J Clin Endocrin Metab. First published ahead of print November 18, 2008 as doi:10.1210/jc.2008-0380

Cigarette smoking during pregnancy is associated with alterations in maternal and fetal thyroid function

Short title: Smoking, pregnancy & thyroid function

Beverley Shields,[1] Anita Hill,[1] Mary Bilous,[2] Beatrice Knight,[1] Andrew T Hattersley,[1] Rudy W Bilous,[2] Bijay Vaidya[1]

(1) Department of Endocrinology, Peninsula Medical School, Royal Devon & Exeter Hospital, Exeter; (2) Department of Endocrinology, James Cook University Hospital, Middlesbrough, UK

Disclosure statement: The authors have nothing to disclose.

Correspondence:

Dr. B. Vaidya, Department of Endocrinology, Royal Devon & Exeter Hospital, Exeter, EX2 5DW, U.K. Tel: (44) 1392 403831. Fax: (44) 1392 402270. Email: bijay.vaidya@pms.ac.uk

Key words:

Thyroid, pregnancy, smoking, fetus

Word count:

Text: 1920, abstract: 245, tables 3, supplementary tables 2, figures 0

Abstract

Context: Studies in the general population have shown lower serum thyrotropin (TSH) levels in smokers as compared to non-smokers.

Aim: To examine whether smoking is associated with changes in thyroid function of pregnant women and their fetus.

Subjects & methods: We examined the relationship between smoking and thyroid function (serum TSH, FT4 and FT3) in two independent cohorts of pregnant women without a history of thyroid disorder or an overt biochemical thyroid dysfunction: (a) first trimester cohort (median gestation 9 weeks) (n=1428), and (b) third trimester cohort (gestation 28 weeks) (n=927). We also analysed the relationship between maternal smoking and thyroid hormone levels in cord serum of 618 full-term babies born to the women in the third trimester cohort.

Results: In smokers compared to non-smokers, median serum TSH was lower (first trimester cohort: 1.02 mIU/l v 1.17 mIUl/l, p=0.001; third trimester cohort: 1.72 mIU/l v 1.90 mIU/l, p=0.037) and median serum FT3 was higher (first trimester cohort: 5.1 pmol/l v 4.9 pmol/l, p<0.0001; third trimester cohort: 4.4 pmol/l v 4.1 pmol/l, p<0.0001). In both cohorts, serum FT4 in smokers and non-smokers were similar. The prevalence of anti-thyroperoxidase antibodies was also similar in smokers and non-smokers in both cohorts. Cord serum TSH of babies born to smokers was lower than of those born to non-smokers (6.7 mIU/l v 8.1 mIU/l; p=0.009).

Conclusions: Cigarette smoking is associated with changes in maternal thyroid function throughout the pregnancy and in fetal thyroid function as measured in cord blood samples.

Introduction

Optimal maternal thyroid function during pregnancy is important for a successful pregnancy outcome (1). Several factors, including thyroid hormone binding proteins, placental human chorionic gonadotrophin, placental deiodinases and dietary iodine, are known to modulate determinants of maternal thyroid function in pregnancy. Autoimmunity is also known to influence maternal thyroid function. In addition, environmental pollutants, such as dioxins and polychlorinated biphenyls, may affect thyroid function in pregnancy (2). In the general population, lower serum thyrotropin (TSH) levels have been found in smokers (3-10). Several studies, but not all, have also found that smokers have higher serum levels of thyroxine (3,5,7), triiodothyronine (11,12), or both (9). It is not known whether smoking is associated with similar changes in thyroid function in pregnancy. Also, little is known whether maternal smoking during pregnancy is associated with changes in fetal thyroid function. In this study, we have examined the influence of smoking during pregnancy on maternal and fetal thyroid function as measured in cord blood.

Subjects and Methods

Subjects

We analysed the relationship between smoking and thyroid hormone levels in two independent cohorts of pregnant women: one during the first trimester, and one at the beginning of the third trimester.

