

The effect of methylxanthines on milk volume and composition, and growth of rat pups

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A study was conducted to investigate the effect of differential dose levels of methylxanthines on lactational performance, with respect to milk volume and composition and pup growth. The methylxanthines; caffeine, theophylline and theobromine, were administered via drinking water in the proportions occurring in tea, at a dose of 50, 1 and 2 mg/kg body-weight respectively to Wistar albino rats throughout pregnancy and for up to the 14th day of lactation. A fourth group received a mixture of all three methylxanthines. Maternal food and fluid intake and weight changes, as well as weight gain in the litter were monitored thrice weekly. Milk samples were collected from the dams on days 7 and 14 of lactation, while milk volume was measured on days 12–13 by a method using tritiated water. Results showed that caffeine and theobromine significantly enhanced litter weight ($P < 0.01$ and 0.05 respectively). In the caffeine group, enhanced litter growth was due to a significant increase ($P < 0.05$) in milk volume, consequent to increased maternal food intake ($P < 0.05$). In the theobromine group there was only a weak association between increased litter weight and milk volume. Theophylline had no effect on the volume or composition of milk, or litter weight. The combination of all three methylxanthines also failed to produce any of the positive effects observed with separate drug treatments.

Methylxanthines: Lactational performance: Growth: Rat

Caffeine, theophylline and theobromine are related purine compounds referred to as methylxanthines. Methylxanthines are commonly consumed dietary components and occur in coffee, tea, cocoa and cocoa products. Other sources are caffeinated soft drinks, kolanut and some patent medicines such as Beechams powder. Coffee contains mainly caffeine, while tea contains caffeine, theobromine and theophylline. Cocoa products contain primarily theobromine.

Methylxanthines are rapidly and completely absorbed when ingested, and have a wide spectrum of effects such as an increase in the metabolic rate, diuresis, stimulation of the central nervous system and relaxation of smooth muscle (Ritchie, 1975; Acheson *et al.* 1980). Studies by other investigators also show that methylxanthines induce lipolysis in adipose tissue through the elevation of catecholamines, resulting in the elevation of plasma free fatty acids (Bellet *et al.* 1968, 1969; Acheson *et al.* 1980; Patwardhan *et al.* 1980).

For many years it was reported (Bellet *et al.* 1969, Daubresse *et al.* 1973, Ritchie, 1975; Patwardhan *et al.* 1980) that methylxanthines exerted their broad spectrum of effects through inhibition of phosphodiesterases, the enzymes involved in the degradation of cyclic AMP. In this way, they would elevate the concentration of cyclic AMP which then mediated the various effects seen. This concept, however, does not seem to explain certain physiological events such as decreased, rather than increased, levels of cyclic AMP in the central nervous system (CNS) produced by theophylline as reported by Daly (1977). Second, Kakiuchi *et al.* (1975) and Smellie *et al.* (1979) have indicated that caffeine and

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theophylline are relatively weak inhibitors of most phosphodiesterase isoenzymes, and never attain brain concentrations (500–1000 μM) sufficient to cause a marked inhibition of such enzymes. Daly *et al.* (1981) have also indicated that caffeine inhibits phosphodiesterase at the high concentration of 0.5–1 mM normally not found in human tissues. Furthermore, analogous inhibitors of phosphodiesterase which cause far more potent inhibition of phosphodiesterase than caffeine or theophylline, have failed to produce more potent stimulation. It has similarly proved difficult to demonstrate any change in tissue or body fluid levels of cyclic AMP following administration of theophylline (Daly, 1977).

The pharmacological effects of methylxanthines are seen at micromolar concentrations; brain and blood levels after coffee consumption are 10–15 μM . In asthma where theophylline is used therapeutically, plasma levels of 40 μM have been observed; however, convulsions occur at concentrations of 200 μM (Gould *et al.* 1984). The Special Report on the Third International Caffeine Workshop (Anon, 1981) indicated measurable levels of 10–280 μM at which various pharmacological effects can be produced. At levels in excess of 400 μM they reported that lethality in humans can be expected.

