Relationship between Milk Composition and Pup's Growth in Mice[1] (42892)

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Abstract. **The effect of lactation numbers (first, second, and third) on milk composition and the relationship between milk composition and pup's growth were examined in four strains of mice (SHN, SLN, C3H/He, and GR/A). Although the percentage of milk fat and moisture increased and decreased, respectively, through the first to third lactations in C3H/He mice, no differences in milk composition among lactation numbers were observed in the other mouse strains. In pooled data derived from the four strains of mice, there was no significant correlation between milk composition and body weight or growth rate of pups on Day 12 or 20 of the first lactation. In contrast, the amount of milk fat and moisture was the positively and negatively, respectively, correlated with the pup's growth parameters during the second and third lactations. This was reflected by a lower fat content and a higher moisture content in the milk of GR/A mice, especially evident during the second and third lactations and reduced pup's growth, when compared with the other mouse strains. Milk lactose and ash content was not correlated with pup's growth parameters. The results of our study stress the importance of milk fat for the growth of mouse pups.** [P.S.E.B.M. 1989, Vol 191]

Composition as well as yield of milk is an essential factor for the growth of offspring in mammals. However, very little information has been published on milk composition in the laboratory mouse (1, 2), and no information is available on the influence of lactation numbers (first, second, third lactations, etc.) on milk composition and the effect of milk composition on pup's growth in this commonly used laboratory animal species. This study was undertaken to examine mouse strain-related differences in the milk composition among the first, second, and third lactations and the relationship between milk composition and pup's growth.

Materials and Methods

Animals. Animals used were SHN/Mei, SLN/Mei, C3H/HeMei, and GR/AMei strains of mice maintained in our laboratory by strict brother × sister mating. At 60–70 days of age, each female was placed with one litter mate male in a plastic cage (16 × 28 × 13 cm) with wood shavings. Only animals that delivered 6–8 pups within 30 days after placing with males were used in this study. All pups were allowed to nurse normally until Day 20 of lactation. Throughout the experiments, all mice were maintained in an animal room, which was air-conditioned (22–24°C and 55–70% relative humidity) and artificially illuminated (14 hr of light from 5:00 to 19:00 hr). All mice were fed a standard commercial diet (Lab MR Breeder; Nihon Nosan Kogyo KK, Yokohama, Japan) and provided tap water *ad libitum.*

Milk Sampling. On the evening of Day 12 of lactation, lactating mice were separated from their pups by a wire net for 14 to 15 hr and milk was collected according to the procedure of Nagasawa (3). Amount of milk collected was 0.3–2.0 ml per mouse and each pooled aliquot of milk (>5.0 ml) represents milk samples collected from three to five mice.

Milk Composition. *Fat.* Fat was determined by the creamatocrit method of Lucas *et al.* (4). About 75 μl of whole milk were drawn into a standard glass capillary tube (75 mm in length, 1.5 mm and 1.2 mm in outside and inside diameters, respectively), centrifuged at 12,000 rpm for 10 min, and fat content was obtained by the following formula.

$$Y = [(X - 0.59)/0.146] \times 0.1$$

where Y = fat content (g/100 ml) and X = percentage

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of cream length against total sample in the tube (creamatocrit percentage).

Protein. Protein was determined by the ultraviolet spectrophotometric method of Saito (5). A mixture of 0.5 ml of whole milk and 10 ml of buffer solution were filtered and the absorbancies of the filtrate were measured at wave lengths of 215 and 225 nm. Protein content was calculated by the following formula.

$$Y = 13.166\ X + 0.267$$

where Y = Kjeldahl crude protein (g/100 ml) and X = Difference in absorbance measured at two wave lengths.

Lactose. Whole milk (250 μl) was diluted with 20 ml of distilled water and filtered at pH 4.6. Lactose was determined by an enzymatic method using the F-kit (Beehringer-Manheim Co., Manheim, FRG).

Ash. Whole milk (1 ml) was incinerated at 550°C until ash was free from carbon and the residue was weighed.

Moisture. Whole milk (1 ml) was weighed in aluminum foil, dried at 99 ± 1°C for 3 hr and the residue was weighed. Moisture content equals the difference between these two weights.

Fat, protein, and lactose determinations were performed in triplicate, and the average was calculated for each sample. Ash and moisture determinations were the result of single measurements due to the limited amount of samples. All values are presented as percentages.

Pup's Growth. Average body weight and the percentage of increase in weights on Days 12 and 20 of age (lactation) were used as the indices of pup's growth (6).

Correlations between Milk Composition and Pup's Growth. Correlation coefficients were calculated between milk composition and pup's growth parameter at the first, second, and third lactations using the pooled data of four strains.

Statistics. Statistical significance of differences of correlation coefficients was determined by the method of Snedecor and Cochran (7). For all other parameters, Duncan's multiple range test was used.