(a) Middlesbrough cohort:

In 2002-3, TSH, free T4 (FT4) and free T3 (FT3) was analysed in 1466 pregnant women without known thyroid disorders during their first antenatal check-up (13). At

the time of sampling, with a questionnaire, women were asked how many cigarettes they smoked a day, within different categories (Table 1), and whether they stopped smoking within the past 12 months.

Women with overt biochemical hypothyroidism (elevated TSH with low FT4; n=10) and hyperthyroidism (fully suppressed TSH with raised FT4 or FT3; n=25) were excluded. Smoking data were missing in three women. Therefore, 1428 women were included in the analysis. Results of anti-thyroperoxidase antibodies (anti-TPO) were available in 1234 (86.4%).

(b) Exeter cohort:

Women were recruited between 1999-2004 as part of the Exeter Family Study of Child Health (EFSOCH) (14). Blood samples were taken at 28 week gestation, when women were asked by the research midwife whether they smoked and if so, how many a day, within different categories (Table 1). The women were also given a questionnaire to fill in which asked whether they had ever smoked, and if so, how many they smoked one month before their pregnancy and within the first three months of their pregnancy.

TSH, FT4, FT3 and anti-TPO was analysed in stored serum samples of 1001 women without known thyroid disorders. We excluded women with overt biochemical hypothyroidism (n=9) and hyperthyroidism (n=1). Smoking data were missing in 41 women. Therefore, 927 women were included in the analysis. Results of anti-TPO were available in 924 (99.7%). TSH, FT4 and FT3 levels in cord serum samples of 618 full-term babies born to these women were also available.

Socioeconomic status in both cohorts was assessed by Townsend scores based on post codes (15). The local research ethics committees approved the study, and all participants gave informed written consent.

Analysis of thyroid function and thyroid antibodies

Serum TSH, FT4 and FT3 were analysed in both cohorts using the electrochemiluminescent immunoassay, run on the Modular E170 Analyzer (Roche, Burgess Hill, UK). The manufacturers' reference ranges were: Middlesbrough, TSH 0.27-4.2mIU/l, FT4 12-23pmol/l and FT3 4-7.8pmol/l; and Exeter, TSH 0.35-4.5mIU/l, FT4 11-24pmol/l and FT3 3.9-6.8pmol/l. In Middlesbrough, anti-TPO was analysed by a manual semi-quantitative microtitre plate agglutination method (Fujirebio Inc., Tokyo, Japan). A reactive pattern detected at a final dilution of 1 in 1600 or greater was considered positive. In Exeter, anti-TPO was analysed using the competitive immunoassay (Roche, Burgess Hill, UK), and a concentration above 34kIU/l was considered positive.

Statistical analyses

Maternal TSH results were not normally distributed despite various transformations of the data. Therefore, analysis was carried out using non-parametric statistics. For consistency, maternal FT3 and FT4 results were also analysed using non-parametric statistics. Comparisons in thyroid function tests between smokers and non-smokers were carried out using Mann-Whitney U tests. Regression analysis was used to examine associations between smoking and thyroid function tests whilst adjusting for parity and socioeconomic status, and residuals were checked for normality. Cord TSH and FT3 concentrations were also positively skewed, but log transformation of the

data approximated normal distribution. Analysis of variance was used to examine associations between maternal smoking and cord thyroid function tests, whilst adjusting for confounders such as gestational age, low Apgar score and mode of delivery (Caesarean, assisted delivery or normal vaginal birth, added to the ANOVA as dummy variables).

Results

Study populations

Demographic characteristics of the pregnant women are shown in Table 1. The prevalence of smokers was higher in the Middlesbrough cohort ($P<0.0001$). Amongst the non-smokers, 248/1022 in the Middlesbrough cohort and 134/794 in the Exeter cohort had stopped smoking during the pregnancy ('previous smokers').

