Breast feeding and smoking hygiene:

Major influences on cotinine in urine of smokers' infants

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SUMMARY The determinants of urine cotinine levels were studied in a group of 101 infants aged 3 months, including 79 infants whose mothers were current smokers. At a pre-arranged home visit the infants' mothers completed an interviewer-administered questionnaire, and samples of maternal urine and breast milk and infants' urine were collected. Cotinine and nicotine levels were determined by gas-liquid chromatography. Infant urine cotinine levels ranged from 0 to 140 µg/l (0–1120 ng cotinine/mg creatinine). A linear dose response relation between mother's smoking rate and infant urine cotinine level was observed among breast-fed infants ($r=0.79$, $p<0.001$). The relation was weaker among infants fed by both breast and bottle ($r=0.56$, $p=0.01$) and was not apparent among bottle-fed infants ($r=0.15$, $p=0.16$). In addition to mode of feeding and mother's smoking rate, mother's smoking "hygiene" (assessed by the reported frequency of smoking while feeding and with infant in same room) was independently associated with infant urine cotinine level. Father's smoking pattern and exposure to smoke outside the household did not relate significantly to infant cotinine levels. We conclude that when mothers smoke, breast feeding is the principal determinant of cotinine in infants' urine. It is likely that most of this cotinine comes from cotinine in mothers' breast milk, but further research is needed to establish how much nicotine is ingested by breast-fed infants of mothers who smoke, and to investigate possible health effects.

There is growing evidence of health risks due to passive smoking, especially in infancy and early childhood.^{1,2} Accurate biochemical measures of passive smoking are needed to identify these risks precisely, and to quantify the benefits of any anti-smoking intervention. Previous work has shown that cotinine, a major metabolite of nicotine, can be measured in blood, saliva, and urine and is a better indicator of exposure to tobacco smoke than is carbon monoxide, nicotine or thiocyanate.^{3,4} However, more information is required before cotinine can be used as a quantitative guide to exposure.

A number of factors are likely to influence cotinine levels in urine. One study has already shown a dose response relation between amount smoked by parents and levels of cotinine in urine and saliva of infants.⁵ Mode of infant feeding may be a very important influence, since it has long been known that nicotine is secreted in the breast milk of mothers who smoke.⁶ However it is not known how large a contribution breast milk nicotine and cotinine make to cotinine levels in infants, compared with that from inhaled tobacco smoke. Style of smoking around the baby

may influence exposure. Some parents often smoke while in close contact with their children (for example, while feeding or together in enclosed spaces), while other parents make a deliberate effort to avoid smoking in the presence of their children.

It has been shown in active smokers that manner of smoking is a more important determinant of nicotine intake than number of cigarettes smoked.⁷ Critical factors include butt length, depth of inhalation of smoke, and interval between puffs. It is not clear exactly how these variables influence the composition of side-stream smoke. It is possible, however that smokers of high nicotine yield cigarettes who puff rather than inhale, and smoke their cigarettes right to the butt, increase the exposure of their infants to nicotine.

There is some indirect evidence that the sex and age of the infant may independently affect observed levels of cotinine. Among active smokers women metabolise nicotine more slowly than do men, so that levels of nicotine in body fluids tend to be higher for the same number of cigarettes smoked.⁸ In a study of adult non-smokers⁹ it was found that women excreted more

nicotine and less cotinine than did men after intravenous injection of a standard dose of nicotine. In the case of infants, age may be important. The urine cotinine half-elimination time in neonates is two to three times that in adults,¹⁰ and it is not known whether it is days, weeks or even months before infants develop the capacity fully to metabolise nicotine.

The purpose of our investigation was to measure the contribution of these factors (with the exception of age) to levels of cotinine in the urine of smokers' infants. All infants were assessed three months after birth.

Methods

The participants in the study included 99 women and 101 infants (including two pairs of twins) involved in a trial of a health education programme to reduce passive smoking (unpublished). The study included women who had smoked during pregnancy and who gave birth at the Queen Victoria Hospital in Adelaide, South Australia, between June and September 1984. These women were first contacted in hospital shortly after the birth and were then visited at home three months later, when a questionnaire was completed and samples were collected for biochemical analysis.

