

## Short reports

### Caffeine secretion into breast milk

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**SUMMARY** Serum and milk concentrations of caffeine were measured in 5 breast-feeding mothers after a standardised oral dose of caffeine. Peak concentrations of caffeine in serum and milk were attained 60 minutes later. Binding of caffeine by constituents of serum and breast milk was low (25 and 3.2% respectively). In breast milk, caffeine binding is associated with the cream layer, and correlates with the butter fat content. Caffeine does not diffuse freely into breast milk and concentrations in milk are lower than in maternal serum.

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Most breast-feeding mothers will ingest daily some quantity of caffeine from coffee, tea, or soft drinks. Information about the passage of caffeine from serum into breast milk, however, is limited (Illingworth, 1953). We studied the relationship between serum and milk concentrations of caffeine after the ingestion of a standardised oral dose.

#### Subjects

Five healthy white women participated in the study. The subjects ranged in ages from 25 to 31 years, weighed 53.1 to 70 kg, and had been nursing their infants for periods of 4 months to one year. The purpose and procedure of the study was explained to each woman and her informed consent obtained. Each was asked to refrain from taking any coffee, tea, or soft drinks in the 24-hour period immediately before the study.

#### Methods

All studies were done approximately 2 hours after a morning meal. 150 or 300 mg caffeine in the form of caffeine sodium benzoate diluted with approximately 2 oz (60 ml) water was taken by each woman within a 3-min period. Blood and milk were collected at

time 0 and at 30, 60, and 120 minutes after taking the caffeine.

The caffeine concentrations in serum and breast milk were measured by gas liquid chromatography. 250  $\mu$ l milk and 100  $\mu$ l serum were pipetted into 12 ml conical tubes into which etidocaine, the internal standard, had previously been pipetted and dried. Samples were capped and stored at  $-20^{\circ}\text{C}$  until analysis. Extraction was carried out by adding 5 ml isopropyl alcohol and chloroform (5:95) to each specimen, mixing for 30 seconds with a Vortex mixer, freezing the aqueous layer by immersion of the tube in a dry ice and acetone bath, and decanting the organic layer. The extraction was repeated, and the organic phases pooled and dried under nitrogen. Each dried extract was redissolved in 50  $\mu$ l chloroform and 2  $\mu$ l was injected into the gas chromatograph. A Hewlett-Packard 5830A gas chromatograph was equipped with a flame ionisation detector and a 1.8 m glass column packed with 3% SE-30 on 100-120 Gas Chrom. Q (Applied Science). The initial oven temperature of  $195^{\circ}\text{C}$  was kept for  $2\frac{1}{2}$  minutes and then it was increased at a rate of  $2^{\circ}/\text{min}$ . Injector temperature was  $250^{\circ}\text{C}$ , and the detector temperature  $300^{\circ}\text{C}$ . Nitrogen flow was 35 ml/min. Retention time of caffeine and etidocaine were 3.44 and 6.8 minutes respectively. Peak height ratios were calculated and compared with drug supplemented standards processed identically for determination of concentrations of caffeine in the samples of milk and serum.

Protein binding of caffeine in milk and serum was determined by equilibrium dialysis. 10  $\mu$ l  $^{14}\text{C}$ -labelled caffeine (40-60 mCi/mmol) (New England Nuclear) was added to 1 ml samples of milk and serum which then were dialysed against 1 ml 0.2 mol/l phosphate buffer, saline solution, pH 7.4 at an osmolality of 290 mmol. After incubation at  $27^{\circ}\text{C}$  for 5 hours, radioactivity was counted in duplicate aliquots of sample and dialysate. The

fraction of unbound drug was calculated by dividing the radioactivity in the dialysate by the radioactivity in the sample. Binding of caffeine in skimmed breast milk and cream was studied by adding radioactive caffeine to whole milk, separating the layers by centrifugation, and performing equilibrium dialysis on both the skimmed milk and cream layers as described above. Butter fat content of the milk was determined by Pevely Dairy Company, St Louis, Missouri.

## Results

The Table summarises the data obtained from the 5 women. After the ingestion of 150 mg caffeine, peak concentrations of caffeine in serum ranged from 2.39 to 4.05  $\mu\text{g/ml}$ , and peak concentrations in milk ranged from 1.4 to 2.4  $\mu\text{g/ml}$  by 60 minutes. Doubling the dose of caffeine to 300 mg resulted in a doubling of the serum caffeine concentration at 30 and 60 minutes and did not alter the milk to serum concentration ratio in the one woman studied. At all time intervals the caffeine concentration in milk correlated with that in serum ( $0 \geq 30 \text{ min}$ ,  $r = 0.93$ ;  $30 \geq 60 \text{ min}$ ,  $r = 0.80$ ;  $60 \geq 120 \text{ min}$ ,  $r = 0.90$ ). For each time interval, the correlation coefficient ( $r$ ) between the concentrations in serum and milk was 0.89. Analysis by linear regression indicated a linear relationship between serum ( $x$ ) and milk ( $y$ ) caffeine concentrations with an intercept ( $b$ ) of 0.342 and slope ( $a$ ) of 0.381 ( $y = ax + b$ ).

Caffeine appeared to equilibrate rapidly between serum and breast milk. No caffeine was detected in baseline samples of milk or serum. The average of the milk to serum concentration ratios was 0.52 (SD 0.098). The ratios were derived from comparisons of the areas under the paired concentration versus time curves (milk divided by serum) and provide a measure of average concentration ratios over time. The one-way analysis of variance was used

to test the possibility that the milk to serum concentration ratio varied significantly at each time interval. No significant differences were found ( $F = 0.972$ ;  $v_1 = 2$ ,  $v_2 = 12$ ).