Effect of smoking during pregnancy on maternal thyroid function

In both cohorts, maternal TSH concentrations were lower in smokers than in non-smokers and FT3 concentrations were higher (Table 2). There was no association between smoking and FT4 concentrations. Removing those with subclinical hypo- or hyperthyroidism made little difference to the results, although in the Exeter cohort, the difference in the TSH concentrations was no longer statistically significant ($p=0.055$). Similarly, removing those who had no anti-TPO status made no difference to the results. In both cohorts, the prevalence of anti-TPO positive women was similar amongst smokers and non-smokers, although median anti-TPO concentration was higher in smokers in the Exeter cohort (Table 2).

When examining only anti-TPO negative women, FT3 concentrations remained higher in the smokers in both cohorts (Table 3). TSH concentrations remained lower in the smokers, however the difference between the smokers and non-smokers was reduced, and the result was only significant in the Middlesbrough cohort.

Adjustment for socioeconomic status and parity weakened the association between smoking and TSH concentrations, but this still remained significant in the Middlesbrough cohort ($p=0.016$) and became borderline significant in the Exeter cohort ($p=0.071$) (Supplementary Table 1). FT3 concentrations remained higher in smokers compared with non-smokers in both cohorts (Middlesbrough, $p=0.007$; Exeter, $p<0.001$).

We analysed, within the non-smokers groups, whether thyroid hormone levels in women who stopped smoking only during the pregnancy ('previous smokers') were different from those in women who did not smoke at all ('long-term non-smokers'). In both cohorts, there were no differences in the thyroid function results between 'previous smokers' and 'long-term non-smokers', except FT4 concentrations were reduced in 'previous smokers' in the Middlesbrough cohort ($p=0.007$) (Supplementary Table 2). In the Exeter cohort, we found no differences in thyroid function tests between those who ceased smoking in the first 12 weeks with those who had never smoked (data not shown).

When examining number of cigarettes smoked per day, we found no effects of a dose response on TSH, FT4, or FT3 concentrations (data not shown).

Effect of paternal smoking on maternal thyroid function

Paternal smoking status was only available in the Exeter cohort. To explore the effect of passive smoking, we compared those families where neither parent smoked (n=590) and those where only the father smoked (n=160). There was no significant difference in maternal TSH or FT4 concentrations between those families where neither parent smoked and those where only the father smoked, but FT3 concentrations were slightly higher in the latter (median (IQR): 4.10pmol/l (3.85, 4.43) v 4.17pmol/l (3.91, 4.55)) (p=0.049).

Effect of maternal smoking on fetal thyroid function

TSH in cord serum samples of babies born to women who smoked during pregnancy was lower than of those born to non-smokers (p=0.008) (Table 2).

Discussion

In this cross-sectional study of two independent cohorts of women at different stages of gestation, we found that women who smoked during pregnancy have lower serum TSH and higher serum FT3 than those who did not smoke. We also found lower TSH concentrations in cord blood of babies born to mothers who smoked during pregnancy. These findings are compatible with the epidemiological studies in the general population, which have also consistently shown low TSH levels in smokers (3-10). Furthermore, several of these studies have shown that smokers have higher serum levels of thyroxine (3,5,7), triiodothyronine (11,12), or both (9).

There are several possible mechanisms by which smoking may affect thyroid hormone levels. Firstly, nicotine or other constituents in cigarette smoke may

stimulate thyroid hormone secretion either directly or through sympathetic activation. However, nicotine infusion in rats did not show any effect on T4, T3 and TSH levels (16). Secondly, thiocyanate (a toxin in the cigarette smoke) may cause intrathyroidal iodine depletion predisposing to increased thyroid nodularity and autonomous thyroid hormone secretion (7). Thirdly, smoking has been shown to be associated with a reduced prevalence of anti-TPO, suggesting that low TSH levels in smokers reflect a decreased prevalence of autoimmune hypothyroidism (8). However, we found similar prevalence of anti-TPO in smokers and non-smokers in our two cohorts (Table 2), and the influence of smoking on thyroid hormone levels were persistent when only anti-TPO negative women were analysed (Table 3). Finally, smoking may alter thyroid hormone levels through its effect on deiodinase activity. Nicotine has been shown to increase type 2 deiodinase activity in cultured rat brain glial cells (17). Our finding of raised FT3 (and not FT4) amongst smokers may be explained by the increased type 2 deiodinase activity associated with smoking, and suggests that this smoking-related change in thyroid function is more likely to be a peripheral phenomenon than thyroid stimulation.