The questionnaire administered on the home visit sought information on both amount of smoking and perceived exposure of infants to tobacco smoke. Smoking rates were assessed by the question, "How many cigarettes a day do you (or your partner) smoke, on average?". Women were also asked to estimate the number of cigarettes that they and other adults had smoked in the house in 48 hours prior to the visit. Exposure of infants to tobacco smoke, or smoking "hygiene", was assessed by questions on how frequently the baby was in the same room when the mother or father was smoking at home, and whether parents smoked while feeding or holding the baby. (Women were asked to choose between "usually", "sometimes", and "never" in answering these questions.)

Urine specimens were collected from infants at the time of the three month home visit using adhesive urine collection bags surrounded by cotton wool and plastic wrap to catch leakage. Specimens of urine and breast milk were also taken from mothers. Where possible at least 2 ml of each fluid was collected. Specimens were placed in a sterile container, frozen, and then transported to the laboratory. The samples were analysed by gas-liquid chromatography using a method described by Kogan *et al.*¹¹ The limits of determination were 5 and 7.5 µg/l for nicotine and cotinine respectively. Calibration curves covering two concentration ranges were used (nicotine 5–50 µg/l and 50–1000 µg/l, cotinine 7.5–50 µg/l and 50–1000

µg/l), and in all cases the calibration data were linear. Interassay variability was less than 7%. Urinary creatinine concentrations were determined by the Jaffé rate method, using reagents supplied by Beckman, on an Astra routine analyser. Urinary pH was determined using a Beckman 44 pH meter, previously calibrated using standard pH solutions.

Data analysis was by Pearson correlation coefficients, two-tailed *t* tests for independent samples, and one way analysis of variance, as appropriate. Multiple linear regression models were used to find important predictors of infant urinary cotinine.

The urinary cotinine concentration was selected as the principal outcome measure, rather than the urinary cotinine:creatinine ratio. This was partly to make full use of the available data. Creatinine measures could not be made in 19% of specimens from infants, as the entire volume of the specimen was required for the cotinine assay. However, in the remainder of cases the infant urine cotinine correlated closely ($r = 0.80$, $p < 0.001$) with the cotinine:creatinine ratio. The statistical analyses presented in this report were repeated with the urinary cotinine:creatinine ratio as the outcome measure, and the results did not differ in any consistent or substantial manner from those reported here.

Another consequence of the small volumes (less than 2 ml) of urine collected from some infants was that low cotinine concentrations often could not be measured precisely. The best estimate of the cotinine level in 18 (17%) of the urine specimens collected from infants was a range of 1–20 µg/l. To make full use of the remaining 83% of findings and retain the information held in the 17% "indeterminate low" measurements, the distribution of these measurements was assumed to be symmetrical around the midrange point, and they were assigned the value of 10 µg/l in the statistical analysis. The usual error degrees of freedom for each test were reduced by one less than the number of cases whose cotinine measurement was treated in this manner. This technique leads to more conservative hypothesis testing procedures than if the constraints on the data were ignored.

Results

Levels of cotinine in the infants' urine ranged from 0 to 140 µg/l. Univariate analysis (table 1) showed that the variables most closely related to infant urinary cotinine concentration were: amount smoked by mother; mode of feeding; presence or absence of other smokers in the household besides the mother; and the two measures of maternal smoking "hygiene"—frequency of smoking in same room as infant, and mothers smoking habit while feeding. Sex of infant,

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Table 1 Mean infant urinary cotinine by maternal smoking, mode of feeding, and other possible influences on exposure

	Mean infant urinary cotinine (µg/l)	P
Number of cigarettes smoked by mother in previous 48 h:		
0 (n=20)	2.1	0.001 +
1-20 (n=45)	18.2	
21+ (n=36)	24.5	
Mode of feeding:		
Breast (n=38)	22.2	0.06 +
Mixed (n=16)	22.1	
Bottle (n=47)	11.7	
Other smokers in household besides the mother:		
Yes (n=80)	20.1	0.03*
No (n=21)	6.3	
Mother smokes while feeding infant:		
Usually (n=5)	47.2	0.02 +
Sometimes (n=14)	26.7	
Never (n=60)	18.0	
Infant in same room when mother smokes:		
Usually (n=27)	25.8	0.01 +
Sometimes (n=43)	18.9	
Never (n=9)	19.9	
Sex of infant:		
Male (n=48)	16.2	0.64*
Female (n=53)	18.3	
Mother usually inhales deeply while smoking:		
Yes (n=46)	17.8	0.81*
No (n=55)	16.8	
Mother usually smokes cigarette to the butt:		
Yes (n=41)	14.1	0.21*
No (n=60)	19.5	

+ one way analysis of variance

* t test

inhalation of cigarette smoke, and butt length did not relate to levels of cotinine observed in infants' urine. Other factors that were tested, but did not show any association with infant urinary cotinine, included: nicotine yield of the brand of cigarette usually smoked by mother; number of cigarettes smoked by other adults in the household; father's smoking "hygiene"; presence or absence of exposure to cigarette smoke outside the house in previous 48 hours; urine pH; and time of urine collection.