In view of such evidence, it is questionable whether methylxanthines might be mediating their effects through inhibition of phosphodiesterase. There is now strong evidence that another mechanism, namely antagonism to endogenous adenosine, could be responsible for certain effects of methylxanthines since adenosine is structurally analogous to methylxanthines and has opposite effects (Daly, 1977; Fredholm, 1978, 1980; Daly, 1981; Fredholm & Persson, 1982; Gould *et al.* 1984).

Caffeine and theophylline at micromolar concentrations have now been found to block the interaction of adenosine with its extracellular receptors. Such adenosine receptors govern a variety of physiological functions (Daly, 1982; Newby, 1982) and appear to represent likely loci for the expression of pharmacological activity of caffeine and theophylline.

A large body of literature indicates that methylxanthines are easily transferred through the plasma and other tissue fluids, including amniotic fluid and cerebrospinal fluid (Axelrod & Reichenthal, 1953; Yurchak & Jusko, 1978; Ogilvie, 1985), through the placenta (Horning *et al.* 1975; Parsons *et al.* 1976) and through breast milk (Resman *et al.* 1977; Tyralla & Dodson, 1979; Berlin, 1981). In recent years there has been an increase in the consumption of beverages containing methylxanthines by all age-groups, including pregnant and lactating women (Graham, 1978). The resurgence of breast feeding (Hartman & Prosser, 1984) means that an increasing number of infants will be exposed to the presence of methylxanthines. What effect these ubiquitous food components might have on breast-fed infants has yet to be established. While studies have indicated possible risk effects of methylxanthines on pregnancy, Hart & Grimble (1990) demonstrated that caffeine and theobromine have growth-promoting effects on rat pups. Zoumas & Tarka (1976) also reported a growth-stimulating effect of theobromine on weanling rats. These findings have motivated the present investigation into the effects of methylxanthines on lactational performance with a view to exploring the mechanism by which litter growth is affected. In the present study milk volume and composition were measured. The study also examined whether methylxanthines change the extent of maternal depot fat mobilization by examining the fatty acid contents of milk and maternal depot fat from animals receiving three different methylxanthines or a mixture of all three.

The various doses of methylxanthines used in the study were chosen on an empirical basis, derived from the results of previous investigations in our laboratory, and on the proportions of methylxanthines found in tea. Animals were not, therefore, exposed to equimolar quantities of each methylxanthine but rather to proportions found in a beverage which contains all three. The different dose levels may be justified on the grounds that the

methylxanthines have varying potencies resulting in differential metabolic effects (Ritchie, 1975). In previous studies the administration of 50 mg caffeine showed positive effects on rats (Hart & Grimble, 1990). The choice of the theobromine level was reinforced by studies on mice in the same laboratory (A. Bakkum and R. F. Grimble, unpublished results), which demonstrated that administration of 1 and 2 mg theobromine/kg body-weight produced significant pup growth, beyond which level (5 mg/kg) no further increase was achieved.

MATERIALS AND METHODS

Caffeine (1,3,7-trimethylxanthine) was obtained from BDH Chemicals Ltd, Poole, Dorset, and theophylline (1,3-dimethylxanthine) and theobromine (3,7-dimethylxanthine) from Sigma Chemical Co, Poole, Dorset. Standard laboratory chow (PRD), obtained from Labsure, Manea, Cambridgeshire, contained 198 g protein/kg and 28 g fat/kg.

Virgin albino Wistar rats, weighing 200–220 g, were housed individually in wired solid-bottomed cages and fed on a pelleted diet (PRD) throughout the study. The rats were mated separately with age-matched male rats, and the day on which a vaginal plug was found was counted as day 0 of pregnancy. The male rats were separated from the females which were then weighed, and the respective drug treatments initiated.

Caffeine, theophylline and theobromine were administered via drinking water in the proportions occurring in tea (Graham, 1978). Rats received 50 mg caffeine, 1 mg theophylline and 2 mg theobromine/kg body-weight per d. To examine the possible effects of tea-drinking on the lactating mother, a further group was included, that received a combination of 50 mg caffeine, 1 mg theophylline and 2 mg theobromine/kg body-weight. At least five rats were used in each group, picked at random and assigned to either caffeine, theophylline, theobromine or a mixture of all three drugs. The appropriate amount of each drug was dissolved in distilled water by gentle heating, made up to volume and stored at 0° in aspirators for no longer than 1 week at a time. A control group received plain drinking water stored under the same conditions as the drug solutions.

