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Milk Fat Concentration and Growth of Rat Pups

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Summary

The relationship between milk fat concentration and the growth of rat pups from birth to the 20th day was investigated. A total of 36 first-time-lactating Wistar rats and 366 pups from litters of 8–12 were used. The concentration of milk fat was determined by the crematocrit method, on the 2nd, 5th, 10th, 15th, 20th and 25th day of lactation. A wide range of milk fat concentration with a great variability in each individual dam was observed during lactation. To correlate milk fat and pup's growth, the mean value of the milk fat of the individual mothers was calculated for the first 10 days from the values of the 2nd, 5th and 10th day of lactation, and for the second 10 days from the values of the 10th, 15th and 20th days. The results show that pups fed higher fat milk in the first 10 days of life grew faster than pups fed lower fat milk. A significant positive correlation ($P<0.001$) was found between the fat concentration of milk and the weight gains of pups in the first 10 days, but not in the older groups. It indicates that the fat concentration of milk is important for the growth of pups mainly in the early postnatal period of life.

Key words

Rat milk – Fat – Protein – Pup growth – Rats

Introduction

The growth rate of suckling pups depends mainly on the supply and composition of their mother's milk. There is considerable evidence that the quantity of milk produced by rat dams is sufficient for the optimal nutrition of about 12 pups in a litter (Kumaresan *et al.* 1967, Hausberger and Volz 1984, Wurtman and Miller 1976). Experimental conditions which modify the availability of milk for pups lead to growth acceleration in overfed and to reduction of growth in underfed animals (Knittle and Hirsch 1968, Cryer and Jones 1980, Faust *et al.* 1980, Kostolansky and Angel 1989). From this aspect the significance of milk quantity for pup growth seems to be clear, but little information is available on the significance on the composition of milk. Rat milk is rich in fat and protein and poor in carbohydrates (Keen *et al.* 1981, Cox and Mueller 1937, Luckey *et al.* 1954). While the proteins and carbohydrates do not change significantly, the fat concentration in the milk varies considerably during lactation (Luckey *et al.* 1954, Godbole *et al.* 1981). Because the energy requirement of pups during the suckling period is covered mainly from milk fat, the variability of its concentration may significantly influence the growth of pups.

This study was undertaken to follow the individual differences in the milk fat concentration, its variability during lactation and its relationship to the growth of suckling rat pups.

Materials and Methods

Animals

Thirty-six first-time-lactating Wistar rat dams (obtained from Velaz, Prague) and 366 pups were used in this experiment. Only dams with a litter of 8 and more pups were used. On the first day after birth, the litters larger than 12 pups were reduced to 12 pups per nest. The litters were individually housed in Plexiglas cages in a temperature-controlled environment (22 °C) with 12:12 light-dark regime. The dams had free access to standard laboratory food (DOS 2b, Velaz, Prague) and tap water. The pups were individually weighed on the 1st, 10th and 20th day. The mean weight of male and female pups from each nest were used for

statistical analysis. The dams were milked on the 2nd, 5th, 10th, 15th, 20th and 25th days of lactation.

Before the experiment, the constancy of milk composition in the samples obtained from different nipples or taken successively from one nipple was examined. The milk fat and protein concentration were estimated in samples obtained from each nipple (2 samples/nipple) of the same dam and in 6 samples obtained from one nipple. Six dams were used altogether. We found an approximately 2 % difference in the milk fat and protein between the samples from the individual nipples of one dam and less than 3 % differences between the samples obtained from one nipple.

Milk sampling

Three hours before milking (at 08.00 h), the mothers were separated from their pups. Then, 10 min before milking, they received an injection of 2 IU oxytocin intraperitoneally. During milking they were anaesthetized with diethyl aether. Milk was obtained by massaging the mammary gland and by subsequent gentle thumb pressure on the nipple. The droplets of milk were collected in "standard glass disposable micropipettes" (20 μl content, 64 mm length, C M S, Houston, Tx). The milking time for obtaining 3 tubes of milk (2 tubes for fat and 1 tube for protein estimation) was about 1-2 min in each rat.

