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Nicotine and cotinine concentrations in serum and milk of nursing smokers

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1 Analysis of 44 milk samples from 23 nursing smokers revealed that there was a linear correlation between nicotine concentrations in serum and in milk ($r = 0.70$). The nicotine concentrations in milk were considerably higher than the corresponding serum concentrations: milk/serum concentration ratio = 2.92 ± 1.09; ($n = 44$).
2 There was also a linear correlation between the cotinine concentrations in serum and in milk ($r = 0.89$). The cotinine concentrations in milk were lower than the corresponding serum concentrations: milk/serum concentration ratio = 0.78 ± 0.19; ($n = 44$).
3 The direct comparison between the half-lives of nicotine in serum and in milk was possible in five nursing smokers. The half-life of nicotine in milk was determined in four additional smoking mothers. The half-life of nicotine in milk $t_{1/2} = 97 \pm 20$ min slightly exceeded the half-life of nicotine in serum $t_{1/2} = 81 \pm 9$ min; the difference between these two values was not statistically significant ($P > 0.05$).
4 Cotinine concentrations remained fairly consistant during a 4 h interval without smoking.

Keywords nicotine cotinine smoking milk serum

Introduction

A number of studies indicate that 20–35% of nursing mothers are smokers (Kuzma & Kissinger, 1981; The health consequences of smoking, US Department of Health, Education and Welfare, 1973). These smoking mothers, like all smokers, inhale thousands of different substances with every puff, including numerous toxic substances (Schmeltz *et al.*, 1975). Little is known about the content of exogenous and potentially harmful substances in milk derived from tobacco smoke.

In a number of earlier studies nicotine concentrations have been assayed in milk by semi-quantitative bioassay techniques (Emanuel, 1932; Hatcher & Cosby, 1927; Perlman *et al.*, 1942; Thompson, 1933). In some more recent studies nicotine as well as its major metabolite cotinine have been measured in milk of nursing smokers as well as passive smokers by specific analytical assay methods using gas liquid chromatography (Ferguson *et al.*, 1976; Hardee *et al.*, 1983). In these studies, however, single milk samples of a limited number of mothers have been investigated only. Comparative measurements in serum samples of the mothers have not been performed. Therefore, little is known on the time course of nicotine and cotinine concentrations in milk and on the extent of transfer of nicotine and cotinine from serum into milk. Particularly the important question of possible accumulation of nicotine and cotinine in milk has not yet been adequately addressed.

The pharmacokinetics of nicotine as well as cotinine have been extensively studied in serum of smokers during the past few years (Benowitz *et al.*, 1982; Isaac & Rand, 1972; Kyerematen *et al.*, 1982, 1983; Langone & van Vunakis, 1975; Russell & Feyerabend, 1978). To gain detailed information on nicotine and cotinine concentrations in milk of nursing smokers we have investi-

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gated both the extent of transfer of nicotine and cotinine from serum into milk and have compared the half-lives of nicotine and cotinine in milk with corresponding half-lives in serum. A specific gas chromatographic assay technique has been used for nicotine and cotinine determinations. The half-lives were determined during a 4 h time interval where the mothers refrained from smoking. During this interval multiple serum and milk samples have been collected.

Methods

The milk samples (volumes 2–5 ml) have been collected by the smoking mothers by pressure on the breast or by pumping the milk into sterile plastic tubes. The samples were frozen at −20°C until analysis. The time difference between the collection of the milk samples and of the venous blood samples (10 ml) was always less than 5 min. The blood samples were centrifuged at 3000 rev/min, the serum was stored at −20°C until analysis. The transfer of nicotine and cotinine from serum into milk was investigated in two series of experiments. In the first series a single milk sample and at the same time a single serum sample were collected from each of 18 nursing smokers. The time difference between the last cigarette smoked and the collection of the samples was between 0.25 h and 4.00 h. The number of cigarettes smoked by these 18 mothers ranged from 5–40 cig/day. In the second series the half-lives of nicotine and cotinine were determined in serum and milk of five nursing smokers. These five mothers smoked until the initiation of the experiment (15.00–17.00 h) the usual number of cigarettes. Then, the mothers abstained from smoking, and milk and serum samples were collected at hourly intervals for a period of 4 h. The time difference between the last cigarette smoked and the first serum and milk sample was at least 15 min to assure that the distribution equilibrium between the nicotine concentrations in serum and milk had been reached. From one mother an additional serum and milk sample was collected 10 min after the first cigarette had been smoked at the end of the experiment to gain further insight into the transfer rate of nicotine from serum to milk.