In both cohorts, we found that women who stopped smoking during the pregnancy ('previous smokers') had thyroid hormone levels similar to non-smokers. Similarly, in the Exeter cohort, women who stopped smoking in the first 12 weeks of pregnancy had thyroid hormone levels similar to non-smokers. Consistent with these findings, a study in the general population has shown that smoking cessation is associated with a reversal of smoking-related changes in thyroid hormone levels(10). Together, these findings suggest that the smoking-related changes in thyroid hormone levels are rapidly reversible in both non-pregnant and pregnant individuals.

This study shows, for the first time, that smoking during pregnancy is also associated with changes in fetal thyroid function as measured in cord blood at the time of delivery. Cord serum TSH of babies born to women who smoked during pregnancy was lower than those born to non-smoker mothers. Previous studies did not find a correlation between maternal smoking and cord thyroid function (18-20), but the studies were smaller and were not sufficiently powered to detect the size of change in our study. We did not find an association between maternal smoking and cord serum FT3. A possible explanation for this finding is that the mild influence of smoking on FT3 (possibly mediated by an increased type 2 deiodinase activity) is counter-balanced by very active placental type 3 deiodinase enzyme (1). One limitation of our study was that we did not corroborate smoking status using laboratory tests; however, a recent study of pregnant women has shown that self-reported smoking information is reliable (21).

There is increasing evidence that even mild abnormalities in maternal thyroid hormone levels during pregnancy are associated with both maternal and fetal adverse outcomes (1). Therefore, finding of factors, such as smoking, which may modulate thyroid hormone levels in pregnancy is important. Although, the magnitude of effect of smoking on thyroid function in our study was small, the smoking-related changes in the thyroid function extended to the fetus, suggesting these changes could have a biological impact on the fetus. Further studies are needed to analyse whether the changes in the maternal and fetal thyroid function associated with maternal smoking results in any adverse pregnancy outcomes.

Acknowledgements

We gratefully acknowledge the help of Sony Anthony and Stewart Hutchison in data collection, John Drury and Maurice Salzmann in the analysis of thyroid function, Elaine Hall and Barbara Woodward in organizing the databases, and Sath Nag in the analysis of socioeconomic status in the Middlesbrough cohort. Thanks also to Rod Taylor for statistical advice. This study was supported by The Wellcome Trust, Endocrine Research Fund, and The Research & Development Directorate, Royal Devon & Exeter NHS Foundation Trust. BS, AH and BK are employed as core staff within the NIHR-funded Peninsula Clinical Research Facility.