Milk composition

Milk fat was determined by the crematocrit method of Lucas *et al.* (1978). About 18 μl of milk (approx. 56-58 mm column) was drawn by capillarity into the glass tubes which were then sealed at the empty end in a flame and centrifuged at 10 000 x *g* for 10 min. The whole column and the crematocrit were measured with an accuracy of 0.05 mm. The fat concentration was expressed in g/100 ml milk by the formula given by Nagasawa *et al.* (1989) – check Milk fat = 0.1 x (crematocrit in % – 0.59)/0.146.

The protein concentration of milk was analyzed by the method of Lowry *et al.* (1951). The glass tubes were filled end to end to contain 20 μl. The values were expressed in g/100 ml milk.

Statistics

The mean milk fat and protein of the individual dams in the first 10 days (each average value calculated from the 2nd, 5th and 10th days collections) and in the second 10 days of lactation (average values from 10th, 15th and 20th days) were correlated with the weight gains of their pups from the 1-10th or 10-20th day, respectively. The litters were arbitrarily divided into high (17 dams) and low (19 dams) milk fat groups according to the mean fat concentration in the milk (above and below the mean value of 14.2 g/100 m) during the first 10 days of lactation. Statistical significance of correlation coefficients was determined by Snedecor and Cochran (1980). For all other variables the Student's t-test was used.

Results

The mean values of milk fat and protein concentration on the 2nd, 5th, 10th, 15th, 20th and 25th day are presented in Table 1. The protein concentration in the milk showed only slight individual differences and no changes of the mean values during lactation. In the milk fat, lower mean values were recorded on the 5th and 20th day and higher on the 10th, 15th and 25th day. A wide range of individual values was found at all the times of estimation.

Comparison of the weight gains of pups in groups receiving high fat milk (17 litters) or low fat milk (19 litters) in the first 10 days shows that pups fed higher fat milk had significantly higher weight gains ($P < 0.001$). This trend also continued in the second 10 days. The differences in weight gains remained significant ($P < 0.05$ males, $P < 0.01$ females) despite the minimal differences in milk fat between groups (Table 2). During both investigated periods, the growth rate differences between males and females were not significant.

Table 1
Fat and protein concentrations in rat milk during lactation

	Age in days					
	2	5	10	15	20	25
Fat (g/100 ml)						
	14.8±0.7	12.8±0.4	15.3±0.5	15.5±0.5	13.9±0.3	15.8±0.5
	(9.7-18.0)	(8.0-19.6)	(7.0-22.1)	(11.0-20.8)	(10.4-18.9)	(11.1-23.3)
Protein (g/100 ml)						
	8.9±0.2	9.2±0.2	9.0±0.1	9.0±0.1	8.9±0.1	9.1±0.1
	(8.2-10.6)	(7.7-10.4)	(8.0-10.5)	(7.8-10.5)	(8.0-10.6)	(8.1-10.6)

Values are means ± S.E.M. (n = 36). Values in parentheses show the range.

Table 2
Weight gain of rat pups fed a higher (group A) or lower (group B) fat milk

Group	Weight gain (g)	Fat g/100 ml	Protein g/100 ml
	First 10 days		
Males A	14.3±0.5***		
		15.7±0.3***	9.1±0.1
Females A	13.8±0.5***		
Males B	9.9±0.5		
		12.7±0.4	9.0±0.1
Females B	10.1±0.5		
	Second 10 days		
Males A	20.2±1.5*		
		15.4±0.4	9.1±0.1
Females A	19.1±1.1**		
Males B	15.9±0.8		
		14.5±0.4	9.0±0.1
Females B	15.2±0.8		

Values are means ± S.E.M. Group A comprised 17 litters; group B 19 litters. Mean milk fat and protein for the first 10 days was calculated in each dam from values obtained in the 2nd, 5th and 10th day of lactation, and for the second 10 days from values obtained in the 10th, 15th and 20th day. Significantly different values from group B: * $P<0.05$; ** $P<0.01$; *** $P<0.001$ (evaluated by Student's t-test).