In both series of experiments 44 milk/serum sample pairs have been collected of 23 smokers. In four additional smokers nicotine and cotinine concentrations in milk were measured during a 4 h interval where the mothers had abstained from smoking to gain additional data on the half-life of nicotine and cotinine in milk.

Nicotine and cotinine were extracted from milk (aliquots of 1 ml) according to methods previously reported (Hengen & Hengen, 1978; Feyerabend & Russell, 1979). The nicotine and cotinine concentrations were then measured by gas liquid chromatographic (g.l.c.) procedures. The conditions of chromatography were set for nicotine as follows: glass column (6 mm diameter × 2 mm length), packed with 10% Apiezon and 2% KOH on Chromosorb WAW mesh 80/100 (Feyerabend & Russell, 1979). Temperature of the injector: 240°C, temperature of the oven: 230°C, temperature of the PN-detector: 300°C. N_2-carrier gas flow: 30 ml/min. Internal standard: modaline (Hengen & Hengen, 1978). The retention times for nicotine and modaline were 0.9 and 1.3 min respectively. The limit of detection for nicotine was 0.2 ng/ml. Standard curves were linear over the entire concentration range studied, 0.2–500 ng/ml.

For the determination of cotinine a specific g.l.c. method was developed: a glass column (6 mm diameter, 2 m length), packed with poly-S 179 (Applied Science) was used. Temperature of the injector: 320°C, temperature of the oven: 280°C, separation with temperature programme: 8°C/min, temperature of the PN-detector: 300°C. N_2-carrier gas flow: 30 ml/min. Internal standard: lignocaine (Feyerabend & Russell, 1980). The retention times for lignocaine and cotinine were 0.9 and 1.2 min respectively. The limit of detection for cotinine was 5 ng/ml. Standard curves were linear over the entire concentration range studied, 5–500 ng/ml.

Results

The range of nicotine and cotinine concentrations as well as the milk/serum concentration ratios found in the 44 milk/serum sample pairs are reported in Table 1. The data shown in Table 1 indicate that the milk/serum concentration ratios of nicotine varied much more than those of cotinine.

In Figures 1a and b the nicotine and cotinine concentrations in the milk of the smoking mothers were plotted against the corresponding serum concentrations. There was a linear correlation between the nicotine and cotinine concentrations in serum and in milk (nicotine: $r = 0.70$, cotinine: $r = 0.89$). The nicotine concentrations in milk were higher than those in serum, while the cotinine concentrations in milk were lower than those in serum.

All milk/serum concentration ratios of nicotine exceeded 1, while for cotinine most milk/serum concentration ratios were below 1. Further, there was no correlation between the

Table 1 Nicotine and cotinine concentrations in serum and milk of nursing smokers

	Patients (n)	*Samples* (n)	*Nicotine (ng/ml)*	*Cotinine (ng/ml)*
Serum	23	44	1.0 – 28.0[1]	16 – 330[1]
Milk	23	44	2.0 – 62.0[1]	12 – 222[1]
Milk/serum concentration ratio			2.92 ± 1.09[2] (1.45 – 6.02)[1]	0.78 ± 0.19[2] (0.60 – 1.20)[1]

[1] range
[2] mean ± s.d.

milk/serum concentration ratios and the time interval between the last cigarette consumed and the collection time. This indicates a rapid distribution of nicotine and cotinine between serum and milk.

The time course of nicotine and cotinine concentrations in milk and in serum of five smoking mothers during a 4 h time interval without cigarette smoking is shown in Figure 2. The time course of nicotine and cotinine concentrations in milk of four additional nursing smokers during a 4 h time interval is shown in Figure 3. The decay of nicotine concentrations in serum and milk followed first order kinetics and allowed graphic determination of half-lives in both fluids (Table 2). The half-lives in milk, $t_{1/2}$ = 97 ± 20 min, slightly exceeded those in serum, $t_{1/2}$ = 81 ± 9 min. There was no statistically significant difference between half-lives of nicotine in serum and milk ($P > 0.05$, Student's t-test). The cotinine concentrations in milk and serum did not decrease sufficiently during these 4 h time intervals to allow determination of the half-life of this substance.