There was no evidence that mothers seriously under-reported the amount that they smoked. Twenty two women claimed to be ex-smokers: cotinine was not detected in urine samples from 16 of these women, and levels in four others were consistent with passive smoking only (being less than 50 µg/l)³. The two women who reported themselves to be ex-smokers, and who had urine cotinine levels greater than 50 µg/l, were categorised as light smokers (1-20 cigarettes in previous 48 hours), but were excluded from the

regression analyses. In general, levels of cotinine in maternal urine correlated strongly with reported smoking rates ($r=0.64$, $p<0.001$).

In table 2, breast milk nicotine and cotinine levels are shown in relation to maternal smoking rate. While cotinine was detected in the breast milk of all smoking mothers with one exception, nicotine was not detected in 13 samples of breast milk, or 48% of samples from smoking mothers.

Mother's smoking habit while feeding, and the presence or absence of other smokers in the household besides the mother, were each closely related to the number of cigarettes smoked by the mother in the previous 48 hours. Women who usually smoked while feeding their infant smoked more heavily than women who never smoked while they were feeding (mean numbers of cigarettes were 70.6 and 20.3 respectively, $p<0.01$ (t test)). Women who lived with other smokers also smoked more cigarettes, on average, than women living in households containing no other smokers (means 25.9 and 11.5, $p<0.01$ (t test)).

Although breast-fed infants had significantly higher mean urinary cotinines than bottle-fed infants, mothers who breast-fed tended to be lighter smokers (the mean number of cigarettes smoked in the previous 48 hours by mothers who were breast feeding was 22.5 compared with a mean of 34.7 in the bottle-feeding group, $p=0.01$ (t test)). According to the smoking "hygiene" responses, breast-feeding mothers were also less likely to expose their infants to tobacco smoke, although this difference was not statistically significant ($\chi^2_{2df}=2.2$, $p=0.34$).

We next examined separately the relation between mother's smoking rate and infant urine cotinine concentration for each mode of feeding (figure). Simple linear regression models applied to the data demonstrated that mother's smoking rate was most strongly related to infant urinary cotinine in the breast-fed group ($r=0.79$, $p<0.001$). Among infants who were fed by both breast and bottle, the correlation

Table 2 Mean breast milk nicotine and cotinine and infant urine cotinine levels ($\pm$ SD), by mother's smoking rate. Breast-fed mothers and infants only.

	Number of cigarettes smoked by mother in previous 48 h		
	0 (n=11)	1-20 (n=20)	21+ (n=7)
Mean levels (µg/l) of:			
(a) Breast milk nicotine	0 (± 0)	8.3 (± 13.0)	32.6 (± 26.6)
Cotinine	0 (± 0)	84.4 (± 93.3)	234.0 (± 110.8)
(b) Infant urine cotinine	0.9 (± 3.0)	22.0 (± 19.9)	56.0 (± 42.0)

Infant urine cotinine concentrations (C), by number of cigarettes smoked by mother in previous 48 hours (S), by mode of feeding

was weaker ($r=0.56$, $p=0.01$), and among infants who were bottle-fed alone cotinine concentrations were not significantly related to mother's smoking rate ($r=0.15$, $p=0.16$). The difference between the regressions in the breast- and bottle-fed groups was highly statistically significant ($z=4.06$, $p<0.001$). The difference between the bottle-fed and mixed feeding groups was not statistically significant at the 5% level ($z=1.35$, $p=0.09$).

Variables were then entered into multiple regression models.¹² The outcome variable (infant urine cotinine concentration) was log transformed to achieve a normal distribution of residuals in the regression analysis. Breast- and bottle-fed infants were examined separately.

