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Impact of maternal iron deficiency on quality and quantity of milk ingested by neonatal rats

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1. The effect of maternal iron deficiency on milk composition and consumption by sucking rats was investigated.

2. Dams (*n* 42) were fed on semi-purified diets with either 8 (Fe−) or 250 (Fe+) mg Fe as ferrous sulphate/kg throughout gestation and lactation. Total milk intake was determined at days 7, 12 and 17 of lactation from the rate of disappearance of ^3H_2O from the total body water pool of pups. Measurements of milk constituents and Fe status of animals also were made.

3. Feeding the Fe− diet led to the development of anaemia in dams and pups and to growth retardation of sucking pups.

4. Concentrations of total lipid and Fe in milk from Fe− dams were significantly lower than those from Fe+ dams. Mean milk intakes (ml/d) of Fe-deficient pups were 21 and 28% less than intakes of Fe-sufficient pups on days 12 and 17 respectively. However, when expressed per kg body-weight, mean milk intakes were similar between groups on days 17 and 12 and increased by 47% in the Fe-deficient group on day 17 of lactation.

5. It is concluded that maternal Fe deficiency affects the quality of milk ingested by neonatal rats. However, Fe-deficient pups are at least partially able to compensate for reduced milk energy and nutrient contents by increasing intake in late lactation.

Iron deficiency, the most common nutritional disorder, is estimated to afflict 10–20% of the population worldwide (Bothwell *et al.* 1979). Infants and pregnant women are particularly vulnerable to developing Fe deficiency because of high demands for Fe during these stages of the life cycle (Dallman *et al.* 1975; Bothwell *et al.* 1979). Infants are at greatest risk because their principal and often only source of nutrition is milk, which is notoriously low in Fe (Picciano & Guthrie, 1976; Picciano *et al.* 1981). In the rat, maternal Fe deficiency results in a decrease in the concentration of milk Fe (Bartholmey & Sherman, 1985). However, the possible effects of maternal Fe deficiency on milk production and concentrations of major milk constituents have not been examined.

Recent results from our laboratory show that folate incorporation into milk is considerably reduced in severely and moderately Fe-deficient animals (O'Connor *et al.* 1987*a*). In addition to the development of anaemia, pups nursed by Fe-deficient dams show marked growth retardation. Whether this growth retardation results entirely from altered milk composition or from the combined effects of poor milk quality and decreased milk intake has not been examined. Provision of protein-deficient diets to lactating rats reduces milk output, demonstrating that maternal nutriture has a direct impact on the quality of milk available to the sucking pups (Venkatachalam & Ramanathan, 1964; Menaker & Navia, 1973). Similar relations between maternal nutrition, milk composition and output are evident in human beings. Jelliffe & Jelliffe (1978) reported that milk production may be reduced by as much as 40% in severe protein, energy, or protein–energy malnutrition. Miranda *et al.* (1983) observed a two-thirds reduction in the protein content of colostrum and a suppression of several immunologic factors in milk samples from malnourished Colombian women.

* For reprints.

Table 1. *Basal diet composition (g/kg)*

Ingredient	Amount
Casein*	220
Sucrose	300
Maize starch	315
Maize oil†	100
Cellulose‡	20
Vitamin mix§	10
Iron-free AIN-76 mineral mix‖	35

* Vitamin-free test casein (Teklad, Madison, WI).

† Mrs Tucker's pure maize oil (Anderson Clayton Foods, Dallas, TX).

‡ Alphacel (ICN Nutritional Biochemical Co., Cleveland, OH).

§ No. 40060 (Teklad) composition of vitamin mix (mg/kg diet): *P*-aminobenzoic acid 110·1, ascorbic acid, coated (97·5%) 991·2, biotin 0·441, vitamin B_{12} 29·7, calcium pantothenate 66·1, choline 0·1443, pteroylmonoglutamic acid 1·98, inositol 110·1, menadione 49·6, riboflavin 22·0, niacin 99·1, pyridoxine hydrochloride 22·0, thiamin hydrochloride 22·0, dry retinyl palmitate 39·6, dry ergocalciferol 4·405, dry tocopheryl acetate 242·3, maize starch 4·7 g.

‖ AIN-76 mineral mix prepared without Fe. Composition of mineral mix (g/kg mineral mix): $CaHPO_4$ dibasic 500·0, NaCl 74·0, potassium citrate monohydrate 220·0, K_2SO_4 52·0, MgO 24·0, $ZnCO_3$ 1·6, $MnCO_3$ 3·5, cupric carbonate 0·485, KIO_3 0·01, $Na_2SeO_3.5H_2O$ 0·01, $CrK(SO_4)_2.12H_2O$ 0·55, sucrose 124·03. $FeSO_4.7H_2O$ was added to the diets at the expense of sucrose to provide the desired Fe concentrations.