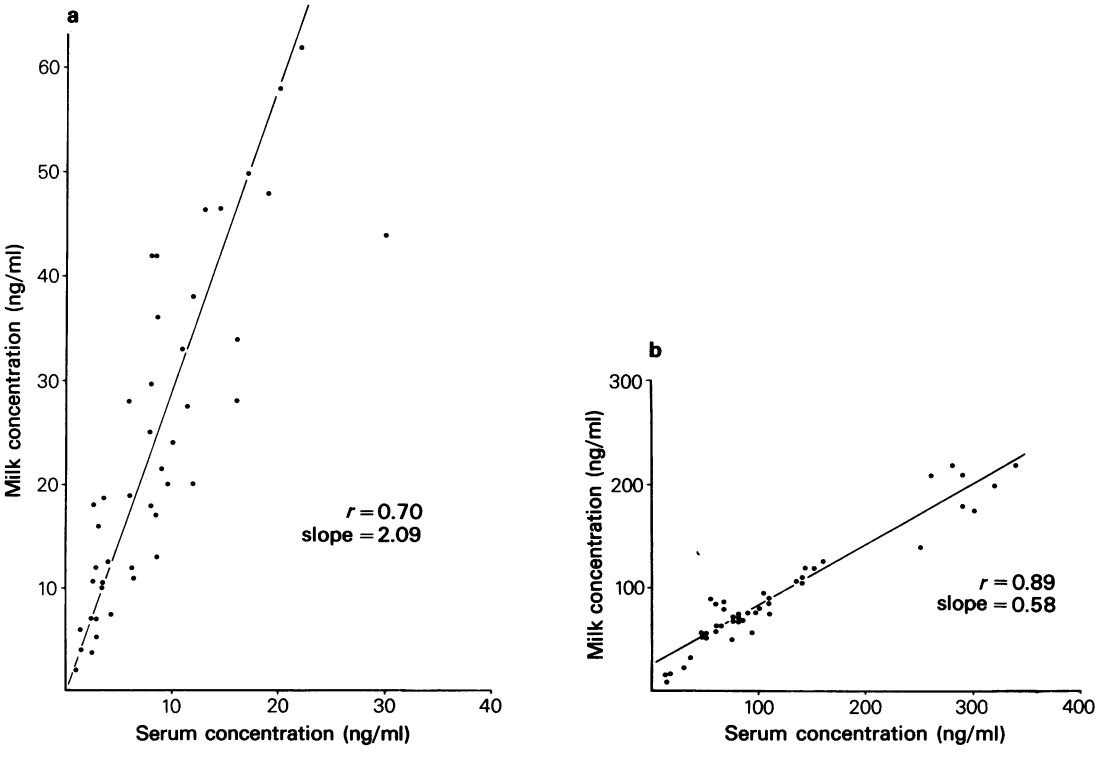

Figure 1 Concentration of (a) nicotine and (b) cotinine in milk against corresponding serum concentrations in nursing smokers.

