Investigator CV	Sue Cooper	30 May 2013
Letter from Sponsor		31 May 2013
Letter of invitation to participant	Participants not consented in clinic V1	28 May 2013
Letter of invitation to participant	Follow up four invite V1	28 May 2013
Letter of invitation to participant	1.1	03 July 2013
Other: Katharine Bowker CV		30 May 2013
Other: CV Tim Coleman		11 April 2013
Other: CV Sarah-Anne Lewis		11 April 2013
Other: Follow up letter 4	1.1	03 July 2013
Other: Request Letter Follow Up	1.1	03 July 2013
Other: Contact Information Sheet	1.1	03 July 2013
Other: Postcard Information	1.1	03 July 2013
Other: Thank you Letter	1.1	03 July 2013
Other: Instruction Sheet Picture	1.1	03 July 2013
Other: Thank you Letter 4	1.1	03 July 2013
Participant Consent Form	1.1	03 July 2013
Participant Information Sheet: Instruction Sheet	1.1	03 July 2013
Participant Information Sheet	1.1	
Protocol	1.1	03 July 2013
Questionnaire: Follow up Questionnaire 1	1.1	03 July 2013
Questionnaire: Follow up Questionnaire 2	1.1	03 July 2013
Questionnaire: Follow up Questionnaire 4	1.1	03 July 2013
Questionnaire: Baseline Questionnaire	1.1	03 July 2013
Questionnaire: Follow Up Questionnaire 3	1.1	03 July 2013

Questionnaire: Screening Questionnaire	1.1	03 July 2013
REC application	1	05 June 2013

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

13/EM/0236	**Please quote this number on all correspondence**

Yours sincerely,



Rachel Nelson
Assistant Committee Co-ordinator

E-mail: NRESCommittee.EastMidlands-Nottingham2@nhs.net

Copy to:	*Dr Sue Cooper* *Dr Paul Cartledge,* *Mrs Charlotte Davies*

8.2.14 Site approval

Nottingham University Hospitals NHS
NHS Trust

Research & Innovation
Nottingham Integrated Clinical Research Centre
C Floor, South Block
QMC Campus
Derby Road
Nottingham
NG7 2UH

Tel: 0115 970 9049

www.nuhrise.org

RSCH 241

DATE: 15/07/2013

Katharine Bowker
12 Queens Drive
Beeston
Nottingham
NG9 2ES

Dear Katharine

Re: Letter of Access for Research

Study Title: Investigating Nicotine Metabolism in pregnant smokers
Principal Investigator at NUH: Prof Jim Thornton
CLRN ID: 125756 R&D Ref: 13OB009
Sponsor: University of Nottingham

This letter confirms your right of access to conduct research through **Nottingham University Hospitals NHS Trust** for the purpose and on the terms and conditions set out below. This right of access commences on **01/08/2013** and ends on **24/10/2013** unless terminated earlier in accordance with the clauses below.

You have a right of access to conduct such research as confirmed in writing in the letter of permission for research from this NHS organisation. Please note that you cannot start the research until the Principal Investigator for the research project has received a letter from us giving permission to conduct the project.

The information supplied about your role in research at **Nottingham University Hospitals NHS Trust** has been reviewed and you do not require an honorary research contract with this NHS organisation. We are satisfied that such pre-engagement checks as we consider necessary have been carried out.

You are considered to be a legal visitor to Nottingham University Hospitals NHS Trust premises. You are not entitled to any form of payment or access to other benefits provided by this NHS organisation to employees and this letter does not give rise to any other relationship between you and this NHS organisation, in particular that of an employee.

While undertaking research through **Nottingham University Hospitals NHS Trust**, you will remain accountable to your employer **University of Nottingham** but you are required to follow the reasonable instructions of **Prof Jim Thornton** in this NHS organisation or those given on her/his behalf in relation to the terms of this right of access.

Where any third party claim is made, whether or not legal proceedings are issued, arising out of or in connection with your right of access, you are required to co-

operate fully with any investigation by this NHS organisation in connection with any such claim and to give all such assistance as may reasonably be required regarding the conduct of any legal proceedings.

You must act in accordance with **Nottingham University Hospitals NHS Trust** policies and procedures, which are available to you upon request, and the Research Governance Framework.