In studying the breast-fed infants, the independent variables included were: the number of cigarettes smoked by mother in previous 48 hours; the two variables describing mother's smoking "hygiene"; breast milk nicotine and cotinine levels; the presence or absence of other smokers in the household; and the presence or absence of exposure to cigarette smoke outside the household in the previous 48 hours. We applied a stepwise linear regression technique, and the variables selected at the 5% probability level were mother's smoking rate and mother's smoking "hygiene" while the infant was in the same room (table 3).

To examine the influence of breast milk nicotine and cotinine on infant urine cotinine levels, the analysis

was repeated, omitting mother's smoking rate because of the high correlation between this variable and milk nicotine and cotinine. In the modified analysis milk cotinine was found to be a better predictor of infant's urine cotinine than was milk nicotine (R^2 change 0.57 and 0.01, $p=0.01$ and 0.89 respectively). This finding was largely unaffected by the order of entry of milk nicotine and cotinine into the model.

Table 3 Variables selected in stepwise multiple regression; outcome is infant urine cotinine concentration

Step*	Variable	Partial regression coefficient	R ² change	Significance of R ² change
(A) Breast-fed infants				
1	Number of cigarettes smoked by mother in previous 48 h	0.660	0.619	0.0001
2	Infant in same room when mother smokes: 1 = usually 2 = sometimes 3 = never	-0.550	0.063	0.019
(B) Bottle-fed infants				
1	Mother smokes while feeding infant: 1 = usually 2 = sometimes 3 = never	-0.572	0.087	0.044

* Probability of F-to-enter < 0.05

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The bottle-fed infants were examined in the same manner, excluding milk nicotine and milk cotinine from the independent variables. The only variable selected in the stepwise analysis at the 5% probability level was mother's smoking "hygiene" while feeding (table 3).

In both the bottle-fed and breast-fed groups the same variables were selected in the regression analyses when mother's urine cotinine level was entered in place of mother's reported smoking rate.

Discussion

In our study population, women who were breast feeding their infants at three months were relatively light smokers and reported greater smoking "hygiene" than women who were bottle feeding, yet their infants had considerably higher levels of cotinine in the urine.

Cotinine in infants' urine may come from three sources: nicotine inhaled by passive smoking; nicotine ingested with breast milk; and maternal cotinine which is ingested with breast milk and excreted by the infant unchanged. Our results, shown in the figure, indicate that when mothers smoke and breast-feed, breast milk is a more important source of cotinine than is passive smoking. (The intermediate slope of the regression line for the mixed feeding group is consistent with the smaller volumes of breast milk consumed by these infants.)

The relative levels of cotinine and nicotine we observed in breast-milk suggest that most of the cotinine in breast-fed infants' urine is exogenous (that is, maternal cotinine). Nicotine is concentrated in breast milk to levels two to three times higher than in plasma, but in spot samples the ratio of breast milk cotinine to nicotine is generally 5:1 or higher.¹³ The results of the regression analyses in the present study also indicate that milk cotinine may be a more important determinant of infant urine cotinine than is breast milk nicotine. It is, however, necessary to be cautious about the conclusions of studies such as ours, which are based on samples of breast milk taken at a single point in time. While cotinine levels in breast milk change little over periods of up to 4 hours without smoking, the half-life of nicotine in breast milk is only 90 minutes.¹³

The short half-life of nicotine in breast milk is the most likely reason for our finding that nicotine was not detected in almost half the samples of breast milk from mothers who smoked. It means also that spot samples are unlikely to provide accurate estimates of the total amount of nicotine ingested by a breast-fed infant. Studies based on 24 hour collections of breast milk would be more helpful, but no such data have yet been published. Nevertheless there is other evidence that breast feeding may contribute to the nicotine exposure

of infants. In a hospital-based survey, Luck and Nau found that neonates who were exposed to cigarette smoke products only by way of breast milk had urinary cotinine and nicotine excretion rates which were significantly greater than those of non-smoking controls, and were less than, but overlapped with, the range of excretion rates observed in older infants exposed to passive smoking alone.¹⁴

Cotinine is pharmacologically inactive,¹⁵ and it is unlikely that cotinine in breast milk has any adverse effects on infants' health. It is not known whether the quantities of nicotine commonly transmitted through breast milk do any harm. Nicotine which is ingested in aqueous solution is largely metabolised in the liver before reaching the systemic circulation.¹⁶ We know of no experimental work on the absorption and metabolism of nicotine in breast milk, where, due to its high lipid solubility, nicotine is likely to be contained chiefly in the fat particles.

