Abstract

Objectives

The aim of this study was to explore the relationships between nausea and vomiting in pregnancy and (a): fetal growth restriction; and (b) maternal caffeine metabolism and fetal growth restriction.

Methods

A cohort of 2643 pregnant women, aged 18-45 years, attending two UK maternity units between 8-12 weeks gestation, was recruited. A validated tool assessed caffeine intake at different stages of pregnancy and caffeine metabolism was assessed from a caffeine challenge test. Experience of nausea and vomiting of pregnancy was self-reported for each trimester. Adjustment was made for confounders, including salivary cotinine as a biomarker of current smoking status.

Results

There were no significant associations between fetal growth restriction and nausea and vomiting in pregnancy, even after adjustment for smoking and alcohol intake. There were no significant differences in the relationship between caffeine intake and fetal growth restriction between those experiencing symptoms of nausea and vomiting and those who did not, for either the first ($p=0.50$) or second trimester ($p=0.61$) after adjustment for smoking, alcohol intake and caffeine half-life**.** There were also no significant differences in the relationship between caffeine half-life and fetal growth restriction between those experiencing symptoms of nausea and vomiting and those who

did not, for either the first trimester (p=0.91) or the second trimester (p=0.45) after adjusting for smoking, alcohol intake and caffeine intake.

Conclusion

The results from this study show no evidence that the relationship between maternal caffeine intake and fetal growth restriction is modified by nausea and vomiting in pregnancy.

Introduction

Just two weeks after fertilisation, nausea and vomiting may be the first symptoms of pregnancy and reports show that 69 to 89% of women may experience these symptoms during this dynamic stage of the lifecycle [1-4]. Although a common symptom, its exact aetiology is unknown. Nausea and vomiting of pregnancy are thought to have a positive effect on pregnancy by: ensuring an optimum balance of nutrition between maternal and fetal tissues [5]; causing avoidance of foods which may harm the mother or embryos [6]; or reflecting a favourable balance of hormones [7]. Timing of nausea and vomiting in pregnancy is of particular interest as while food restriction (which coincides with nausea and vomiting in pregnancy) during the first trimester may be beneficial to the developing fetus, this may not be the case later in pregnancy [5, 8].

There have been several studies of the role of nausea in pregnancy and its interaction with alcohol, caffeine and smoking [4, 9, 10]. Caffeine, in particular, has received much interest in recent years. Studies have shown that maternal caffeine consumption may increase the risk of adverse pregnancy outcomes [11-15]. It has been suggested that women who experience nausea may be less likely to consume caffeine [16]. The secretion of human chorionic gonadotrophin, which has been suggested as the most likely cause of nausea and vomiting in pregnancy [17], has been negatively associated with coffee consumption [18, 19]. It is therefore postulated that unchanged or heavy caffeine consumption may be a marker of suboptimal placental hormone synthesis [7], and consequently, a vulnerable implantation. This hypothesis has been difficult to prove as the few studies which have examined the association between maternal caffeine

intake, nausea and vomiting in pregnancy and poor pregnancy outcome have produced mixed results [10, 20-22].

In order to examine the complex relationship between pregnancy outcome, nausea and vomiting in pregnancy and caffeine intake, it is clear that a deeper exploration is needed. Inconsistent results from previous studies may be due to a number of methodological flaws. These flaws include an imprecise or biased assessment of caffeine exposure, failure to consider changes during pregnancy e.g. changes in intake and smoking habits, possible confounders such as alcohol and smoking [23, 24] and individual differences in metabolism.

The Caffeine and Reproductive Health (CARE) study [11] addressed the methodological issues described above through a prospective, longitudinal observational study of the relationship between maternal caffeine intake, metabolism and fetal growth restriction. The results of this study showed that maternal caffeine consumption throughout pregnancy was associated with an increased risk of fetal growth restriction and that this association was stronger among women with a faster caffeine metabolism than those with a slower caffeine metabolism. Nausea has been associated with increased estrogen levels [4, 25, 26] which in turn have been negatively associated with CYP1A2 activity, the primary enzyme implicated in caffeine metabolism [25, 27]; therefore nausea may have a role to play in the association found in the CARE study. Indeed, a recent review has stressed the importance of examining the 'pregnancy signal i.e. a reduction to coffee consumption and aversion to other odours and tastes [28]. Therefore the results of the CARE study warranted further investigation of the relationship between fetal growth restriction, nausea and vomiting of pregnancy, maternal caffeine intake and metabolism.