You are required to co-operate with **Nottingham University Hospitals NHS Trust** in discharging its duties under the Health and Safety at Work etc Act 1974 and other health and safety legislation and to take reasonable care for the health and safety of yourself and others while on **Nottingham University Hospitals NHS Trust** premises. You must observe the same standards of care and propriety in dealing with patients, staff, visitors, equipment and premises as is expected of any other contract holder and you must act appropriately, responsibly and professionally at all times.

You are required to ensure that all information regarding patients or staff remains secure and *strictly confidential* at all times. You must ensure that you understand and comply with the requirements of the NHS Confidentiality Code of Practice (http://www.dh.gov.uk/assetRoot/04/06/92/54/04069254.pdf) and the Data Protection Act 1998. Furthermore you should be aware that under the Act, unauthorised disclosure of information is an offence and such disclosures may lead to prosecution.

You should ensure that, where you are issued with an identity or security card, a bleep number, email or library account, keys or protective clothing, these are returned upon termination of this arrangement. Please also ensure that while on the premises you wear your ID badge at all times, or are able to prove your identity if challenged. Please note that this NHS organisation accepts no responsibility for damage to or loss of personal property.

We may terminate your right to attend at any time either by giving seven days' written notice to you or immediately without any notice if you are in breach of any of the terms or conditions described in this letter or if you commit any act that we reasonably consider to amount to serious misconduct or to be disruptive and/or prejudicial to the interests and/or business of this NHS organisation or if you are convicted of any criminal offence. Your substantive employer is responsible for your conduct during this research project and may in the circumstances described above instigate disciplinary action against you.

Nottingham University Hospitals NHS Trust will not indemnify you against any liability incurred as a result of any breach of confidentiality or breach of the Data Protection Act 1998. Any breach of the Data Protection Act 1998 may result in legal action against you and/or your substantive employer.

If your current role or involvement in research changes, or any of the information provided in your Research Passport changes, you must inform your employer through their normal procedures. You must also inform your nominated manager in this NHS organisation.

Yours sincerely –

Hannah Finch
Senior RM&G Manager

cc: HR department of the substantive employer

8.2.15 List of self-reported medication use

Follow up gestation	*Medication*	*Main cytochrome involved in metabolism*	*Influence on nicotine metabolism*
18 to 22 weeks	*Fluoxetine x2*	*CYP2D6*	*Unknown*
	Citalopram	*CYP2C19*	*Unknown*
	Amoxicillin	*CYP2C19*	*Unknown*
	Omeprazole	*CYP2C19*	*Unknown*
	Hay fever tablets	*--*	
	Not stated	*--*	
32 to 36 weeks	*Citalopram*	*CYP2C19*	*Unknown*
	Cyclizine	*CYP2C9*	*Unknown*
	Gaviscon	*CYP2C19*	*Unknown*
	Omeprazole	*CYP2C19*	*Unknown*
	Lamotrigine	*CYP2D6*	*Unknown*
4 weeks postpartum	*Citalopram*	*CYP2C19*	*Unknown*
	Sertraline	*CYP2D6*	*Unknown*
	Doxycycline	*CYP3A4*	*Unknown*
	Lamotrigine	*CYP2D6*	*Unknown*
	Contraception x 3	*CYP3A4*	*Increases nicotine metabolism due to hormonal influence on cytochrome CYP2A6*
12 weeks postapartum	*Contraceptive x 5*	*CYP3A4*	*Increases nicotine metabolism due to hormonal influence on cytochrome CYP2A6*
	B12 Injections	*CYP1A1*	*Unknown*
	Citalopram	*CYP2C19*	*Unknown*
	Fluoxetine	*CYP2D6*	*Unknown*
	Sertralinex2	*CYP2D6*	*Unknown*
	Lamotrigine	*CYP2D6*	*Unknown*
	Antibiotic – not stated	*--*	

8.2.16 Multi-level model output from Chapter Three, section 3.3.11

The model shows the effect sizes representing the difference in NMR over-time (between time points). The coefficients show that compared to time point 5 (12 weeks postpartum) the NMR significantly increased over time. The chi-squared distribution is 0.0004, which indicates the variable time is a significant factor in change in NMR.