A significant positive correlation ($P<0.001$) was found between the weight gain of pups and the milk fat concentration for the first 10 days of life but not for the second period (Table 3).

Discussion

The micromethods used in our experiment enabled us to perform an easy and rapid milking operation and simple but precise estimation of the milk fat and protein concentration in individual dams. The mean values of milk fat and protein concentration recorded in the experiment are in agreement with those reported by others (Cox and Mueller 1937, Luckey *et al.* 1954, Godbole *et al.* 1981). Only a small range of individual values was found in milk protein, but a large one in milk fat. The highest values of milk fat were practically twice as high (on the 10th day three times) as the lowest one. Moreover, a large variability in milk fat of individual dams was recorded during lactation. From this point of view, it is clear that the relationship between the growth of pups and the milk fat concentration must be evaluated individually. Because of the great individual variability in milk fat during lactation, the mean value of fat concentration had to be also recalculated for the observed period.

Our results show a significant positive correlation between the milk fat concentration and weight gains of pups in the first 10 days of life and no correlation in the second 10 days. The arbitrary division of litters into two groups according to the milk fat concentration in the first 10 days also showed that the pups fed higher fat milk grew faster than pups on lower fat milk. The higher growth rate of these pups also continued in the second 10 days, although the milk fat concentration of these groups did not differ substantially at this time. It would indicate that pups who obtain more dietary energy – whether it is *via* a higher milk fat concentration, higher milk volume, or both – grow faster, suckle more vigorously and stimulate their dams to produce still more milk. It was demonstrated that the production of milk is regulated by the number of pups in the nest and increases proportionaly from 2 to 12 pups (Kumersan *et al.* 1967). The milk intake of pups reared in normal litters (8 pups/nest) gradually increase from birth to the 15th day (Babický *et al.* 1970) The increased milk production of dams and high milk fat, recorded in our experiment on day 15, enable optimal growth and development of rat pups during this time.

The decrease of milk fat concentration on the 20th day of lactation and subsequent increase on the 25th day are probably related to the changes in feeding of pups after the 15th day. It was demonstrated that the consumption of solid food by pups gradually increases and milk intake decreases from the 15th day of lactation (Babický *et al.* 1971, 1972). The milk fat concentration that is high on the 25th day may be important from the nutritional aspect despite of a low intake of milk by pups at this time. On the 25th day, the intestinal wall matures and digestion and resorption are stabilized at adult values (Hahn and Koldovský 1966), but the production of bile by pups is insufficient for digestion of fat from solid food (Moltz and Lee 1981). This handicap is compensated by coprophagy of special dam's faeces rich in bile acids which is maximal on 25th day (Nováková and Babický 1989). These two mechanisms – coprophagy that enable utilisation of less digestible fats from solid food, and supplement of easily digestible milk fat provide optimal conditions for the development of pups before weaning.

Table 3
Correlation of weight gain with milk fat and protein concentration in the milk

Group	Weight gain (g)	Fat g/100 ml	Correlation coefficient	Protein g/100 ml	Correlation coefficient
			First 10 days		
Males	11.9±0.5		0.66***		−0.18
		14.2±0.3		9.1±0.1	
Females	11.8±0.4		0.65***		−0.23
			Second 10 days		
Males	17.9±0.8		0.16		−0.18
		15.0±0.3		9.0±0.1	
Females	17.1±0.7		0.08		−0.26

Weight gains are means ± S.E.M. of male and female pups from 36 litters. Correlation was done between mean values of milk fat and weight gains of pups in 36 litters. Mean milk fat and protein for the first 10 days was calculated in each dam from values obtained on the 2nd, 5th and 10th day of lactation, and for the second 10 days from values obtained on the 10th, 15th and 20th day. *** $P<0.001$

The obtained positive correlation between the milk fat concentration and the weight gains of pups in the first 10 days of life and its absence in the second 10 days shows that the fat concentration of milk is important for the growth of pups, mainly in the early postnatal period.

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