Hence our aim here was to use the valuable data collected by the CARE study [11] to explore the relationships between nausea and vomiting of pregnancy and (a): fetal growth restriction; and (b) maternal caffeine metabolism and fetal growth restriction.

Methods

Participants

Pregnant women assessed as at low risk of complications were prospectively recruited from the maternity units of two large UK teaching hospitals (Leeds and Leicester) from September 2003 to June 2006. Women aged 18-45 years with singleton pregnancies were recruited. Those with prior chronic disease, psychiatric illness, HIV or hepatitis B infection were excluded. Following screening of pre-booking maternity notes, eligible women were sent study information and were asked to return a reply slip indicating their willingness to take part in the study. Personal contact was made with those who agreed to take part and the initial visit was conducted at the hospital, the volunteer's general practice or home by a clinical research fellow. The demographic details of the participants were recorded by a questionnaire (age, parity, maternal height, weight, socio-economic status and gestational age). Local ethics committee approval was obtained at both centres and written informed consent was collected before recruitment.

Measurements

Caffeine intake

Detailed methods on the caffeine assessment are described elsewhere [11]. A validated caffeine assessment tool (CAT) to record habitual caffeine intake was administered at booking (8-12 weeks) by a clinical research fellow or research midwives, and completed

by the participants during weeks 13-28, and finally weeks 28 to the end of pregnancy. The questionnaire assessed caffeine consumption from all potential dietary sources and over the counter medications. Brand names, portion sizes, methods of preparation, and quantity and frequency of intake were recorded for different gestational periods so that precise caffeine intakes could be estimated using an in-house program [29]. Each CAT also collected details of potential confounders like smoking and alcohol intake.

Caffeine metabolism

Caffeine half-life was assessed from a caffeine challenge test [29] performed within two weeks of recruitment. The participants were required to fast overnight, complete the challenge at home the following morning (a drink of 500 mL diet cola, containing 63.5 mg caffeine ingested over a period of 20 minutes), and avoid any other caffeine consumption during the challenge. Saliva samples were then collected at approximately one and five hours post-challenge using a Salivette® (Sarstedt, Aktiengesellschaft & Co., Loughborough, UK). Participants recorded times of sample collection along with details of drinks or food consumed during the test period in a questionnaire. The samples were returned in a pre-paid envelope to the corresponding laboratory at Leeds and Leicester, where the saliva was isolated from the Salivettes by centrifugation and stored at -80 °C. At Leicester, saliva samples were sub-aliquotted and sent, on dry ice, to the University of Leeds for analysis. Half-life for caffeine was calculated from salivary caffeine concentrations recorded at one and five hours post-challenge. Saliva samples were also used for determining nicotine exposure. Salivary cotinine concentrations were quantified by an enzyme linked immunosorbent assay (ELISA) kit (Cozart Bioscience, Oxfordshire,

UK) according to the manufacturer's instructions in samples taken at recruitment and in a sub-sample at 28 weeks gestation (n=488). Participants were categorized as active smokers (>5ng/ml), passive smokers (1-5ng/ml), or non-smokers (<1ng/ml).

Pregnancy details

For each trimester, participants reported whether they experienced nausea or vomiting (yes/no). Information regarding antenatal pregnancy complications and delivery details were obtained from the electronic maternity databases. Gestational age was dated using crown rump length ultrasound measurements performed in the first trimester. The primary outcome measure was fetal growth restriction and has been reported elsewhere and in the statistical methods below [11].

Statistical methods

This analysis examined data collected by the first two CATs (≤28 weeks gestation) so that nausea and vomiting in pregnancy during the first and second trimesters could be assessed. The data from the final CAT, which was completed post pregnancy, was not used for the current analyses to reduce the influence of recall bias. Caffeine consumption was expressed in mg/day averaged over the individual trimesters and grouped into categories of intake (<200mg/day; ≥200mg/day) which are in line with current guidance for pregnant women in both the United Kingdom and United States [30, 31].