All animals had free access to food and fluid, with a lighting schedule of 14 h light and 10 h dark. Room temperature was maintained at $22 \pm 2^\circ$. Food and fluid consumption and weight changes in the dams were recorded every 2 d throughout the gestation and lactation periods. About 3 d before the expected date of delivery, the dams were transferred to Macrolon boxes with bedding and nesting materials. Litters born before 16.00 hours on any day were designated birthdate 0.

At 3 d post partum litter sizes were adjusted to eight pups per dam. This standardization is in accordance with the findings of Wilde & Kuhn (1979) who demonstrated that litter size affects mammary tissue weight and, thus, milk production which will ultimately influence litter growth.

Weight changes in the dams and litter, and food and fluid consumption of the dams were monitored thrice weekly until 14 d post partum. The dams were then separated from their litter and decapitated for further studies.

Maternal carcass fat was determined after evisceration of the carcass, and oven-drying at 105° to obtain a constant weight. The loss of weight gave the water content. The dried carcass was then finely ground with 2–2.5 times its weight of anhydrous sodium sulphate to obtain a homogeneous mixture. A portion of each sample was transferred to a Soxhlet thimble and further re-dried in the oven to remove any water taken up during grinding. Fat was extracted from the dried carcass samples in duplicate with light petroleum (b.p. 60–80°) in a Soxhlet apparatus. The thimble was then drained of all solvent and further dried in an oven. Carcass fat was calculated from the difference in weight of the thimble before and after extraction.

Milking procedure

Separate groups of dams were milked on days 7 or 14 of lactation under diethyl ether anaesthesia. Milk (2 ml) was collected by means of manual expression of the ventral thoracic and abdominal teats, and stored at -20° until analysed for total protein, fat and lactose.

Milk volume measurement

Milk volume was measured between 10.00 hours on day 12 and 10.00 hours on day 13 of lactation using a modification of the method of Rath & Thenen (1979). The method involved the separation of the pups from their mothers, and the intraperitoneal injection of 0.2 ml (10 mCi/ml) of tritiated water into the dam. After 1 h the pups were replaced and allowed to suck. The $^3\text{H}_2\text{O}$ content of the dam's blood was maintained at a plateau over a 24 h period by replacement of drinking water with 17 mCi tritiated water/ml (plus the appropriate dose of methylxanthine). At the end of this period a blood sample was taken from the mother and the pups weighed, killed and homogenized in distilled water.

The ^3H content in the dam's plasma and pups' homogenate was calculated to derive the volume of milk produced by the mother and the milk intake of the pups. This measurement is based on the assumption that all radioactivity in the pup comes from the milk obtained from the mother. It also assumes that all or most of the radioactivity stays in the body water. The validity of the latter assumption was tested by estimating the radioactivity that was: (a) lost in the pups' urine, (b) incorporated into the pups' body lipids, and (c) taken up into milk solids. Losses from these routes amounted to less than 8% of the administered dose.

Determination of radioactivity

Radioactivity in samples was measured by liquid scintillation counting using Phillips liquid-scintillation analysers, model PW4700. Counting efficiency was 64% for ^{14}C and 28% for all ^3H . Variations in quenching were corrected by external standardization using programme quench curves and the counts expressed as disintegration per minute (dpm). The scintillants used were tritoscint or Beckman Ready-Solve (High Wycombe, UK) as appropriate.

Determination of total protein, lactose and triacylglycerol concentration of milk

A portion (100 μl) of rat milk was precipitated with 2.5 ml 1 M-trichloroacetic acid (TCA). The resultant supernatant fraction was reserved for the assay of lactose. The precipitate was washed with 5 ml TCA (50 g/l) dissolved at 37° in 2 ml 0.3 M-potassium hydroxide, and assayed for total protein by the method of Lowry *et al.* (1951).