Number of observations included in the model=301
Number of groups=100
Observations per group: Min=1
Average=3
Max=5

Wald chi2=20.43
Prob > chi2= 0.004

NMR time point	Coefficient	P value	95% CI	
5 (12 weeks postpartum)	--	--	--	--
4 (4 weeks postpartum)	0.0142	0.857	-0.140	0.169
3 (32-36 weeks)	0.255	0.001	0.100	0.409
2 (18-22 weeks)	0.234	0.001	0.090	0.378
1 (8-14 weeks)	0.199	0.004	0.064	0.333

8.3 Understanding pregnant smokers' adherence to Nicotine Replacement Therapy during a quit attempt: a qualitative study

8.3.1 Participant information sheet

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

INFORMATION ABOUT THE RESEARCH
(Final version 1.1:12/03/2014)

Experiences of pregnant women who use NRT in pregnancy as part of a quit attempt

Name of Researchers: Katharine Bowker, Sue Cooper, Tim Coleman, Sarah Lewis

We would like to invite you to take part in a short telephone survey about your experience of using Nicotine Replacement Therapy (NRT) during your pregnancy. Depending on your answers we may then ask you to take part in a more detailed telephone interview. Before you decide whether you would like to take part, we would like you to understand why the research is being done and what it would involve for you. One of our team will go through this information sheet with you and answer any questions you have. Talk to others about the study if you wish. Ask us if there is anything that is not clear. This study is part of a research degree (PhD) being undertaken by researcher Katharine Bowker.

What is the purpose of the study?
We know that pregnant women, like you, are often given NRT (such as nicotine patches, inhalators, gum etc.) to help them stop smoking. We want to find out more about the experiences of pregnant women using NRT, so we can have a better understanding about the ways in which pregnant women use NRT, and how pregnant women feel NRT supports a quit attempt.

Why have I been chosen?
We are asking you to take part because you have been prescribed NRT to help you with a quit attempt during your pregnancy.

Do I have to take part?
No, this is entirely up to you. You are free to leave the study at any time without giving a reason, and it would not affect the care you receive.

What will happen to me if I take part? What will I have to do?
A researcher will contact you by telephone a few days after sending you this information sheet. If you are happy to take part, they will ask you a few questions about how you used NRT. These questions will take between 5-10 minutes to complete. Depending on your answers you may then be asked to take part in a longer telephone interview. In this longer interview, the researcher will ask you some further questions to find out about your experience of using NRT in more detail. Again, it is up to you if you want to do this second interview, and the researcher will make sure you understand everything, answer any questions you have, and are happy to do this before this interview takes place. This interview can either take place straight after the telephone survey, or the researcher can arrange to call you back at a more suitable time. This interview will be audio recorded and will last approximately 30-60 minutes. You can change your mind and stop the interview at any time, but if this happens we would still like to use the information you have provided until that point.

What are the benefits of taking part?
There will be no direct benefit to you, but you will be helping to improve stop smoking support for future pregnant women. If you participate in the second longer telephone interview, to thank you for your time and any inconvenience of taking part you will receive a £20 high street shopping voucher.

What are the possible disadvantages and risks of taking part?
There are no disadvantages or risks in taking part in this study, other than giving up your time to take part in the study.

What if there is a problem?
If you have a concern about any aspect of this study, you should ask to speak to the researcher who will do her best to answer your questions. The researcher's contact details are given at the end of this information sheet. If you remain unhappy and wish to complain formally, you can do this by contacting NHS Complaints.

Will taking part in this study be kept confidential?
Yes. We will keep all information that is collected about you during the research strictly confidential. The interview responses will be anonymous and will not have your name and address attached, only a code. Only the researchers and people allowed to check that the research is carried out correctly could have access to your personal information.

What will happen if I don't want to carry on with the study?
Nothing, your participation is voluntary and you are free to withdraw at any time, without giving any reason, and without your legal rights being affected. However, we wouldn't be able to erase the information you had already given us and we may still use this in the study.

What will happen to the results of the research study?
We will publish the study results but you will not be identified in any report. Findings will help the NHS understand how pregnant women use NRT as part of their quit attempt.

Who is organising and funding the research?
This research is being funded by the School of Medicine at the University of Nottingham and the National Institute of Health Research, National School of Primary Care.

Who has reviewed the study?
To protect your interests, an independent group of people, called a Research Ethics Committee review the study; West of Scotland Research Ethics Committee 4 have reviewed and approved this study.

If you would like further information, please contact Katharine Bowker (researcher) on Katharine.bowker@nottingham.ac.uk or telephone 0115 748 4040

THANK YOU FOR TAKING THE TIME TO READ THIS INFORMATION SHEET

8.3.2 Telephone screening survey

Telephone survey

Participant ID number / Initials	
Date returned	
Researcher's initials	

Thank you for agreeing to take part in this short survey. Explain the study. Has the woman read the PIS? If not verbally explain what information was on the PIS.