Unconditional logistic regression modelling was used to examine the associations between fetal growth restriction, caffeine intake and potential confounding factors, using STATA v 10.0 (College Station Texas, USA). Firstly, the association between fetal

growth restriction and nausea and vomiting of pregnancy was assessed. Fetal growth restriction was defined as birthweight below the 10th centile on a customised centile chart which incorporated the main non-pathological factors affecting fetal growth - maternal height, weight, ethnicity, parity at booking, gestation at delivery and gender of the neonates [32]. Ethnicity was categorised as follows: European (includes British Isles and those of European origin elsewhere e.g. Australia); Indian; Pakistani; Bangladeshi; Afro-Caribbean; African (sub-Sahara); Middle Eastern (including North Africa); and South East Asian. Parity was defined as the number of times a participant gave birth to a fetus with a gestational age of 20 weeks or more. In addition to the covariates adjusted by the use of the customised growth centiles, statistical adjustment was also made in all models for salivary cotinine levels and self-reported alcohol consumption assessed at recruitment and at 28 weeks, if available. Secondly, we formally tested whether the association between fetal growth restriction and maternal caffeine intake differed according to experience of nausea and vomiting in pregnancy, by including an interaction term in the model and adjusting for smoking, alcohol intake and caffeine half-life. Finally, we repeated the latter analyses examining caffeine half-life rather than caffeine intake adjusting for smoking, alcohol, and caffeine intake. Caffeine half-life was not normally distributed therefore women were categorised as having a short half-life (fast caffeine clearance) or long half-life (slow caffeine clearance) using the median value as a cut-off. For this analysis we wished to focus on those with stronger symptoms of pregnancy, therefore we assessed nausea and vomiting as a combined symptom (i.e. those who experienced both nausea and vomiting - NVP). However, we also analysed nausea and

vomiting in pregnancy separately as both symptoms may occur independently and have different aetiologies.

Results

A total of 13,071 eligible women were invited to participate and 2,643 (20%) consented. Eight women were excluded after their pregnancies were terminated. Of the remaining 2,635 pregnancies, 2,519 had data on caffeine intake and nausea and vomiting for the first and/or second trimester. Data on caffeine intake and *both* nausea and vomiting (NVP) were not available on all women (1^{st} trimester caffeine intake and NVP (n=1,513); 2^{nd} trimester caffeine intake and NVP (n=1,583)). Half-life data were available on 1,520 women. The median (IQR) for caffeine metabolism half-life was 6.9 (4.5 to 11.7) hours. The mean (SD) maternal age was 29.9 (5.9) years. Just over half of the cohort gave birth to boys (n=1269, 52%) and 1191 (48%) women were primiparous. Thirteen per cent of women (n=336) had a growth restricted baby. The median (IQR) caffeine intake over the first and second trimesters was 116.5 (45 to 225) mg/day and 106 (37 to 206) mg/day, respectively. **Table 1** presents the number of women who reported nausea and vomiting during pregnancy among those who had data on caffeine intake, nausea and vomiting for the first and/or second trimester (n=2,519). Most women experienced nausea during the first trimester, with fewer women reporting vomiting**.** Almost half (45%) of the women reported both nausea and vomiting in pregnancy in the first trimester and 21% in trimester two.

Only the logistic regression analyses conducted on those with stronger symptoms (both nausea and vomiting experienced) are presented in this current paper and were

conducted among those who had data on caffeine intake, nausea and vomiting for the first and/or second trimester (n=2,519). The logistic regression analyses conducted for nausea and vomiting separately produced results similar to the combined analyses and are therefore not shown. Results from logistic regression showed no significant associations between fetal growth restriction and nausea and vomiting in pregnancy, even after considering the covariates included in the customised growth charts and additional adjustment for smoking and alcohol intake **(Table 2).** Approximately 70% of women who reported nausea and vomiting in the first trimester reported caffeine intakes less than 200mg/day, with similar results found among those who reported nausea and vomiting in the second trimester (Table 3). Further analyses showed that women who reported nausea in the first trimester had significantly lower caffeine intakes compared to those who did not (difference: 22 mg/day (95% CI: 6 to 39), $p<0.01$). However, there were no significant differences in the caffeine intake and fetal growth restriction relationship between those experiencing symptoms of nausea and vomiting for either the first ($p=0.50$) or second trimester ($p=0.61$) and those who did not, after consideration of the covariates in the customised growth charts in addition to the adjustment for smoking, alcohol intake and caffeine half-life **(Table 3).** The models presented in this table illustrate the interaction term indirectly.