References

1. **Glinoer D** 1997 The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology. Endocr Rev 18:404-433
2. **Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lutkeschipholt IJ, Van der Paauw CG, Tuinstra LG, Brouwer A, Sauer PJ** 1994 Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. Pediatr Res 36:468-473
3. **Eden S, Jagenburg R, Lindstedt G, Lundberg PA, Mellstrom D** 1984 Thyroregulatory changes associated with smoking in 70-year-old men. Clin Endocrinol (Oxf) 21:605-610
4. **Ericsson UB, Lindgarde F** 1991 Effects of cigarette smoking on thyroid function and the prevalence of goitre, thyrotoxicosis and autoimmune thyroiditis. J Intern Med 229:67-71
5. **Fisher CL, Mannino DM, Herman WH, Frumkin H** 1997 Cigarette smoking and thyroid hormone levels in males. Int J Epidemiol 26:972-977
6. **Georgiadis E, Papapostolou C, Korakis T, Evagelopoulou K, Mantzoros C, Batrinos M** 1997 The influence of smoking habits on thyroid gland volume: an ultrasonic approach. J R Soc Health 117:355-358
7. **Knudsen N, Bulow I, Laurberg P, Ovesen L, Perrild H, Jorgensen T** 2002 Association of tobacco smoking with goiter in a low-iodine-intake area. Arch Intern Med 162:439-443
8. **Belin RM, Astor BC, Powe NR, Ladenson PW** 2004 Smoke exposure is associated with a lower prevalence of serum thyroid autoantibodies and thyrotropin concentration elevation and a higher prevalence of mild thyrotropin concentration suppression in the third National Health and Nutrition Examination Survey (NHANES III). J Clin Endocrinol Metab 89:6077-6086
9. **Jorde R, Sundsfjord J** 2006 Serum TSH levels in smokers and non-smokers. The 5th Tromso study. Exp Clin Endocrinol Diabetes 114:343-347
10. **Asvold BO, Bjoro T, Nilsen TI, Vatten LJ** 2007 Tobacco smoking and thyroid function: a population-based study. Arch Intern Med 167:1428-1432
11. **Christensen SB, Ericsson UB, Janzon L, Tibblin S, Melander A** 1984 Influence of cigarette smoking on goiter formation, thyroglobulin, and thyroid hormone levels in women. J Clin Endocrinol Metab 58:615-618
12. **Karakaya A, Tuncel N, Alptuna G, Kocer Z, Erbay G** 1987 Influence of cigarette smoking on thyroid hormone levels. Hum Toxicol 6:507-509
13. **Vaidya B, Anthony S, Bilous M, Shields B, Drury J, Hutchison S, Bilous R** 2007 Detection of thyroid dysfunction in early pregnancy: Universal screening or targeted high-risk case finding? J Clin Endocrinol Metab 92:203-207
14. **Knight B, Shields BM, Hattersley AT** 2006 The Exeter Family Study of Childhood Health (EFSOCH): study protocol and methodology. Paediatr Perinat Epidemiol 20:172-179
15. **Townsend P, Phillimore P, Beattie A** 1988 Health and Deprivation: Inequality in the North. London: Croom-Helm
16. **Colzani R, Fang SL, Alex S, Braverman LE** 1998 The effect of nicotine on thyroid function in rats. Metabolism 47:154-157
17. **Gondou A, Toyoda N, Nishikawa M, Yonemoto T, Sakaguchi N, Tokoro T, Inada M** 1999 Effect of nicotine on type 2 deiodinase activity in cultured rat glial cells. Endocr J 46:107-112

18. **Chanoine JP, Toppet V, Bourdoux P, Spehl M, Delange F** 1991 Smoking during pregnancy: a significant cause of neonatal thyroid enlargement. Br J Obstet Gynaecol 98:65-68
19. **Gasparoni A, Autelli M, Ravagni-Probizer MF, Bartoli A, Regazzi-Bonora M, Chirico G, Rondini G** 1998 Effect of passive smoking on thyroid function in infants. Eur J Endocrinol 138:379-382
20. **Herbstman J, Apelberg BJ, Witter FR, Panny S, Goldman LR** 2008 Maternal, infant, and delivery factors associated with neonatal thyroid hormone status. Thyroid 18:67-76
21. **George L, Granath F, Johansson AL, Cnattingius S** 2006 Self-reported nicotine exposure and plasma levels of cotinine in early and late pregnancy. Acta Obstet Gynecol Scand 85:1331-1337

Table 1. Demographic characteristics of pregnant women

			Middlesbrough cohort	Exeter cohort	P-value
N			1428	927	
Mean (SD) maternal age at recruitment			27.0 (6.0)	30.3 (5.3)	<0.0001
Median (IQR) gestational age at recruitment (weeks)			9 (8, 11)	28*	<0.0001
Ethnicity:					
Whites (%)			1314 (92)	927 (100)	
South Asian (%)			50 (3.5)	-	
Other races (%)			48 (3.4)	-	
Not known (%)			16 (1.1)	-	
Smokers (%)			406 (28.4)	133 (14.3)	<0.0001
Number of cigarettes smoked (n %)**:					
	Middlesbrough	Exeter			
1	Occasional	1-4 a day	19 (1.3)	35 (3.8)	
2	1-5 a day	5-9 a day	112 (7.8)	38 (4.1)	
3	6-10 a day	10-14 a day	161 (11.3)	32 (3.5)	
4	11-20 a day	15-19 a day	99 (6.9)	17 (1.8)	
5	-	20+ a day	-	11 (1.2)	
6	>20 a day	-	15 (1.1)	-	