Acute nicotine toxicity in breast-fed infants has been reported,¹⁷ but there are no data on how frequently this occurs. Early work, which concluded that it was safe for mothers who were light to moderate smokers (less than 15 cigarettes per day) to breast feed, was based on the weight adjusted dose of nicotine needed to produce acute toxicity in adults.⁶ However, it seems possible from animal studies¹⁸ that infants may be much less tolerant of nicotine than expected, especially in the first months after birth. Subtoxic doses of nicotine affect many important physiological functions, including vasomotor tone, thermoregulation, and lipid metabolism.¹⁹ It is not known what long term effects, if any, there may be in infants chronically exposed to low levels of nicotine.

Epidemiological studies have found the principal health risks from parental smoking to be an increase in lower respiratory illness.²⁰ This may be due to other substances than nicotine in cigarette smoke, although nicotine has been shown *in vitro* to have a specific inhibitory effect on immune function²¹ which could lead to increased susceptibility to respiratory infections. In our study, nicotine served as a marker of exposure to smoke (and one which is likely to underestimate exposure to vapour phase components of tobacco smoke).²² Other factors in side-stream smoke can affect the respiratory tract. For example, formaldehyde, acrolein, and other respiratory irritants inhibit alveolar macrophages²³ and increase permeability of the respiratory tract mucosa.²⁴ These substances are present, together with nicotine, in tobacco smoke but have not been demonstrated in breast milk. Further studies are needed to determine whether other smoke products in addition to nicotine and cotinine are present in breast milk, and whether these substances influence the health of breast-fed infants.

The effects of smoking on breast milk may be important for health education programmes intended to reduce smoking among parents. (Such programmes have traditionally presented information on indirect and delayed effects of smoking, which are generally weak motivating factors for behaviour change.) Apart from mode of feeding and amount smoked by the mother, the other major influence on infant urine cotinine levels in this study was mothers' smoking habit while feeding her infant, and frequency of smoking with her infant in the same room. It follows that, if heeded, advice to mothers to avoid smoking around their infant could reduce children's exposure to nicotine, even if this advice did not lead to cessation of smoking. The results showed that smoking by other adults in the household contributed little to infants' cotinine levels. This finding fits with the results of epidemiological studies, which have found risk of respiratory illness in infancy related to smoking by infant's mother but not to smoking habit of father.²

We cannot explain the absence, among the bottle-fed infants, of a relation between maternal smoking rate and infant urine cotinine level, as was reported by Greenberg *et al.*⁵ This may be due to the more sensitive radioimmunoassay method of analysis used by Greenberg *et al.* or, at least in part, to inaccuracies in smoking rates reported by mothers in our study. When we examined maternal urine cotinine level in place of self-reported smoking rate we found a weak correlation with infant urine cotinine level ($r=0.33$, $p=0.01$). Other factors included in this study—manner of smoking, sex of infant, exposure to cigarettes outside the house, time of sampling, and urine pH—were not found to be related to infant urine cotinine levels. Further research is needed to show whether the numbers in this study were too small to make significant non-linear relations apparent, or whether there are important interaction effects in addition to that between maternal smoking and mode of feeding.

While previous studies in this area have frequently reported only the urinary concentration of cotinine,²⁵ others have presented their findings in terms of the cotinine:creatinine ratio, to allow for any influence of urine flow on urinary cotinine levels.²⁶ We repeated the multivariate analyses in this study, using the urinary cotinine:creatinine ratio as an alternative outcome variable. (The ratio data were obtained from 82 infants and ranged from 0 to 1720 ng cotinine:mg creatinine.) The results were very similar to those obtained previously. The same three factors shown in table 3 were selected in the stepwise regression models as significant independent influences on urinary cotinine excretion. To check whether recoding the indeterminate cotinine values had greatly influenced the analysis, we repeated the multiple regressions

using aggregated cotinine data (0, 1–20 ng/ml, 21–40 ng/ml, 41–60 ng/ml, etc.) as the outcome variable. Again the results did not differ substantially from those shown in table 3.

In our total study population of infants approximately 60% of the variance between individuals in cotinine concentrations could not be explained statistically with the independent variables we had selected. This may reflect in part variation between individuals in rate of nicotine metabolism.²⁷ Important questions for future studies include the amount of nicotine ingested in breast milk, its effects on infant health, and the metabolism of nicotine in infants.

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