Researcher's initials

Does the woman give verbal consent to take part in the telephone survey? ☐

1. What is your age?

☐

2. (REMINDER TO WOMAN) *This survey is being requested because you recently saw your smoking advisor and you were prescribed NRT.*

   a. In your most recent quit attempt what type or types of NRT were you prescribed by your smoking advisor? (go through the list)

   b. Did you use any of the NRT that was prescribed on at least one occasion? (go through the list)

	Prescribed (Y/N)	Used (Y/N)		Prescribed (Y/N)	Used (Y/N)
Patches	☐	☐	Gums	☐	☐
Lozenges	☐	☐	Spray (mouth or nasal)	☐	☐
Nicotine inhaler	☐	☐	Microtab	☐	☐

3. a. Have you used any other NRT products (including e-cigarettes) that were not supplied by stop smoking advisor during your most recent quit attempt?

Yes		No	

b. if YES what did you use?

Patches		Gums	
Lozenges		Spray (mouth or nasal)	
Nicotine inhaler		Microtab	
E cigarette		Other	
Please state if other-			

4. Have you used NRT for more than one day during your most recent quit attempt?

Yes		No	

5. During your most recent quit attempt did you use an NRT product?

Everyday or almost everyday		Every other day	
Less than every other day			

6. a If you currently use or have previously used patches during your most recent quit attempt, did you usually wear the patch for 16 hours a day?

YES		NO	

b. If NO

How many hours did you wear the patch for on a typical day?

7. How many days has it been since you first started using the NRT prescribed by your adviser in your most recent quit attempt?

8. How many days have you used NRT for during your most recent quit attempt?

9.a. If you currently use or have previously used in your must recent quit attempt any of the quick acting forms of NRT (gum, spray, tablets, inhaler, lozenges) on a typical day how many times did/do you use it?

Times used on a typical day	

b. If using an E cigarette how many times on a typical day did/do you use it?

10. How many cigarettes did you smoke per day before your most recent quit attempt?

11. During your most recent quit attempt, have you ever smoked one or more cigarettes while you were using NRT, *so using both NRT and smoking at the same time*?

Yes		No	

12. Are you currently using NRT?

Yes		No	

13. a Are you currently smoking?

Yes		No	

b. If not smoking, how many days have you quit for?

14. In your most recent quit attempt would you have liked more advice about any of the following when you saw your advisor (yes/no options for below):

The safety of using NRT in pregnancy		The benefits of using NRT in pregnancy	
How to use your selected NRT product(s)		Side effects and how to avoid or minimise these	
Using more than one NRT product at the same time		Other NRT products	
Ways to help you remember to take your NRT product at the right times			

Eligible for interview

Used NRT for more Than one day (excluding E cigarette use)	

And one of the following

Smoking and using NRT (yes to Q12 and Yes to Q13)	
Smoking and not using NRT (yes to Q12 and No to Q13)	
Not smoking and not using NRT (No to Q12 and No to Q13)	
Not currently smoking and using NRT but not as recommended. (No to Q13 yes to one of the following) For example 1. Not wearing patches for 16 hours a day (No. Q6) 2. if only using short acting medication, not using it for each smoking urge (measured by comparing the number of cigarettes used to smoke per day with number of times used NRT) 3. Number of days used patch not equivalent to number of days since prescribed (Q7 & Q8)	

If yes willing to take part?

Yes			No		

8.3.3 Interview topic guide

Aim – describe the experiences of women who have used but not adhered or discontinued using NRT.

- To explore women's expectations about using NRT as part of a quit attempt
- To understand women's experiences of using NRT including reasons for not using this as instructed or early discontinuation
- To explore women's thoughts on how important using NRT was to their quit attempt

1. **INTRODUCTION** – consent, confidentiality, honesty, purpose, audio etc

2. **SMOKING BEHAVIOUR and EXPECTATIONS of NRT**

a) Before trying to quit how did you feel about your smoking? Probes, general, pre-pregnancy, smoking? In pregnancy?
b) How were you referred for help? Probe- community, hospital GP, self.
c) What support did you receive? Probe-face to face/telephone/individual group
d) How did you feel about the support you received?
e) Had you heard of NRT or experienced using it before it was suggested to you? Probe-what was your experience or family/friends experience?
f) How did you feel about the opportunity to use NRT to aid your quit attempt? Probe Were you expecting it? Positive or negative feelings?
g) What did you think about the information and advice you were given about using NRT? Probe- who gave it, was it helpful, in what ways could it have been more helpful?
h) What level of choice did you feel you had in regards to what NRT product you were given? Probe did you have a preference, if so how come?
i) Did you discuss using NRT with family, friends or another health professional i.e. midwife/GP? Probe if so, was this helpful?
j) Did you receive any advice from the pharmacist about using NRT?
k) Once being prescribed NRT what were your thoughts about using it? Probe- motivation to use it? Level of choice?