Women who experienced nausea and vomiting in pregnancy did not have significantly increased odds of having a slow caffeine metabolism after consideration of the covariates in the customised growth charts in addition to the adjustment for smoking, alcohol, caffeine intake (first trimester nausea and vomiting in pregnancy: OR: 0.87, 95% CI:0.67 to 1.13; second trimester nausea and vomiting in pregnancy: OR: 0.84, 95%

CI:0.52 to 1.36) . **Table 4** shows that there were no significant differences in the caffeine half-life and fetal growth restriction relationship between those experiencing symptoms of nausea and vomiting for either the first trimester (p=0.91) or the second trimester (p=0.45) and those who did not after consideration of the covariates in the customised growth charts in addition to the adjustment for smoking, alcohol intake and caffeine intake**.** The models presented in this table illustrate the interaction term indirectly.

Discussion

Main findings

In our cohort, the incidence of fetal growth restriction (FGR) was 13%, a figure higher than that often quoted, however similar to the low birth weight rates reported for the areas assessed in this current study[33]. However, it has been shown that using standard birth weight measurements for gestational age and gender alone, underestimates growth restricted babies by as much as 50% [34]. We therefore believe that our findings reflect the true incidence in our population since we used customised charts. We did not find a relationship between nausea and vomiting in pregnancy and fetal growth restriction. Although caffeine intake has been implicated in poor pregnancy outcomes [11-15], and in this current study, women who experienced nausea in the first trimester had significantly lower caffeine intakes, nausea and vomiting did not modify associations between fetal growth restriction and maternal caffeine intake or metabolism.

Nausea and vomiting in pregnancy and fetal growth restriction

Most of the studies assessing the relationship between nausea and vomiting in pregnancy and pregnancy outcome have focussed on spontaneous miscarriage and suggest a protective effect of nausea and vomiting in pregnancy [4, 35, 36]. It is however, still well recognised that nutrient restriction throughout pregnancy results in fetal growth restriction [5]. The Dutch Famine Study [37] suggests that the degree of fetal growth restriction is associated with the timing of nutrient restriction. The results from this current study do not show any associations between fetal growth restriction and nausea and vomiting in pregnancy experienced during the first or second trimester.

Nausea and vomiting in pregnancy, fetal growth restriction and maternal caffeine intake

To the best of our knowledge, no previous studies have examined if the association between maternal caffeine intake and fetal growth restriction differs depending on whether the mother experiences nausea and vomiting in pregnancy. Again, studies have tended to explore spontaneous miscarriage as an outcome. Fenster *et al* (1991) found an association between caffeine intakes >300mg/day and spontaneous miscarriage among nauseous women (adjusted OR: 2.10, 95% CI: 1.20 to 3.70), while no association was seen among the non-nauseous women [10]. The study by Wen *et al* (2001) examined the associations between maternal caffeine intake and nausea before pregnancy and during the first trimester and spontaneous miscarriage [21]. They found that maternal caffeine consumption >300mg/day during the first trimester after nausea started might increase the risk of spontaneous miscarriage (RR:5.4, 95% CI:2.0 to 14.6), however these results were only based on four cases. On the contrary, Savitz *et al* did not find nausea or vomiting to be effect modifiers in the relationship between caffeine consumption and

spontaneous miscarriage [20]. Similarly, this current study did not find nausea and vomiting to modify the association between caffeine intake and fetal growth restriction.

Nausea and vomiting in pregnancy, fetal growth restriction and maternal caffeine metabolism

Although caffeine is commonly consumed during pregnancy, its elimination from the blood is slower in pregnant women, probably due to the increased levels of progesterone and estrogen [38]. Caffeine concentrations in the fetus are believed to be in equilibrium with maternal caffeine concentrations [38], however caffeine clearance in infants only approaches adult values at approximately four and a half months after birth [39]. Variations in maternal caffeine metabolism have been associated with fetal growth restriction [40].