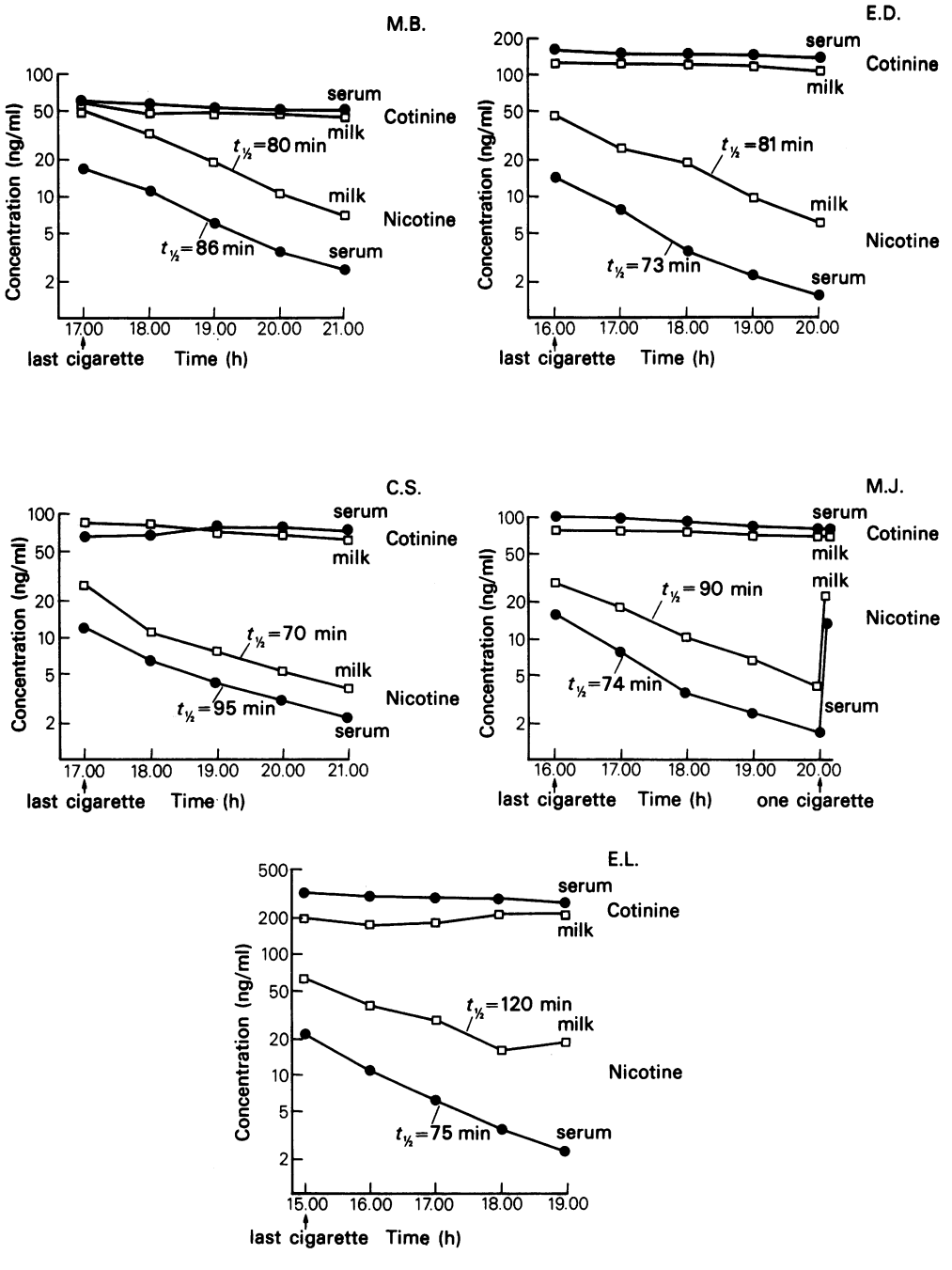

Figure 2 Nicotine and cotinine concentrations in milk and serum of five smoking mothers (subjects E.L., M.B., M.J., C.S., E.D.) during a 4 h time period where no cigarettes have been consumed.

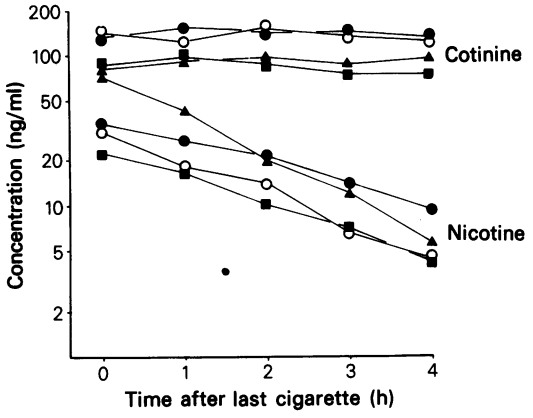

Figure 3 Nicotine and cotinine concentrations in milk of four smokers (S.P. ■, D.P. ▲, D.B. ●, S.N. ○) during a 4 h time interval where no cigarettes have been consumed.