3. **Experience of using NRT**

a) How prepared/well instructed did you feel when you first started to use NRT? Probe know when and how to take, know about the side effects.
b) How soon after being prescribed NRT did you take it? Probe- Did you plan this and set a quit date?
c) How did you find using the patches (or gum etc.?) Probe- How easy was it to use? How did you feel about doing it?
d) On a typical day can you describe how you use/used NRT? (go back to survey to see what products they were using) Probe- frequency you use NRT? For how long? - What influences this?
e) What impact, if any did using NRT have on your daily routines? Probe- did you remember to take it?
f) How do you feel NRT assisted/is assisting your quit attempt? Probe with any cravings or urges to smoke?
g) Did you experience any side effects of using NRT? Can you describe these?
h) Did you use NRT as it was recommended by your smoking advisor? For example if you were given patches did you wear them as advised? Probe if you didn't-what did you do and why?
i) What was it like giving up smoking while you used NRT? Probe was it how you expected, if not, how did it differ?

4. **Current situation and reflections** (clarify from the survey questions what the current situation is i.e. from the survey I can see that you are still using NRT, but do not wear the patch for the recommended 16 hours, would you say this applies to you… or… you said you were currently smoking and not using NRT anymore, is this correct? etc.)

Using NRT not adhering ☐

Not using NRT returned to smoking ☐

Using NRT and smoking ☐

Not using NRT and NOT smoking ☐

Those who are using NRT but not adhering

a) Do you think NRT is helpful in aiding your quit attempt?
b) How long do you intend to use NRT for?
c) Can you tell me more about how you use NRT on a daily basis?
d) Are there any particular reasons why you use NRT in the way you have just described?
e) Have you smoked while using NRT? If so why?
f) Have you talked with anyone about how you finding your quit attempt? I.e. smoking advisor/family/friend.
g) Do you feel you had enough support? Probe If yes why is that, if no- who would you like more support from?

Those who are not using NRT returned to smoking

a) Do you think NRT was helpful in aiding your quit attempt?
b) How long did you use NRT for?
c) After beginning NRT can you remember when you first started to smoke again? Probe- did you experience cravings to smoke, was there anything that triggered you to smoke?
d) Can you describe why you stopped using NRT?
e) What did it feel like to smoke again?
f) Are your smoking habits now the same as before your quit attempt?
g) Do you plan to try and quit again in the future? Probe If yes would you use NRT again and is there anything you would do differently? If no, why?
h) Were there any particular reasons why you stopped using NRT?
i) Did you talk with anyone about stopping NRT? I.e. smoking advisor/family/friend.
j) Did you feel you had enough support to stop smoking?
k) What most describes your situation

1) you stopped using NRT and then started to smoke again

OR

2) you started smoking before you stopped using NRT

If yes to 1.

I. Were there any particular reasons why you stopped using NRT and then returned to smoking?

If yes to 2.

I. How did you feel about using both NRT and smoking? Probe-Did you have any concerns about smoking and using NRT?
II. Can you tell me how you used NRT and smoked on a typical day? Probe- did you smoke your regular number of cigarettes?
III. Was there anything that lead you to eventually stopping using NRT and return to smoking only?

Those who are using NRT (regardless of adherence) and are smoking

a) Do you think NRT is helpful in aiding your quit attempt?
b) After beginning NRT can you remember when you first started to smoke again? Probe- did you experience cravings to smoke, was there anything that triggered you to smoke?
c) On a typical day can you describe how you use NRT and your smoking habits? Probe- do you smoke your regular number of cigarettes? What NRT product did you use?
d) Are there any particular reasons why you use NRT and smoke?
e) Do you plan to quit smoking? Probe if yes when and why, if no why not what is your plan?
f) How do you feel about using both NRT and smoking? Probe-Do you have any concerns about smoking and using NRT?
g) Do you feel NRT is helpful to you? Probe- how, why?
h) How long do you intend to use NRT for?
i) Do you intend to quit smoking?
j) Have you talked with anyone about your experience if using NRT? I.e. smoking advisor/family/friend.
k) Do you feel you had enough support? Probe If yes why is that, if no- who would you like more support from?