The present study was designed to investigate the influence of maternal Fe deficiency on the quality and quantity of milk delivered to sucking rat pups. Specifically, we measured milk intake, and milk contents of protein, amino acids, total lipid, ash and Fe and calculated energy intakes of Fe-sufficient and Fe-deficient rat pups.

METHODS AND MATERIALS

Animal care and dietary treatments

Virgin Sprague-Dawley rats (Harlan Industries, Indianapolis, IN), weighing 150–170 g, were housed in suspended, stainless-steel, wire-mesh cages in a room with controlled temperature, humidity (less than 40% relative humidity at 22°) and light (12 h light cycle). Rats were acclimatized for 2 weeks before breeding and fed on a non-purified ration (Purina Rodent Chow; Ralston Purina Co., St Louis, MO) and tap water. Animals weighing between 180 and 220 g were mated. Pregnancy (day 1) was confirmed by the presence of vaginal plugs and sperm. On day 1 of pregnancy, rats (*n* 42) were randomly assigned to one of two experimental dietary treatments: Fe-sufficient (Fe+) and Fe-deficient (Fe−).

The two experimental diets (Table 1) were formulated to be nutritionally adequate for reproduction in the rat, except for Fe (National Research Council, 1978). Direct analyses confirmed dietary Fe concentrations of 8 mg/kg (Fe−) and 250 mg/kg (Fe+). Demineralized water (Nanopure, Barnstead, Boston, MA) and the experimental diets were fed *ad lib*. At 2 d before the expected date of delivery, dams were placed in large maternity cages in which a smaller pup cage containing Fe-free bedding was placed (San-i-cel; Paxton Processing Co., Paxton, IL). Litters were culled to seven pups (four males and three females) on day 2 of lactation. Dams were permitted to nurse their young (seven pups per litter) until day 7, 12 or 17 of lactation (seven litters per dietary treatment at each stage of lactation measured). Food and water were positioned exterior to the inner pup cage so that only the dams had access to them.

Milk intake determination

On the mornings of days 4, 9 and 14, immediately following the 12 h dark cycle, pups were separated from dams and ear-notched to enable identification. Five pups in each litter were given a 30–50 μl subcutaneous injection (amount depending on age) of a tritiated physiological saline (9 g sodium chloride/l) solution (50 Ci/l; Amersham, Arlington Heights, IL). Two pups in each litter were sham injected with unlabelled physiological saline. This dose of radioactivity was chosen to permit a reliable measurement of isotope recycling among dams and pups. At 24, 48, and 72 h after injection, each pup was weighed and a 10 μl tail blood sample taken and placed in 10 ml scintillation cocktail (Beckman LS9000, Fullerton, CA). This procedure resulted in a total water intake measurement for two consecutive days. Immediately following the collection of tail blood, two litters in each treatment group at each experimental period were killed by cervical dislocation, cut open, frozen and freeze dried (Labconco Co., Kansas City, MO) for determination of total body water. Total water intake was estimated as described by Coward *et al.* (1982), using the following equation to calculate the disappearance of ^3H_2O from the total body water pool:

$$\text{Total water intake} = \frac{Q_1 - Q_2}{\log Q_1/Q_2} \times \log C_1/C_2 ,$$

where Q represents total body water volume of the pup, C the specific activity of ^3H_2O in whole blood at the beginning (Q_1, C_1) and end (Q_2, C_2) of the water intake measurement period. Since packed cell volume values did not change more than 2·5% in both control and experimental groups over the period of measurement, it was not necessary to alter this equation for the determination of total water intake in the Fe-deficient group. For the measurement of total milk intake, values for total water intake were corrected for the water produced from the oxidation of protein, fat and carbohydrate in the milk samples (Bergmann *et al.* 1974).

Milk and tissue collection

Approximately 1 h after tail blood collection from pups, dams were injected with ketamine hydrochloride (Ketaset; Bristol Laboratories, Syracuse, NY; 1·0 ml/kg) and oxytocin (Sigma Co., St Louis, MO; 2·5 UPS units/kg) to facilitate milking. Milk was expressed from the mammary gland by gentle massaging and collected by flexible capillary tubing using a suction apparatus. The tube in which milk was collected was kept on ice. Samples of milk and tissues were partitioned among several containers and stored at −70° before analyses. After milking, dams and remaining litter mates were anaesthetized with chloroform and blood collected by cardiac puncture. Livers were removed and rinsed with demineralized water, blotted dry and weighed.