Discussion

The considerable accumulation of nicotine in milk (Figure 1a, Figure 2) can be explained by the chemical properties of this substance as well as the pH gradient between milk and serum. Nicotine is a basic substance: $pKa_1 = 7.8$, $pKa_2 = 3.1$ (Yamamoto, 1965) with low plasma protein binding: 4.9% (Rosenberg *et al.*, 1980) and good lipid solubility (Oldendorf, 1974). Therefore, nicotine would be expected to rapidly diffuse from serum to milk. The pH of milk is usually lower than the pH of serum and also varies considerably more than the pH of serum. While serum pH was reported to be 7.40 ± 0.03 (Documenta Geigy, 1975), the pH of milk was reported to vary between 6.75–7.45, with a mean value of 7.06 ± 0.16 (Ansell *et al.*, (1977). If equilibrium between serum and milk can be assumed and the nonionized form of nicotine has reached equal concentrations in serum and milk, the ionized concentrations and therefore also the total concentrations of nicotine can be expected to be much higher in milk than in serum. The milk/serum concentration ratios can be predicted from the Henderson-Hasselbalch equation: In Table 3 such calculated milk/serum ratios are listed for a serum pH of 7.4 and a range of pH values for milk.

Table 2 Half-lives of nicotine in serum and milk determined during a 4 h time interval of non-smoking

Subject	$t_{1/2}$ *of nicotine (min)*	
	Serum	*Milk*
M.B.	86	80
E.L.	75	120
C.S.	95	70
E.D.	73	81
M.J.	74	90
D.B.	n.d.[1]	96
S.N.	n.d.[1]	127
S.P.	n.d.[1]	120
D.D.	n.d.[1]	70
Mean ± s.d.	81 ± 9 ($n = 5$)	95 ± 22 ($n = 9$)

[1] not determined

Table 3 Milk/serum concentration ratios for nicotine predicted for various pH values of milk. Serum pH 7.40

pH value of milk	*Milk/serum concentration ratio*
6.4	7.43
6.5	5.95
6.6	4.78
6.7	3.84
6.8	3.13
6.9	2.54
7.0	2.07
7.1	1.71
7.2	1.41
7.3	1.18
7.4	1.00

The values of milk/serum concentration ratios predicted from the pH gradient between milk and serum (Table 3) agree well with values reported in the present study (mean ± s.d. = 2.92 ± 1.09). The data in Table 3 show that small changes of the pH value of milk can result in considerable changes of the milk/serum concentration ratios. This strong dependence on the pH gradient explains the considerable range of the milk/serum concentration ratios for nicotine (1.45–6.02). The simultaneous increase of nicotine concentrations in serum and in milk (see Figure 2, subject M.J.) after smoking a cigarette shows that nicotine is transferred rapidly from serum into milk. The similar decay of nicotine concentrations in serum and in milk shows that nicotine diffuses rapidly in both directions (Table 2 and Figure 2).

Further proof for the importance of the pH gradient for the distribution of nicotine from serum into extravascular compartments has been presented by Russell & Feyerabend (1978). These authors found a simultaneous increase of nicotine concentrations in serum and in saliva. Because of the low pH of saliva (pH ≈ 5.5), nicotine cumulates considerably in this fluid (saliva/serum = 12.7). The half-life of nicotine in saliva was, as found for milk in the present

study, in the same range as those in serum (appr. 70 min).

The time course of serum nicotine concentrations found in the present study agrees well with the previously reported data: the highest nicotine concentrations were found immediately after smoking. The decay of nicotine concentrations was found to be biphasic with a short distribution phase ($t_{½,\alpha}$ = 5–10 min) and a longer elimination phase showing considerable interindividual variation ($t_{½,z}$ = 70–140 min) (Benowitz *et al.*, 1982; Kyerematen *et al.*, 1983; Rosenberg *et al.*, 1982; Russell & Feyerabend, 1978).