Nausea has been associated with increased estrogen levels [4, 25, 26] which in turn have been negatively associated with CYP1A2 activity, the primary enzyme implicated in caffeine metabolism [25, 27]. It may be that the increased risk of spontaneous miscarriages found in the studies discussed above [10, 21] was due to decreased caffeine metabolism among these women and the subsequent fetal concentrations of this xenobiotic. However results from another study found that neither estrogen nor progesterone altered CYP1A2 levels in 35 pregnant women [41]. In this current study, women who experienced nausea and vomiting in pregnancy did not have significantly increased odds of having a slow caffeine metabolism after adjusting for smoking, alcohol, caffeine intake, mothers age and body mass index. There was also no significant

modification of the relationship between caffeine metabolism and fetal growth restriction by symptoms of nausea and vomiting during either the first or second trimesters.

It is evident from this discussion that many studies investigating the relationship between pregnancy symptoms and poor pregnancy outcome, only focus on nausea as the symptom and spontaneous miscarriage as the outcome. It has been suggested that nausea in early pregnancy may be associated with a decreased risk of fetal growth restriction, however nausea later in pregnancy may be more detrimental to the fetus [5, 8]. This study is the first to explore whether nausea and vomiting of pregnancy modifies the relationships between fetal growth restriction, maternal caffeine intake and metabolism.

This current study is not without its limitations however. Regarding the measurements, recall bias may have been introduced as the CATs were completed retrospectively, however as the study was primarily examining the effects of caffeine intake, it is unlikely that participants underreported their pregnancy symptoms. The half-life was measured in the first trimester for some participants and so may not be applicable to the second trimester analyses as it has been reported that the caffeine half-life increases from approximately 5.6 hours in the first trimester to 8.9 hours between 12 and 20 weeks gestation [42]. As the aim of the CAT was to primarily examine the association between maternal caffeine intake and fetal growth restriction, our study included a limited assessment of self-reported nausea and vomiting in pregnancy which is weakly correlated to actual physical symptoms [43]. The CAT did not assess the severity of nausea and vomiting of pregnancy; however the CARE study only recruited low-risk pregnant women unlikely to have experienced hyperemesis. This current study did examine duration of nausea, however only 17% of the sample experienced nausea and vomiting of

pregnancy in both the first and second trimester making it difficult to infer any relationship between duration of nausea and vomiting of pregnancy and fetal growth restriction.

Regarding the sample size, the sample sizes calculated for the CARE study were not designed to examine interactions. While there was a low response rate, the results may not be different from that in the general population as several confounders were considered. While the reasons for recruitment decline were not documented due to lack of ethical approval to do so, it is thought that parts of this study may have been perceived as too invasive hence leading to a low response rate e.g. home visits could take up to one hour to complete. The strengths include the detailed assessment of maternal caffeine intake and examination of maternal caffeine metabolism, which have been relatively unexplored to date [10, 11].

The results from our previous analysis of this study showed an association between caffeine intake and increased risk of fetal growth restriction, however the results from this current analysis show no evidence that this relationship is modified by nausea and vomiting in pregnancy.

A reduction of caffeine intake before and during pregnancy is however, warranted to optimise birthweight [11]. Future studies could examine the role of hormones in pregnancy outcome and the interaction between nausea and vomiting in pregnancy and other pregnancy outcomes.

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Reference List

1. Gadsby R, Barnie-Adshead AM, Jagger C. (1993). A prospective study of nausea and vomiting during pregnancy. *The British Journal of General Practice, 43*, 245-248.

2. Lacroix R, Eason E, Melzack R. (2000). Nausea and vomiting during pregnancy: A prospective study of its frequency, intensity, and patterns of change. *American Journal of Obstetrics and Gynecology, 182*(4), 931-937.

3. O'Brien B, Naber S. (1992). Nausea and vomiting during pregnancy: effects on the quality of women's lives. *Birth, 19*(3), 138-143.

4. Weigel RM, Weigel MM. (1989). Nausea and vomiting of early pregnancy and pregnancy outcome. A meta-analytical review. *British Journal of Obstetrics and Gynaecology, 96*(11), 1312-1318.

5. Huxley RR. (2000). Nausea and vomiting in early pregnancy: its role in placental development. *Obstetrics and Gynecology, 95*(5), 779-782.

6. Flaxman S, Sherman P. (2000). Morning sickness: a mechanism for protecting mother and embryo. *The Quarterly Review of Biology, 75*, 113-148.