Lactose was measured in the neutralized supernatant fraction by an enzymic method using lactose-galactose kits supplied by Boehringer (Mannheim, West Germany).

Milk triacylglycerol was measured after extraction in propan-2-ol by AutoAnalyzer (Technicon Instruments Co. Ltd, 1971), Triolein was used as the standard.

Determination of the fatty acid composition of milk, maternal uterine fat and stock diet fat

Fatty acid composition was determined on milk samples taken on days 7 and 14 for comparison with the uterine depot fat and stock-diet fat.

Lipid extraction was carried out using a mixture of chloroform-methanol, (2:1, v/v). The fatty acid composition was then estimated by gas-liquid chromatography. The system used (Pye Unicam 305) was equipped with a 50 m \times 0.22 mm internal diameter fused silica capillary column coated with CP sil 88, 0.18 thick (Chrompak) using a hydrogen carrier.

The peak areas were determined by computerized electronic integration using the

Table 1. *Food and fluid intakes and weight changes of dams administered methylxanthines during pregnancy and lactation*

(Mean values with their standard errors)

Treatment group†	n	Food intake (g/dam per d)				Fluid intake (ml/dam per d)				Body-wt changes			
		Pregnancy		Lactation		Pregnancy		Lactation		Pregnancy (g/dam per d)		Lactation (g/dam per 10 d)	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	7	24	0.9	36	0.6	32	1.5	49	1.5	6	0.3	9	4.3
Caffeine	5	26	0.9	40**	1.0	37*	1.4	66**	3.6	5	0.1	10	13.0
Theophylline	5	23	0.4	34	0.6	29	2.5	54	6.1	5	0.5	9	4.0
Theobromine	5	24	0.6	36	1.5	27	0.8	46	1.7	6	0.2	2	4.8
Mixture	5	27	0.9	40	2.4	32	1.9	54	3.8	6	0.4	11	2.8

Mean values were significantly different from those of the control group (Student's *t* test): * $P < 0.05$, ** $P < 0.01$.

† For details, see p. 341.

following fatty acid standards: C_{10:0}, C_{12:0}, C_{14:0}, C_{14:1}, C_{16:0}, C_{18:0}, C_{18:1}, C_{18:2}, C_{18:3}, C_{20:4}, C_{20:5}, C_{20:6}. Results are expressed as fatty acid percentage composition by weight.

Statistical analysis

Results are expressed as mean value with their standard errors. Tests of significance between control and individual-methylxanthine groups were carried out using Student's unpaired *t* test.

RESULTS

The results of food and fluid intake are presented in Table 1. Food intakes during pregnancy were not significantly different between control and the methylxanthine-treated groups. During lactation the caffeine group consumed significantly more food than the controls ($P < 0.01$). The group receiving the mixture of methylxanthines had a larger mean food intake than the control group, although the difference did not reach statistical significance.

Fluid intake was significantly greater in the caffeine group during pregnancy and lactation, compared with the control group ($P < 0.05$ and $P < 0.002$ respectively).

Body-weight changes of the dams (Table 1) showed no significant differences between groups during pregnancy or lactation. Litter weights (Table 2) were similar at day 3 of lactation; however, they were significantly greater in the caffeine and theobromine groups ($P < 0.01$ and $P < 0.05$ respectively) at 13 d post partum. Litter growth showed a significant increase in the caffeine group ($P < 0.005$). The theobromine group, however, showed no significant change.

Caffeine treatment significantly enhanced milk volume ($P < 0.05$; Table 3), but the total daily production of protein, lactose and triacylglycerols in milk were not significantly altered in any of the groups.

Values for the fatty acid composition of milk fat sampled on days 7 and 14 are presented in Table 4. Methylxanthine treatment produced no significant differences in the milk fatty acid profile.