Those who are not using NRT and have stopped smoking

a) Do you think NRT was helpful in aiding your quit attempt?
b) How long did you use NRT for?
c) Were there any particular reasons why you stopped using NRT?
d) How did it feel the first day you stopped using NRT? Probe did you notice any difference? Does it feel different now?
e) Did you find using NRT helpful? Probe if so in what way? If not why not?
f) Did you talk with anyone about stopping NRT? I.e. smoking advisor/family/friend.
g) Did you feel you had enough support to stop smoking?
h) How long have you been quit
i) Do you intend to stay quit?

Background questions

Pregnancy gestation

How old are you?

Which ethnic group do you belong to (circle)

- White
- Black Caribbean
- Black African
- Black – other Black groups
- Indian
- Pakistani
- Bangladeshi
- Chinese
- Other

Partner

Do you have a partner? Married?

If so, do they smoke?

GENERAL OPINIONS AND SUGGESTIONS

a) Having experienced using NRT in pregnancy, is there anything you think could be improved or changed which may help pregnant women who use NRT in an attempt to quit smoking in their pregnancy?

b) What kind of help or support might have made it easier for you to use NRT/keep using NRT? Probe: How might additional detailed advice about any of the following help?:

   I. The risks and benefits of NRT use in pregnancy
   II. Guidance on how to use your selected NRT product to maximise its benefit
   III. Information on side effects and how to avoid these?
   IV. Reminders for taking NRT at particular times or in particular situations
   V. Information on alternative NRT products in case your first one was not working out for you
   VI. Weekly monitoring of how much NRT you are using and feedback and advice about your usage
   VII. Ways to help you remember to take your NRT product at the right times

c) (If participant felt any of the above may have helped) What would be the best way of receiving this type of advice or support for you? Probe: what do you think of receiving this support on your mobile phone, either by SMS text message or as part of an app?

INTERVIEW CLOSE – Thanks, questions etc.

8.3.4 Thank you letter

The University of Nottingham
UNITED KINGDOM · CHINA · MALAYSIA

Smoking and Pregnancy Research Office
University of Nottingham
Tower Building, Rm 1406
University Park Campus
Nottingham
NG7 2RD
Tel: 0115 7484040
Email: Katharine.bowker@nottingham.ac.uk

Date

Dear

Thank you for completing a telephone interview for this study. Please find enclosed a £20 shopping voucher to thank you for this. We really do appreciate your help.

Once again, thank you for your support.

With best wishes,
Yours sincerely

Katharine Bowker

8.3.5 NHS R&D approval

positive

Nottinghamshire Healthcare NHS
NHS Trust
Positive about integrated healthcare

Research Support Services
Institute of Mental Health
University of Nottingham Innovation Park
Triumph Road
Nottingham
NG7 2TU

E-mail: Shirley.mitchell@nottshc.nhs.uk
Direct Line: 0115 748 4321

Date of NHS permission: 09/04/2014

Dr Sue Cooper
University if Nottingham
1406 Tower Building
University Park
Nottingham
NG7 2RD

Dear Dr Cooper

Study title: Experiences of Pregnant Women Who Use NRT in Pregnancy as Part of a Quit Attempt
IRAS/REC ID: 148547/ 14/WS/0069
Sponsor: University of Nottingham

Thank you for submitting your project to the Nottinghamshire Healthcare NHS Trust's R&D Department. The project has now been given NHS permission for PIC activity by:

Dr Gopi Krishnan: R & D Director, on behalf of Nottinghamshire Healthcare NHS Trust and Michelle Bateman, Research Lead, Health Partnerships (community provider services)

NHS permission for the above research has been granted on the basis described in the application form, study protocol and supporting documentation. The following documents were reviewed:

Document	Version
REC Approval dated	14 March 2014
Participant Information Sheet	v1.1 12 March 2014
Participant Consent Form	v1.1 12 March 2014
Covering Letter	03 March 2014
Letter from Sponsor	03 March 2014
REC application	03 March 2014
Protocol	12 March 2014
Evidence of insurance or indemnity	31 July 2013
Interview Schedules/Topic Guides	v1.0 28 February 2014