* All women in the Exeter cohort recruited at 28 weeks gestation so no IQR given

**Categorisation of number of cigarettes smoked different for Middlesbrough and Exeter cohorts

SD, standard deviation; IQR, interquartile range

Table 2. Effect of maternal smoking during pregnancy on maternal and fetal thyroid hormone levels

	Smokers	**Non-smokers**	**P-value**
Maternal thyroid function in the first trimester (Middlesbrough cohort)*			
N	406	1022	
TSH (mIU/l)	1.02 (0.72, 1.42)	1.17 (0.74, 1.76)	**0.001**
FT3 (pmol/l)	5.1 (4.7, 5.7)	4.9 (4.5, 5.5)	**<0.0001**
FT4 (pmol/l)	14.6 (13.3, 15.9)	14.6 (13.2, 16.1)	0.90
Anti-TPO (n positive)	24/348 (6.9%)	65/886 (7.3%)	0.79
Maternal thyroid function in the third trimester (Exeter cohort)*			
N	133	794	
TSH (mIU/l)	1.72 (1.32, 2.23)	1.90 (1.38, 2.52)	**0.037**
FT3 (pmol/l)	4.35 (4.01, 4.66)	4.13 (3.86, 4.43)	**<0.0001**
FT4 (pmol/l)	12.00 (11.29, 13.13)	12.03 (11.16, 12.98)	0.72
Anti-TPO (n positive)	10/133 (7.5%)	51/791 (6.4%)	0.64
Anti-TPO concentration (kIU/l)***	10.66 (8.50, 12.93)	9.52 (7.57, 12.07)	**0.002**
Thyroid hormone levels in cord blood (Exeter cohort)*			
N	90	528	
TSH (mIU/l)	6.65 (5.8, 7.6)	8.02 (7.51, 8.56)	**0.008**
FT3 (pmol/l)	1.96 (1.86, 2.08)	1.90 (1.85, 1.95)	0.26
FT4 (pmol/l)	14.16 (13.98, 14.35)	14.52 (14.14, 14.91)	0.07

*Maternal values are shown as median (interquartile range) for TSH, FT4, FT3 and anti-TPO concentration, and numbers positive/total numbers tested (%) for anti-TPO.

**Fetal thyroid hormone levels (only available in the Exeter cohort) are adjusted for gestational age, mode of delivery and low Apgar score at birth. Values are shown as (geometric) mean and 95% CI for TSH and FT3, and mean (95% CI) for FT4

***Anti-TPO concentration available only for the Exeter cohort.

Table 3. Effect of smoking on thyroid hormone levels in the anti-TPO negative pregnant women

	Smokers	**Non-smokers**	**P-value**
First Trimester (Middlesbrough cohort)			
N	320	809	
TSH (mIU/l)	0.98 (0.7, 1.38)	1.12 (0.72, 1.70)	**0.005**
FT3 (pmol/l)	5.2 (4.7, 5.7)	4.9 (4.4, 5.5)	**<0.001**
FT4 (pmol/l)	14.6 (13.4, 16.0)	14.6 (13.2, 16.1)	0.557
Third trimester (Exeter cohort)			
N	121	740	
TSH (mIU/l)	1.72 (1.32, 2.23)	1.86 (1.36, 2.49)	0.105
FT3 (pmol/l)	4.35 (4.02, 4.69)	4.13 (3.86, 4.44)	**<0.001**
FT4 (pmol/l)	12.03 (11.16, 13.46)	12.05 (11.16, 12.99)	0.676

Values are shown as median (interquartile range) for TSH, FT4 and FT3.