7. Stein Z, Susser M. (1991). Miscarriage, caffeine, and the epiphenomena of pregnancy: the causal model. *Epidemiology, 2*(3), 163-167.

8. Griggio MA, Luz J, Gorgulho AA, Sucasas CM. (1997). The influence of food restriction during different periods of pregnancy. *International Journal of Food Sciences and Nutrition, 48*(2), 129-134.

9. Barkai G, Reichman B, Ries L, Lusky A, Lipitz S, Goldman B. (1994). The association between alpha-fetoprotein and beta hCG levels prior to and following chorionic villus sampling in cases that spontaneously miscarried. *Prenatal diagnosis, 14*(9), 793-798.

10. Fenster L, Eskenazi B, Windham GC, Swan SH. (1991). Caffeine consumption during pregnancy and spontaneous abortion. *Epidemiology, 2*(3), 168-174.

11. Boylan SM, Cade JE, Dolby VA, Greenwood DC, Hay AW, Kirk SF, Shires S, Simpson N, Thomas JD, Walker J, White KL, Wild CP. (2008). Maternal caffeine intake during pregnancy and risk of fetal growth restriction: a large prospective observational study. *British Medical Journal, 337*, a2332.

12. Ford RP, Schluter PJ, Mitchell EA, Taylor BJ, Scragg R, Stewart AW. (1998). Heavy caffeine intake in pregnancy and sudden infant death syndrome. New Zealand Cot Death Study Group. *Archives of Disease in Childhood, 78*(1), 9-13.

13. Fortier I, Marcoux S, Beaulac-Baillargeon L. (1993). Relation of caffeine intake during pregnancy to intrauterine growth retardation and preterm birth. *American Journal of Epidemiology, 137*(9), 931-940.

14. Giannelli M, Doyle P, Roman E, Pelerin M, Hermon C. (2003). The effect of caffeine consumption and nausea on the risk of miscarriage. *Paediatric and Perinatal Epidemiology, 17*(4), 316-323.

15. Greenwood DC, Alwan N, Boylan S, Cade JE, Charvill J, Chipps KC, Cooke MS, Dolby VA, Hay AW, Kassam S, Kirk SF, Konje JC, Potdar N, Shires S, Simpson N, Taub N, Thomas JD, Walker J, White KL, Wild CP. (2010). Caffeine intake during pregnancy, late miscarriage and stillbirth. *European Journal of Epidemiology, 25*(4), 275-280.

16. Klebanoff MA, Levine RJ, DerSimonian R, Clemens JD, Wilkins DG. (1999). Maternal serum paraxanthine, a caffeine metabolite, and the risk of spontaneous abortion. *The New England Journal of Medicine, 341*(22), 1639-1644.

17. Furneaux EC, Langley-Evans AJ, Langley-Evans SC. (2001). Nausea and vomiting of pregnancy: endocrine basis and contribution to pregnancy outcome. *Obstetrical and Gynecological Survey, 56*(12), 775-782.

18. Petridou E, Katsouyanni K, Spanos E, Skalkidis Y, Panagiotopoulou K, Trichopoulos D. (1992). Pregnancy estrogens in relation to coffee and alcohol intake. *Annals of Epidemiology, 2*(3), 241-247.

19. Lawson CC, LeMasters GK, Levin LS, Liu JH. (2002). Pregnancy hormone metabolite patterns, pregnancy symptoms, and coffee consumption. *American Journal of Epidemiology, 156*(5), 428-437.

20. Savitz DA, Chan RL, Herring AH, Howards PP, Hartmann KE. (2008). Caffeine and miscarriage risk. *Epidemiology, 19*(1), 55-62.

21. Wen W, Shu XO, Jacobs DR, Jr., Brown JE. (2001). The associations of maternal caffeine consumption and nausea with spontaneous abortion. *Epidemiology, 12*(1), 38-42.

22. Weng X, Odouli R, Li DK. (2008). Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. *American Journal of Obstetrics and Gynecology, 198*(3), 279-8.

23. Srisuphan W, Bracken MB. (1986). Caffeine consumption during pregnancy and association with late spontaneous abortion. *American Journal of Obstetrics and Gynecology, 154*(1), 14-20.