Permission is granted on the understanding that the study is conducted in accordance with the Research Governance Framework, ICH GCP [ONLY if applicable], and NHS Trust policies and procedures available http://www.nottinghamshirehealthcare.nhs.uk/contact-us/freedom-of-information/policies-and-procedures/

The research sponsor or the Chief Investigator, or the local Principal Investigator at a research site, may take appropriate urgent safety measures in order to protect research participants against any immediate hazard to their health or safety. The R&D office should be notified that such measures have been taken. The notification should also include the reasons why the measures were taken and the plan for further action. The R&D Office should be notified within the same time frame of notifying the REC and any other regulatory bodies. All amendments (including changes to the local research team) need to be submitted in accordance with guidance in IRAS.

Please note that the NHS organisation is required to monitor research to ensure compliance with the Research Governance Framework and other legal and regulatory requirements. This is achieved by random audit of research.

Yours Sincerely



Shirley Mitchell
Head of Research Management and Governance

CC:

Sponsor: Nottingham University

Trent CLRN

Student: Miss Katherine Bowker

8.3.6 Ethics approval

WoSRES
West of Scotland Research Ethics Service

NHS
Greater Glasgow and Clyde

Dr Sue Cooper
Senior Research Fellow & NIHR Programme Manager, Faculty of Medicine & Health Science
University of Nottingham
1406 Tower Building
University Park
Nottingham
NG7 2RD

West of Scotland REC 4
Ground Floor, Tennent Building
Western Infirmary
38 Church Street
Glasgow
G11 6NT
www.nhsggc.org.uk

Date 19 March 2014
Direct line 0141-211-1722
Fax 0141-211-1847
e-mail Wosrec4@ggc.scot.nhs.uk

Dear Dr Cooper

Study title:	**Experiences of pregnant women who use NRT in pregnancy as part of a quit attempt**
REC reference:	**14/WS/0069**
Protocol number:	**14024**
IRAS project ID:	**148547**

Thank you for your email of 12 March 2014, responding to the Proportionate Review Sub-Committee's request for changes to the documentation for the above study.

The revised documentation has been reviewed and approved by the Lead Reviewer.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Ms Evelyn Jackson, wosrec4@ggc.scot.nhs.uk.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations.

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list

of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The documents reviewed and approved by the Committee are:

Document	*Version*	*Date*
Covering Letter	-	03 March 2014
REC application	-	03 March 2014
Protocol	1.1	12 March 2014
Investigator CV	-	
Participant Information Sheet	1.1	12 March 2014
Participant Consent Form	1.1	12 March 2014
Evidence of insurance or indemnity		31 July 2013
Interview Schedules/Topic Guides	1.0	28 February 2014
Letter from Sponsor	-	03 March 2014
Other: Thank you letter	1.0	28 February 2014
Other: Letter re funding	-	30 January 2014
Other: Katharine Bowker's CV	-	
Other: Sarah Anne Lewis's CV	-	11 April 2013
Other: Tim Coleman's CV	-	11 April 2013
Other: Telephone Survey	1.1	12 March 2014
Response to Request for Further Information	-	12 March 2014

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

14/WS/0069	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely



Dr Jackie Riley
Alternate Vice-Chair

Enclosures: *"After ethical review – guidance for researchers"*

Copy to: *Paul Cartledge, University of Nottingham*
Shirley Mitchell, Nottinghamshire Health Care NHS Trust

8.4 Postgraduate Training Courses and prizes

Course Title	*Credit*
Preparing for the viva	1
Regression analyses in STATA	1
Teaching Induction for Postgraduate Research Students and Research Staff	1
Creating and Managing Long Documents in Microsoft Word	1
Research Methods for Clinical Trials	4
Getting going on your thesis	1
Academics' administrators' professional personal and leadership experience	2
More Functions in Excel	2
Systematic review (Medicine and Health Sciences Faculty)	1
Using Excel as a Database	2
Preparing your first year report and writing scientific abstracts	1
Presentation Skills: Practicing and Reviewing	1
Essential information skills for new researchers in medicine and health sciences	1
NVivo® Part 1: Preparing to use NVivo9® (standalone online learning course)	1
Total Credits	20

Travel Prize

NIHR CLAHRC East Midlands PhD Travel/Research Prize Application awarded 1st June 2015 * due to change in circumstances did not attend conference with this prize money.