Milk and tissue biochemical analyses

The total water content of milk was determined by weighing 500 μl of milk before and after drying overnight (60°). Ash content was determined after the dried milk sample was oxidized in a muffle furnace for 12 h at 550°. The protein content of milk samples was estimated using the BCA protein assay (Pierce Chemical Co., Rockford, IL) and bovine plasma no. 909 as the reference standard (National Bureau of Standards, Gaitherburg, MD) (Keller & Neville, 1986). Amino acid analyses were performed after 21 h hydrolysis of milk samples in 6 M-hydrochloric acid at 100° using a Beckman 119CL amino acid analyser (Fullerton, CA). Milk fat determinations were measured using the creamatocrit method as described by Lucas *et al.* (1978). The latter procedure was standardized using a

Table 2. *Mean body-weight and measurements of iron status of dams and pups*
(Mean values with their standard errors for seven dam samples and five pooled litter (seven pups/litter) samples)

	Dietary Fe (mg/kg)	Post-natal day 7 Mean	7 SEM	12 Mean	12 SEM	17 Mean	17 SEM
Dams:							
Initial body-wt (g)	250	191	3	193	3	194	4
	8	196	6	202	4	190	2
Final body-wt[a,b] (g)	250	245	5	226	10	254	5
	8	261	5	270***	6	245	6
Haemoglobin[a] (g/l)	250	132	2	135	6	141	2
	8	89***	3	97***	4	101***	2
Liver Fe (μg/g dry wt)	250	516·3	40·4	495·6	48·4	555·8	67·6
	8	115·3***	26·7	178·2***	26·3	140·8***	17·5
Pups:							
Day 2 body-wt (g)	250	6·7	0·1	6·1	0·4	6·5	0·2
	8	6·0	0·3	5·9	0·2	6·5	0·6
Final body-wt[a,b] (g)	250	14·4	0·2	25·8	1·0	43·4	1·7
	8	11·5*	0·8	19·0***	1·0	22·0***	1·6
Haemoglobin[a,b] (g/l)	250	72	3	89	5	93	3
	8	49***	2	39***	3	50***	2
Liver Fe (μg/g dry wt)	250	293·9	28·4	166·9	14·9	91·0	15·0
	8	64·1***	23·2	74·8*	21·1	92·1	39·0

Analysis of variance (2 × 3 factorial): [a] significant ($P < 0·05$) time effect, [b] significant ($P < 0·05$) interaction. Comparisons between treatments were made using least significant difference tests. Differences between dietary treatment groups at each time were significant: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

modified Folch method for total lipid analyses (Timmen & Dimick, 1972). Carbohydrate content was calculated by difference for the purposes of correcting water intake values for the measurement of milk intake and for the estimation of pup energy intake.

Haemoglobin (Hb) concentrations were determined in whole blood by the cyanomethaemoglobin method (Richterich, 1969) using prepared reagents and standards (Sigma Diagnostics, St Louis, MO). Livers were dried to a constant weight (90°) and ashed (550°) for a total of 24 h. Nitric acid and two drops of a potassium sulphate solution (100 g/l) were used to facilitate ashing. Ashed diets, milk and livers were analysed for Fe using atomic absorption spectrophotometry (Allied Analytical Systems Model no. 951; Waltham, MA). Accuracy and reproducibility of analytical procedures for Fe determinations were assessed using orchard leaves no. 1571 and bovine liver no. 1577 as standard reference materials (National Bureau of Standards).

Statistical analyses

Results were evaluated using analysis of variance statistics (2 × 3 factorial), *F* tests, least significant difference tests and *t* tests (Steel & Torrie, 1980; SAS Institute Inc., 1985). Where not shown otherwise, a value of $P < 0·05$ was taken to be statistically significant.

Table 3. *Mean proximate constituents and amino acid profiles following acid hydrolysis of milk samples from Fe-sufficient and Fe-deficient rats during lactation*

(Statistical analyses indicated no significant effect of stage of lactation on nutrient composition, therefore, pooled treatment means are presented for purposes of clarity. Values are for thirteen to twenty samples with their standard errors except for amino acid profiles which were performed on four samples/treatment)