The average binding of caffeine to constituents of serum and whole breast milk was 25.1% (SD 2.97) and 3.2% (SD 2.04) respectively. After centrifugation of the breast milk, all of the binding was accounted for by the creamy layer, no binding being detected in the skimmed breast milk. The butter fat content of the milk correlated with caffeine binding ( $r = 0.62$ ). Analysis of the relationship of the fat content of milk ( $x$ ) to the percentage of caffeine bound in milk ( $y$ ) by linear regression indicated an intercept ( $b$ ) of 0.22 and a slope ( $a$ ) of 1.47. The fat content of the five samples ranged from 1.8 to 6.1%. An average of 4.2% (SD 2.4) of the caffeine in cream was nondialysable, a percentage which agrees well with the 3.2% binding determined in whole breast milk.

## Discussion

After the newborn period, nursing infants are likely to ingest caffeine in breast milk. Caffeine, a methyl xanthine present in coffee, tea, and soft drinks (Abramowicz, 1977), is a neurological and myocardial stimulant and may cause coronary artery dilatation, smooth muscle relaxation, skeletal muscle contraction, or diuresis (Ritchie, 1975).

The administration of 2.5 to 5 mg/kg caffeine to preterm infants once or twice daily after a loading dose of 10 mg/kg produces a significant decrease in apnoeic spells (Aranda *et al.*, 1977). There is little information however, about other specific effects, or about the toxicity of caffeine in infants. One 12-month-old child who took between 1 and 1.5 g developed agitation, hyperglycaemia, hypertension, tachycardia, haematemesis, and diuresis (Sullivan, 1977).

Table Caffeine concentrations in serum and breast milk after an oral dose of 150 mg

Subject	Serum concentration ( $\mu\text{g/ml}$ )				Milk concentration ( $\mu\text{g/ml}$ )			
	0 min	30 min	60 min	120 min	0 min	30 min	60 min	120 min
1	ND	1.6	2.4	1.4	ND	1.11	1.43	1.1
2	ND	3.2	2.9	1.6	ND	1.73	1.25	0.54
3	ND	4.0	4.0	1.9	ND	2.29	2.41	1.16
4	ND	2.9	3.1	2.3	ND	1.4	1.3	1.02
5	ND	3.5	3.2	1.9	ND	1.4	1.08	0.81
$\bar{x}$		3.05	3.1	1.6		1.58	1.49	0.926
SD		0.925	0.605	0.536		0.451	0.527	0.253
5a*	1.0	5.8	6.5	4.6 (150 min)	ND	2.8	2.5	2.1

ND = Not detectable. \*Caffeine dose, 300 mg.

Newborn infants eliminate caffeine more slowly than adults and could develop high concentrations if comparable amounts were taken regularly. The half-life of transplacentally acquired caffeine in term newborn babies averaged 80 hours (Parsons *et al.*, 1976) (SD 26 hours). For preterm infants, caffeine elimination is even slower; an average half-life of 97.5 hours was reported in one study (Aranda *et al.*, 1977). Both of these values for half-life are much longer than the average of 3.5 hours reported for adults (Parsons *et al.*, 1976). The average half-life of caffeine for infants after the newborn period is unknown. If caffeine intake was sizeable and if caffeine half-life was comparable with the values found for newborn infants, toxic concentrations might accumulate in infants of nursing mothers with large caffeine intakes.

Our studies show that although caffeine rapidly enters breast milk, the concentrations in milk are less than expected at equilibrium after diffusion. If caffeine diffused freely from serum to milk, caffeine binding to milk and serum constituents should determine the distribution ratio. Considering that 75% of serum caffeine was unbound and available for diffusion into milk, the predicted milk to serum concentration ratio is 0.78\*. The lower ratio (0.52) we observed could indicate either that the caffeine-equilibrated milk was diluted by one-third with milk secreted before caffeine dosage, or that some additional barrier reduces the diffusion of caffeine into milk. If the former were true, at later times the ratio of caffeine concentrations in milk versus serum should have increased as the dilution effect was reduced. We tested this possibility, and the differences in the average ratios at different times were insignificant, indicating no evidence of dilution. We conclude that the data indicate that the diffusion of caffeine from serum into milk is influenced by factors other than the degree of protein binding.

The property of reduced binding of caffeine by constituents of milk relative to those in serum may be an additional factor influencing drug concentration in milk. Decreased binding in milk relative to serum has been demonstrated for the related methyl xanthines, theophylline (Yurchak and Jusko, 1976), and theobromine (Resman *et al.*, 1977). This would tend to reduce the chances of an infant ingesting toxic amounts of these and other drugs that behave in a similar manner.

Can an infant acquire a significant concentration of caffeine by nursing? Our studies indicate that the

nursing infant would receive only a low dose of caffeine after the mother drank a single cup of coffee (150 mg caffeine). A litre of breast milk collected during the first hour after the mother's dose would contain approximately 1.5 mg caffeine. With repeated maternal caffeine ingestion, the caffeine accumulated in the infant would depend on the average concentration in maternal serum and breast milk over time, the volume of milk ingested, and particularly, the infant's clearance rate for caffeine. If older infants have prolonged caffeine elimination rates comparable with newborns, they might accumulate significant amounts of caffeine, although the concentration of caffeine in breast milk is relatively low. Additional studies among infants nursing from mothers who routinely ingest more caffeine than we gave, and studies of caffeine clearance in older infants, are necessary to answer the question.

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\*If the unbound fractions diffuse freely and are in equilibrium, and if the concentration in serum is unity, the expected ratio of concentrations in milk versus serum is calculated  $(0.75 \text{ (unbound in milk)} + 0.03 \text{ (bound in milk)}) \div 0.75 \text{ (unbound in serum)} + 0.25 \text{ (bound in serum)} = 0.78$ .