The ratios of nicotine concentrations between milk and serum showed inter- and intraindividual variation. In one case (Figure 2, subject E.L.), the milk/serum concentration ratio rose from 2.8 at the beginning of the experiment to 6.0 after 4 h; therefore, the half-life of nicotine in milk, $t_{½}$ = 120 min, was considerably greater in this patient than the half-life in serum, $t_{½}$ = 75 min. In the other four smokers these ratios remained relatively constant and therefore, half-lives in milk and serum were very similar. Changes in the milk/serum concentration ratios of nicotine probably result from changes of the pH of milk. Little is known on the time course and regulation of milk pH in nursing mothers. Hall (1975) showed, that the pH increases during the course of one feeding meal by 0.1–0.2 units. Ansell *et al.* (1977) demonstrated great intraindividual day-to-day variations of the pH values of milk. On the averagbe, the half life of nicotine in milk, $t_{½}$ = 97 ± 20 min, (n = 9) were found to be only slightly longer than in serum, $t_{½}$ = 81 ± 9 min (n = 5, see Table 2).

The relatively small variation of the milk/serum concentration ratios of cotinine can be explained as follows: this substance is a weak base. pKa_1 = 4.5 (Yamamoto, 1965). Therefore, cotinine is present both in serum and in milk predominantly as a nonionized molecule. The pH difference between milk and serum is therefore not expected to influence the cotinine concentration in milk.

Cotinine concentrations remained rather constant in serum of smoking mothers (see Figure 2) which agrees with the findings of Kyerematen *et al.* (1982). The low intraindividual variations of cotinine levels are the result of the prolonged half life of cotinine in serum of smokers, $t_{½}$ = 6–30 h (Langone *et al.*, 1975; Kyerematen *et al.*, 1982). The range of cotinine concentrations in serum was between 14–330 ng/ml which agrees with the values reported previously in the literature (10–800 ng/ml) (Benowitz *et al.*, 1983; Hill *et al.*, 1983; Rickert *et al.*, 1981).

A comparison of nicotine and cotinine concentrations in milk found in our study (Table 1) with those reported previously in literature is only possible with the studies of Ferguson *et al.* (1976) and Hardee *et al.* (1983), because only in these two studies specific analytical assay techniques were used (gas liquid chromatography): Ferguson *et al.* (1976) reported nicotine concentrations in a range of <20–512 ng/ml in 28 milk samples of nine nursing mothers who smoked 10–30 cig/day. The nicotine concentrations in 26 of the 28 milk samples were in the range of <20–114 ng/ml; in two samples nicotine concentrations were 277 and 512 ng/ml, respectively. Hardee *et al.* (1983) reported nicotine concentrations in milk of three smoking mothers in a range of 20–150 ng/ml and cotinine concentrations in a range of 50–300 ng/ml. In the milk of passive smokers the nicotine concentrations ranged between 1–7 ng/ml and the cotinine concentration between 2–10 ng/ml.

Serum nicotine concentrations within a range of 1–100 ng/ml have been reported in a number of previous studies. In the majority of smokers serum nicotine concentrations were below 60 ng/ml, only in isolated cases nicotine concentrations reached 100 ng/ml (Armitage *et al.*, 1975; Benowitz *et al.*, 1982; Isaac *et al.*, 1972; Russell & Feyerabend, 1978; Russell *et al.*, 1975, 1980). The milk/serum concentration ratios found in the present study as well as the maximum nicotine concentrations (100 ng/ml) which can be reached in serum of smokers indicate that the two highest values reported by Ferguson *et al.* (1976) (277 ng/ml and 512 ng/ml) should be considered as extreme values. In a previous study where we have analysed 239 milk samples the highest nicotine concentration in milk was 120 ng/ml (Luck & Nau, in preparation). It should be pointed out in this respect that contamination of milk samples, particularly by the fingers of the smoking mothers, must be assiduously avoided in such studies.

In conclusion, we have found a linear correlation for the concentrations of both, nicotine and cotinine, between serum and milk of nursing smokers. Nicotine concentrations are considerably higher in milk than in serum (milk/serum concentration ratio = 2.92 ± 1.09) and cotinine concentrations were lower in milk than in serum (milk/serum concentration ratio = 0.78 ± 0.19). The rapid transfer of nicotine from serum into milk and the short half-lives of nicotine in milk indicate, that mothers who cannot refrain from smoking during the nursing period, should attempt to prolong the time between the last cigarette smoked and breast feeding to minimize exposure of the nursing infant by this substance.

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