The major fatty acids in all groups were: palmitate (C_{16:0}), oleate (C_{18:1}), linoleate (C_{18:2}) and the medium-chain fatty acids (C_{10:0}–C_{14:0}). With the progression of lactation from day 7 to 14, there was an increase in the proportion of the medium-chain fatty acids and a

Table 2. *Effect of methylxanthines on litter weight in rats*
(Mean values with their standard errors)

Treatment group†	n	Litter wt (g)				Litter growth rate (g/d)	
		Day 3		Day 13		Mean	SE
		Mean	SE	Mean	SE		
Control	7	66	1	183	5	11.7	0.6
Caffeine	5	69	3	213**	7	14.4***	0.5
Theophylline	5	63	1	189	2	12.6	0.3
Theobromine	5	66	2	199*	2	13.3	0.5
Mixture	5	60	2	192	16	13.3	0.3

Mean values were significantly different from those of the control group (Student's *t* test): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

† For details, see p. 341.

Table 3. *Effect of methylxanthines on the volume and total 24 h production of protein, lactose and triacylglycerols in milk of rats at day 13 of lactation*
(Mean values with their standard errors)

Treatment group† ... n...	Control 6		Caffeine 6		Theophylline 5		Theobromine 5		Mixture 5	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Milk volume (ml/dam per d)	45.3	2.7	53.9*	3.7	45.0	2.4	51.7	2.6	47.7	3.8
Water (ml/l)	755	12	733	9	774	14	733	12	723	12
Protein (g/dam per d)	3.4	0.3	4.3	0.3	3.1	0.3	3.5	0.1	3.5	0.4
Lactose (g/dam per d)	0.67	0.1	0.91	0.1	0.68	0.08	0.89	0.10	0.71	0.1
Triacylglycerols (g/dam per d)	9.8	0.7	10.8	1.1	9.0	0.6	10.9	0.8	11.5	1.2

Mean value was significantly different from control group (Student's *t* test): * $P < 0.05$.

† For details, see p. 341.

decrease in the long-chain unsaturated fatty acids ($C_{18:1}$ – $C_{18:3}$). These results are in agreement with the findings of Chalk & Bailey (1979).

Comparison of the fatty acid composition of milk fat with those of the uterine fat and the stock-diet fat (Table 5) may indicate its source. The palmitate content of milk fat on days 7 and 14 resembled that of uterine fat (between 25 and 27% in milk fat compared with 27% in the uterine fat). The linoleate ($C_{18:2}$) content of milk fat on days 7 and 14 in all groups was also similar to that of the uterine depot fat. Milk linoleate was also likely to have come from the maternal depot fat, since the proportion of this fatty acid was lower in the former than in the latter. If the diet was a significant source, then a higher proportion would be expected in the milk fat. The source of the depot fat linoleate is dietary, since no known mammal synthesizes this fatty acid (Jenness, 1974).

DISCUSSION

The results presented in the present study showed that caffeine produced a significant increase in food intake during lactation ($P < 0.05$). Similarly, fluid intake was significantly

Table 4. *The effect of methylxanthines on the fatty acid composition (%) of rat's milk taken from milk samples on days 7 and 14 of lactation*

Fatty acid... Treatment group†	C _{10:0}		C _{12:0}		C _{14:0}		C _{16:0}		C _{16:1}		C _{18:0}		C _{18:1}		C _{18:2}		C _{18:3}	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
	<i>Day 7 milk</i>																	
Control	11.0	0.6	9.9	0.2	10.0	0.8	25.1	0.8	2.5	0.4	2.9	0.1	20.4	0.5	17.1	0.1	1.2	0.2
Caffeine	9.7	0.9	9.4	0.0	9.4	0.2	26.0	0.7	2.0	0.2	2.8	0.2	23.9	0.6	15.9	0.2	0.9	0.2
Theophylline	8.8	0.6	9.0	0.5	9.6	0.8	26.3	0.8	2.4	0.5	3.1	0.1	22.4	0.7	17.3	0.7	1.1	0.1
Theobromine	10.9	0.6	10.2	0.3	11.3	0.3	25.8	0.5	2.1	0.3	2.9	0.2	20.1	0.8	15.5	0.0	1.0	0.1
Mixture	11.6	0.7	11.1	0.7	11.8	0.1	26.2	0.1	2.0	0.2	2.8	0.1	18.1	0.1	15.5	0.3	0.9	0.1
	<i>Day 14 milk</i>																	
Control	12.0	0.8	11.1	0.6	11.3	0.8	26.3	0.5	2.0	0.1	3.1	0.1	19.8	0.4	13.6	0.8	0.8	0.1
Caffeine	13.4	0.6	11.9	0.5	13.3	0.7	26.2	0.8	1.2	0.1	3.0	0.1	16.3	0.4	14.4	0.4	1.0	0.2
Theophylline	12.2	0.9	10.9	0.6	11.8	0.8	26.9	0.7	1.7	0.2	3.3	0.2	18.9	0.5	13.3	0.4	1.0	0.1
Theobromine	14.3	0.1	12.6	0.7	12.4	0.7	25.9	0.8	1.7	0.2	2.7	0.1	17.1	0.2	12.2	0.3	1.1	0.2
Mixture	10.9	0.2	12.2	0.6	12.4	0.5	27.2	0.0	1.6	0.1	3.1	0.2	18.4	0.7	13.2	0.8	0.9	0.1