24. Weathersbee PS, Olsen LK, Lodge JR. (1977). Caffeine and pregnancy. A retrospective survey. *Postgraduate Medicine, 62*(3), 64-69.

25. Bernstein L, Depue RH, Ross RK, Judd HL, Pike MC, Henderson BE. (1986). Higher maternal levels of free estradiol in first compared to second pregnancy: early gestational differences. *Journal of the National Cancer Institute, 76*(6), 1035-1039.

26. Jarnfelt-Samsioe A, Eriksson B, Waldenstrom J, Samsioe G. (1985). Some new aspects on emesis gravidarum. Relations to clinical data, serum electrolytes, total protein and creatinine. *Gynecologic and Obstetric Investigation, 19*(4), 174-186.

27. Le Marchand L, Franke AA, Custer L, Wilkens LR, Cooney RV. (1997). Lifestyle and nutritional correlates of cytochrome CYP1A2 activity: inverse associations with plasma lutein and alpha-tocopherol. *Pharmacogenetics, 7*(1), 11-19.

28. Brent RL, Christian MS, Diener RM. (2011). Evaluation of the reproductive and developmental risks of caffeine. *Birth Defects Res B Dev Reprod Toxicol, 92*(2), 152-187.

29. Boylan SM, Cade JE, Kirk SF, Greenwood DC, White KL, Shires S, Simpson NA, Wild CP, Hay AW. (2008). Assessing caffeine exposure in pregnant women. *British Journal of Nutrition, 100*(4), 875-882.

30. Committee on toxicity of chemicals in food consumer products and the environment. (2008). *Statement on the reproductive effects of caffeine.* UK: Food Standards Agency.

31. March of Dimes. (2010). Caffeine in pregnancy. http://www.marchofdimes.com/pregnancy/nutrition_caffeine.html#. Accessed 29-3-2011

32. Gardosi J. (2004). Customized fetal growth standards: rationale and clinical application. *Semininars in Perinatology, 28*(1), 33-40.

33. Office for National Statistics. *Key population and vital statistics 2006*. Great Britain: Palgrave Macmillan, 2008.

34. Gardosi J, Kady SM, McGeown P, Francis A, Tonks A. (2005). Classification of stillbirth by relevant condition at death (ReCoDe): population based cohort study. *BMJ, 331*(7525), 1113-1117.

35. Brandes JM. (1967). First-trimester nausea and vomiting as related to outcome of pregnancy. *Obstetrics and Gynecology, 30*(3), 427-431.

36. Tierson FD, Olsen CL, Hook EB. (1986). Nausea and vomiting of pregnancy and association with pregnancy outcome. *American Journal of Obstetrics and Gynecology, 155*(5), 1017-1022.

37. Lumley L. (1989). Compensatory placental growth after restricted maternal nutrition in early pregnancy. *Placenta, 19,* 105-111.

38. Aldridge A, Bailey J, Neims AH. (1981). The disposition of caffeine during and after pregnancy. *Seminars in Perinatology, 5*(4), 310-314.

39. Aranda JV, Collinge JM, Zinman R, Watters G. (1979). Maturation of caffeine elimination in infancy. *Archives of Disease in Childhood, 54*(12), 946-949.

40. Grosso LM, Rosenberg KD, Belanger K, Saftlas AF, Leaderer B, Bracken MB. (2001). Maternal caffeine intake and intrauterine growth retardation. *Epidemiology, 12*(4), 447-455.

41. Tracy TS, Venkataramanan R, Glover DD, Caritis SN. (2005). Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A Activity) during pregnancy. *American Journal of Obstetrics and Gynecology, 192*(2), 633-639.

42. Nolen GA. The developmental toxicity of caffeine. In: Kalter H, editor. *Issues and Reviews in Teratology*.New York: Plenum Press, 1988: 305-50.

43. Chandra K, Magee L, Koren G. (2002). Discordance between physical symptoms versus perception of severity by women with nausea and vomiting in pregnancy (NVP). *BMC Pregnancy and Childbirth, 25*.