Results

Milk Composition. No significant difference was observed among lactation numbers in milk composition in all mouse strains as well as in pooled data, except in the C3H/He mouse strain (Table I). In C3H/He, the contents of milk fat and moisture increased and decreased, respectively, with the advance of lactation number ($P < 0.05$). GR/A had lower and higher contents of milk fat and moisture, respectively, than C3H/He mouse strain at the third lactation ($P < 0.01$); SLN mice had a higher milk fat content than did GR/A mice ($P < 0.05$). Milk composition values in each strain and pooled data were similar to those previously reported (1, 2).

Pup's Growth. In all mouse strains as well as in pooled data, there were no significant differences between lactation numbers in any growth parameter (Ta-

Table I. Milk Composition in Each Strain (Mean ± SEM)

	SHN	SLN	C3H/He	GR/A	Pooled	Jenness and Sloan (1)[a]	Baverstock *et al.* (2)[b]
Fat							
I[c]	14.8 ± 2.2 (3)[d]	15.2 ± 0.9 (5)	14.2 ± 1.3 (5)	13.2 ± 0.5 (5)	14.3 ± 0.6 (18)	13.1 (5)	21.4 ± 3.9 (3)
II	15.5 ± 0.3 (4)	16.0 ± 0.7 (4)	17.8 ± 1.5 (5)	11.7 (2)	15.9 ± 0.7 (15)		
III	17.5 (2)	16.3 ± 1.8 (3)*	23.0 ± 1.0 (5)*†	11.9 ± 0.9 (4)	17.6 ± 1.3 (14)*		
Protein							
I	10.2 ± 0.7 (3)	10.2 ± 0.5 (5)	10.5 ± 0.5 (5)	10.5 ± 0.3 (5)	10.3 ± 0.2 (18)	9.0 (5)	6.4 ± 0.6 (3)
II	9.3 ± 0.2 (4)	10.0 ± 0.2 (4)	11.0 ± 0.3 (5)	9.9 (2)	10.1 ± 0.3 (15)		
III	10.3 (2)	11.0 ± 0.8 (3)	12.1 ± 0.6 (5)	10.5 ± 0.4 (4)	11.2 ± 0.3 (14)		
Lactose							
I	2.6 ± 0.3 (3)	2.2 ± 0.1 (5)	1.8 ± 0.1 (5)	2.2 ± 0.1 (5)	2.2 ± 0.1 (18)	3.0 (5)	2.3 ± 0.2 (3)
II	2.3 ± 0.2 (4)	2.2 ± 0.1 (4)	1.4 ± 0.2 (5)	2.1 (2)	2.0 ± 0.1 (15)		
III	2.4 (2)	2.0 ± 0.1 (3)	1.9 ± 0.1 (5)	2.0 ± 0.1 (4)	2.0 ± 0.1 (14)		
Ash							
I	1.8 ± 0.1 (3)	1.8 ± 0.1 (5)	1.9 ± 0.1 (5)	1.8 ± 0.1 (5)	1.8 ± 0.1 (18)	1.3 (5)	
II	1.7 ± 0.1 (4)	1.7 ± 0.1 (4)	1.8 ± 0.1 (5)	1.7 (2)	1.7 ± 0.1 (15)		
III	1.8 (2)	1.8 ± 0.1 (3)	1.8 ± 0.1 (5)	1.8 ± 0.1 (4)	1.8 ± 0.1 (14)		
Moisture							
I	69.8 ± 1.4 (3)	68.3 ± 1.0 (5)	66.4 ± 0.9 (5)	68.4 ± 0.9 (5)	68.0 ± 0.6 (18)	70.7 (5)	63.5 ± 3.1 (3)
II	67.7 ± 0.5 (4)	67.5 ± 0.5 (4)	64.1 ± 1.3 (5)	70.7 (2)	66.8 ± 0.7 (15)		
III	66.4 (2)	65.7 ± 2.3 (3)	59.8 ± 1.2 (5)*†	69.6 ± 1.2 (4)	64.8 ± 1.2 (14)*		

[a] In White Swiss strain.
[b] Mean of the values obtained from three periods during the first lactation in BALB/c mice.
[c] Lactation number.
[d] Number of samples.
* Significantly different from GR/A at $P < 0.05$ or 0.01. † Significantly different from I at $P < 0.05$.

ble II). Pups of the GR/A strain grew at a reduced rate when compared with pups of all other mouse strains, a finding consistent with our previous report (6).

Correlation Coefficients between Milk Composition and Pup's Weight and Growth Rate. At the first lactation, there were no significant correlations between pup's weight or growth rate and milk composition (Table III). At the second lactation, significantly ($P < 0.05$ or 0.01) positive and negative correlations were observed between pup's growth rate (on both Days 12 and 20) and the content of fat and moisture, respectively. Similarly, at the third lactation, content of fat and moisture were positively and negatively correlated, respectively, with pup's weight and growth rate ($P < 0.05$ or 0.01).

No other milk component shows a significant correlation with any pup's growth parameter regardless of the lactation number.