† For details, see p. 341.

Table 5. *The effect of methylxanthines on the fatty acid composition of uterine fat from lactating rats. The fatty acid composition of the stock diet (PRD; Labsure, Manea, Cambridgeshire) fat is also given*
(Mean values with their standard errors)

Fatty acid ... Treatment group†	C _{14:0}		C _{16:0}		C _{16:1}		C _{18:0}		C _{18:1}		C _{18:2}		C _{18:3}		C _{20:4}		C _{22:6}	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Control	1.2	0.1	27.3	0.1	5.7	0.5	3.3	0.2	35.3	0.2	25.0	0.2	1.2	0.1	0.5	0.1	0.7	0.2
Caffeine	0.9	0.1	26.2	0.2	4.4	0.1	3.7	0.1	36.4	0.6	26.6	0.4	1.2	0.1	Tr	—	Tr	—
Theophylline	0.9	0.2	26.4	0.5	5.1	0.3	3.6	0.2	35.9	0.6	35.9	0.6	1.2	0.0	—	—	Tr	—
Theobromine	1.2	0.2	27.4	0.8	4.2	0.3	3.6	0.1	36.6	0.7	36.6	0.6	1.1	0.0	—	—	—	—
Mixture	1.2	0.2	27.6	0.8	4.4	0.2	3.3	0.2	35.3	0.6	35.3	0.8	1.2	0.1	Tr	—	Tr	—
Fatty acid ...	C _{15:0} 1.2		C _{16:0} 16.6		C _{16:1} 0.8		Fatty acid composition of stock diet fat		C _{18:1} 21.3		C _{18:2} 51.3		C _{18:3} 4.7		C _{20:4} 0.8		C _{22:6} 0.7	
							C _{18:0} 2.5											

Tr, trace.

† For details, see p. 341.

greater in the caffeine group during pregnancy and lactation ($P < 0.05$ and $P < 0.01$). Although the group receiving the mixture of methylxanthines showed an elevated mean food intake, the value did not achieve statistical significance.

Milk volume was augmented by caffeine. This phenomenon suggests a relationship between enhanced litter growth and increased milk production, consequent to increased food consumption. In the theobromine group, there was only a weak association between increased litter weight at day 13 and milk volume. The total production of protein, lactose and fat showed an upward trend which did not attain statistical significance.

This is not surprising in spite of the increased maternal food intake, as milk composition is seldom affected except under extreme maternal dietary restrictions: however, milk volume may be affected (Mansaray, 1985).

While caffeine and theobromine had positive effects on litter growth, food and fluid intake and milk production, theophylline provoked no effect on any of these variables. This could be as a result of the low dosage administered to the dams. The mixture of methylxanthines similarly failed to produce any of the stimulatory effects observed with caffeine or theobromine. What is the explanation for this paradoxical effect?