Table 1: Symptoms, caffeine intake, caffeine metabolism, smoking and alcohol intake during the first and second trimester

	First trimester		Second trimester		Both trimesters	
	count	**%**	**count**	**%**	**count**	**%**
Nausea	2030	81	842	33	769	31
Vomiting	1188	47	613	24	490	19
Both nausea and vomiting	1122	45	527	21	426	17
Caffeine intake (mg/day)						
<200	1744	70	1421	74	-	-
>200	736	30	512	26	-	-
Caffeine metabolism[a]						
Fast	-	-	765	50	-	-
Slow	-	-	755	50	-	-
Smoke[a]						
No	1777	74	415	85	-	-
Passive	285	12	17	3	-	-
Yes	339	14	56	12	-	-
Alcohol intake (ml/day??)						
0	581	23	695	36	-	-
0.1-0.5	1127	46	932	48	-	-
≥0.5	772	31	306	16	-	-

[a]Few participants took part in the caffeine challenge test during the first trimester (n=4).

[b] Smoking based on salivary cotinine levels: >5ng/ml (smoker), 1-5ng/ml (passive-smoker), <1ng/ml (non-smoker). Salivary cotinine measurements were taken at recruitment and at 28 weeks gestation.

Table 2: Unadjusted and adjusted odds ratios for fetal growth restriction according to the presence of both nausea and vomiting in pregnancy during the first and second trimesters[a]

	n (%)	Unadjusted OR	(95% CI)	p-value	Adjusted OR	(95% CI)	p-value
Both nausea and vomiting during first trimester	1122 (45%)	0.96	(0.69, 1.33)	0.8	0.96	(0.68, 1.35)	0.8
Both nausea and vomiting during second trimester	527 (21%)	0.93	(0.69, 1.25)	0.6	0.85	(0.53, 1.35)	0.5

[a] Separate models developed for each trimester

[b]Adjusted using the customised growth charts for maternal weight, height, ethnicity, parity, gestational age at delivery, sex of baby, and additional adjustment for smoking status (salivary cotinine concentrations) and alcohol intake.

Table 3: Adjusted odds ratios for fetal growth restriction according to caffeine intake and nausea and vomiting[a] symptoms during pregnancy stratified by trimester

Caffeine intake (mg/day)	n (%)	Adjusted OR (95% CI)	p-value
1st trimester	***1513***		
Without nausea and vomiting	*401*		
<200	266 (66)	1	
≥200	135 (34)	0.90 (0.39,2.11)	
With nausea and vomiting	*1112*		
<200	782 (70)	0.86 (0.51, 1.47)	
≥200	330 (30)	1.21 (0.52, 2.81)	0.8
2nd trimester	***1583***		
Without nausea and vomiting	*1056*		
<200	756 (72)	1	
≥200	258 (24)	0.95 (0.45,2.01)	
With nausea and vomiting <200	*527*		
≥200	369 (70)	1.18 (0.62,2.28)	
	149 (28)	0.86 (0.30,2.44)	0.5

[a] Presence of both nausea and vomiting

[b] Adjusted using the customised growth charts for maternal weight, height, ethnicity, parity, gestational age at delivery, sex of baby, and additional adjustment for maternal caffeine half-life, smoking status (salivary cotinine concentrations) and alcohol intake.

Table 4: Adjusted odds ratios for fetal growth restriction according to caffeine half life and nausea and vomiting[a] symptoms during pregnancy stratified by trimester

Caffeine half-life	n (%)	Adjusted OR (95% CI)	p-value
1st trimester	***1513***		
Without nausea and vomiting	*401*		
Short[c]	123 (50)	1	
Long[c]	123 (50)	1.18 (0.55,2.55)	
With nausea and vomiting	*1112*		
Short	325 (51)	0.95 (0.49,1.82)	
Long	316 (49)	1.00 (0.53,1.88)	0.91
2nd trimester	***1583***		
Without nausea and vomiting	*1056*		
Short	371 (52)	1	
Long	348 (48)	1.38 (0.71,2.66)	
With nausea and vomiting	*527*		
Short	173 (53)	0.85 (0.37,1.95)	
Long	154 (47)	1.31 (0.61,2.81)	0.45

[a] Presence of both nausea and vomiting

[b] Adjusted using the customised growth charts for maternal weight, height, ethnicity, parity, gestational age at delivery, sex of baby, and additional adjustment for maternal caffeine intake, smoking status (salivary cotinine concentrations) and alcohol intake.

[c]Short caffeine half life (≤median value), long half-life ≥median value)