Discussion

These results show a positive and a negative correlation between the amount of fat and moisture, re-

Table II. Pup's Growth in Each Strain (Mean ± SEM)

		SHN	SLN	C3H/He	GR/A	Pooled
Pup's weight (g)	Day 12					
	I[a]	7.8 ± 0.2 (3)[b]	7.8 ± 0.2 (5)	7.4 ± 0.5 (5)	5.1 ± 0.1 (5)	6.9 ± 0.3 (18)
	II	7.9 ± 0.3 (4)[b]	8.4 ± 0.8 (4)	7.5 ± 0.3 (5)	5.3 (2)	7.5 ± 0.2 (15)
	III	7.3 (2)	7.3 ± 0.2 (3)	7.3 ± 0.2 (5)	4.9 ± 0.1 (4)	6.6 ± 0.3 (14)
	Day 20					
	I	8.7 ± 0.6 (3)	9.8 ± 0.3 (5)	8.8 ± 0.4 (5)	5.6 ± 0.3 (5)	8.1 ± 0.4 (18)
	II	8.7 ± 0.6 (4)	10.1 ± 1.0 (4)	9.0 ± 0.4 (5)	6.2 (2)	8.8 ± 0.4 (15)
	III	8.0 (2)	8.8 ± 0.6 (3)	9.2 ± 0.4 (5)	5.1 ± 0.2 (4)	7.7 ± 0.5 (14)
Pup's growth rate (%)	Day 12					
	I	424 ± 26 (3)	402 ± 17 (5)	426 ± 20 (5)	266 ± 6 (5)	374 ± 18 (18)
	II	404 ± 9 (4)	357 ± 37 (4)	405 ± 15 (5)	270 (2)	374 ± 16 (15)
	III	363 (2)	342 ± 43 (3)	409 ± 18 (5)	234 ± 9 (4)	338 ± 21 (14)
	Day 20					
	I	483 ± 26 (3)	532 ± 22 (5)	539 ± 30 (5)	300 ± 24 (5)	461 ± 28 (18)
	II	454 ± 26 (4)	450 ± 43 (4)	504 ± 24 (5)	303 (2)	499 ± 22 (15)
	III	406 (2)	437 ± 37 (3)	524 ± 18 (5)	256 ± 17 (4)	411 ± 32 (14)

[a] Lactation number.
[b] Mean of average values of pup's weight obtained from litters of mother mice from which milk was pooled and made one sample. Thus, the number in the parentheses is the same as that of milk samples. Values with no ±SEM are the mean of only two litters.

Table III. Correlation Coefficients between Milk Composition and Pup's Growth Parameters in Mice (Pooled Data of Four Strains)

		Fat (%)	Protein (%)	Lactose (%)	Ash (%)	Moisture (%)
Pup's weight (g)	Day 12					
	I[a]	0.14[b]	−0.29	−0.14	−0.09	0.17
	II	0.38	−0.04	0.04	−0.35	−0.27
	III	0.72**	0.27	0.16	−0.27	−0.59*
	Day 20					
	I	0.35	−0.11	0.13	0.12	−0.03
	II	0.39	0.12	−0.04	−0.03	−0.35
	III	0.74**	0.38	0.07	−0.23	−0.66**
Pup's growth rate (%)	Day 12					
	I	0.01	−0.35	−0.06	−0.06	−0.03
	II	0.55*	0.20	−0.27	0.01	−0.58*
	III	0.84**	0.42	0.01	−0.08	−0.72**
	Day 20					
	I	0.26	−0.16	−0.02	0.16	−0.19
	II	0.59*	0.39	−0.37	0.04	−0.70**
	III	0.83**	0.50	−0.07	−0.07	−0.76**

[a] Lactation number.
[b] Number of samples for correlation coefficients are 18, 15, and 14 at the first, second, and third lactations, respectively.
* or ** Statistically significant at $P < 0.05$ or 0.01.

spectively, in mouse milk and pup's growth parameters. This indicates that the fat content of milk is quantitatively important in the pup's growth and that the more aqueous the milk, the more suppressed the pup's growth. This contention is supported by the data showing that GR/A mice have reduced pup's growth compared with the other mouse strains and have lower and higher contents of fat and moisture, respectively, in the milk. Jenness (8) claimed, "No general relationship has been adduced between milk composition and the growth of young. A reasonably linear relationship holds between percentage of milk energy derived from protein and the logarithm of days required to double birth weight in some species. The species range from human, with 125 days to double birth weight and 7% of milk energy as protein, to rats, rabbits and carnivores with 7 days and 30%. This relation does not hold for aquatic and arctic mammals, whose milk energy is derived largely from fat." The results of our study suggest that mice may more closely resemble the latter species. Furthermore, our present data and the previous observations (8) suggest that the similarity of milk composition between species does not always reflect the similarity of major composition used for pup's growth.

The reason(s) why there was no significant correlation between milk fat content and pup's growth parameters at the first lactation is not clear. The present results suggest that the importance of fat in pup's growth may increase with the advance of lactation number, since the correlation coefficients between these parameters increased from the first to third lactations. Although this study shows that milk fat concentration is important in pup's growth, the contribution of other factors such as total milk production and genetic growth potential of pups must also be considered in this process.

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