It may be speculated that since theophylline is the most potent antagonist of adenosine, it could result, in the present study, in a lowering of the effects seen in the mixed methylxanthine group. For example, if theophylline bound to a mammary cell receptor and produced little or no effect, it may exclude that receptor from binding to caffeine or theobromine which proved to be more potent drugs as regards stimulation of lactational performance. This proposition is supported by the work of McDaniel *et al.* (1977) who reported an inhibition of caffeine uptake as well as caffeine displacement by theophylline in isolated pancreatic islet cells, indicative of competition for a common binding site or transport carrier, or both. Although the present study used different dose levels of methylxanthines, using equal doses of the drugs, Aeschbacher & Wurzner (1975) reported significant stimulation of hepatic microsomal enzyme activity when rats were pre-treated orally with higher doses (150 mg/kg) of either caffeine, theophylline or theobromine. They observed that stimulation was dose-dependent; thus, while 75 mg/kg of each drug produced some enzyme induction, a lower dose of 37.5 mg/kg produced no changes, even with a combination of all three drugs amounting to 112.5 mg methylxanthines/kg. Although the design of these workers' experiment does not tally with the study being reported, the phenomenon observed in the present study is indicative of possible competition at the receptor site to reduce the positive effect seen with individual methylxanthine treatment.

Methylxanthines exerted no influence on milk fatty acid composition. Fatty acid profiles of milk on days 7 and 14 were not significantly different between control and the drug-treated groups. Comparison between milk fatty acids and the uterine fatty acids showed the former to have a profile typical of rat milk fat. If methylxanthines had increased mobilization of maternal body lipids to augment milk production, milk fatty acid composition would have reflected that of the uterine depot fat and showed an enhanced content of palmitate and oleate, with diminished medium-chain fatty acid content. However, it did not.

On a normal laboratory chow, Williamson (1980) indicated that 50% of the lipids are synthesized within the mammary gland (*de novo* synthesis). Among the rodents, rats produce considerable short-chain acids due to the ability to synthesize fat from carbohydrates by conversion of citrate to acetyl CoA by means of ATP-citrate lyase (EC 4.1.3.8) (Jeness, 1974). The remainder of the milk lipids is derived from triacylglycerols and non-esterified fatty acids taken up from the blood through the diet and from endogenous sources (liver and adipose tissue). Physiological adaptations within the

maternal tissue involve increased cardiac output, with greatly enlarged mammary glands, as well as organs that supply substrates to the latter organ such as the liver and gastrointestinal tract. These changes result in diversion of a high proportion of the substrates available in circulation. Glucose, triacylglycerols, free fatty acids and ketone bodies are all directed to the mammary gland for the production of milk (Chatwin *et al.* 1968; Widdowson, 1976; Williamson, 1980). Thus, during lactation some of the components for milk synthesis are supplied directly by the diet, and some from the mother's reserve which ultimately must have come from the diet. In addition to these adaptations in the rat, Naismith *et al.* (1982) demonstrated a cycle of fat retention during gestation, followed by up to a 60% mobilization of the dam's body fat during lactation to augment milk production. If methylxanthines were effecting lipolysis as reported by Bellet *et al.* (1969) and Patwardhan *et al.* (1980), the released fatty acids would have been directed towards the mammary glands, resulting in a preponderance of milk fatty acids reflecting those of the uterine depot fat. This would have subsequently decreased the need for *de novo* synthesis. A previous study by Hart & Grimble (1990) had shown no significant decrease in the carcass fat content of the drug-treated animals, reinforcing the conclusion of a lack of effect of methylxanthines on lipid mobilization and implying that no contribution from adipose tissue that deviated from normal occurred. In summary then, these findings have clearly demonstrated that the fatty acid composition of milk from all groups was a typical profile of rat's milk, derived partly from *de novo* synthesis (medium-chain fatty acids). The rest of the fatty acids, particularly palmitate and oleate were derived from maternal lipids as a normal physiological process, while others such as linoleate originated from dietary sources, as no known species synthesize this fatty acid.

The deductions from the present study suggest a stimulatory influence of theobromine and caffeine on pup weight. These effects may be due to a combination of factors. In the case of theobromine, there was only a weak association between milk production and enhanced litter weight. The factor responsible for the growth stimulation remains obscure. In the case of caffeine, however, the evidence given in the present study links elevated litter growth with increased maternal milk volume, which in turn may result from increased maternal food intake. Factors responsible for the increased food consumption in the caffeine group, and the mechanism effecting growth stimulation will need to be investigated further.

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