	Dams fed			
	250 mg Fe/kg		8 mg Fe/kg	
Milk	Mean	SEM	Mean	SEM
Water (g/kg)	604·7	11·5	634·1	13·6
Fat (g/kg)	269·5	11·3	204·5*	10·0
Ash (g/kg)	1·6	0·1	1·9	0·1
Fe (mg/kg)	8·7	2·2	3·7*	0·4
Protein (g/kg)	142·8	12·5	128·1	9·9
Amino acid profile (nmol/l)				
Aspartic acid	75·6	4·8	72·6	3·9
Threonine	38·5	2·6	36·3	1·7
Serine	87·0	5·0	83·6	4·7
Glutamic acid	162·6	10·6	152·8	8·8
Proline	63·3	8·4	48·3	9·1
Glycine	22·0	1·7	20·5	1·4
Alanine	64·5	3·7	52·2	7·9
Valine	44·1	3·0	38·3	2·7
Methionine	11·9	0·8	16·4	4·2
Isoleucine	35·2	2·1	30·1	3·7
Leucine	82·0	4·7	89·1	11·0
Tyrosine	22·4	1·3	21·4	0·9
Phenylalanine	28·2	1·6	26·3	1·1
Histidine	17·5	0·9	16·5	0·8
Lysine	45·6	3·1	43·5	1·9
Arginine	22·3	1·6	21·1	0·9

Differences between dietary groups were significant (t test): * $P < 0·05$.

RESULTS

Maternal feed intake, body- and organ weights

Values summarizing changes in maternal and pup body-weights during lactation are shown in Table 2. Dietary Fe concentration had no effect on food consumption or body-weight of dams during reproduction except at day 12 of pregnancy when Fe-deficient dams weighed 19% ($P < 0·001$) more than control animals. Pups in the experimental group (Fe−) were smaller than those in the control group (Fe+) on days 7, 12 and 17, with Fe− pups weighing 20 ($P < 0·05$), 26 ($P < 0·001$) and 49% ($P < 0·001$) less than age-matched Fe+ pups respectively. Mean liver weights (values not shown) of Fe− pups were also reduced by 16·1–39·3% ($P < 0·01$). In contrast, no differences in body-weight were found between Fe+ and Fe− pups on day 2 of lactation.

Fe status

Mean Hb concentrations for both dams and their pups strongly reflected the Fe concentration of the diets fed to dams (Table 2). Both dams and pups in the experimental group (8 mg Fe/kg) showed evidence of severe Fe deficiency anaemia with mean Hb

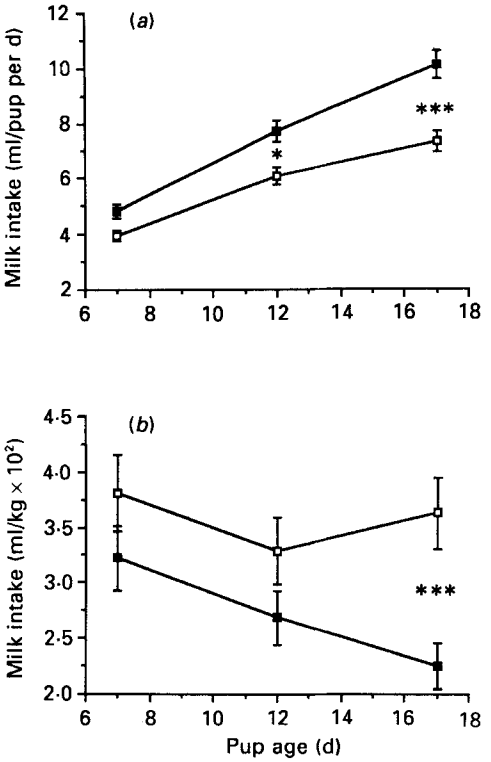

Fig. 1. Mean daily milk intake expressed as (*a*) ml/pup and (*b*) ml/kg body-weight of Fe-sufficient (■) and Fe-deficient (□) sucking rats. Points are means with their standard errors represented by vertical bars. Differences between dietary treatment groups were significant: * $P < 0.05$, *** $P < 0.001$.

concentrations less than 30% of animals in the control group ($P < 0.001$). Hb concentrations for both Fe+ and Fe− dams and Fe+ pups increased as lactation progressed. In contrast, Hb concentrations decreased between days 7 and 14 in Fe− pups. Except on day 17, mean liver Fe concentrations in Fe− pups were 20% less than concentrations in control pups ($P < 0.05$).

Milk composition

Mean milk Fe concentration from Fe− dams was 57% ($P < 0.05$) lower than that from control animals (Table 3). Proximate analysis of milk samples showed that milk from Fe− dams was 24% lower in fat than milk from Fe+ dams ($P < 0.05$). Measurements of water, ash, protein and amino acids of milk samples from control and experimental animals were similar.

Milk intake

Daily milk intake expressed as ml/pup and ml/kg body-weight is summarized in Fig. 1. Milk consumption (ml/pup) of both groups increased linearly over time ($P < 0.001$). When milk intake was expressed as ml/pup per d, Fe+ pups ingested significantly more milk than did Fe− pups ($P < 0.001$). However, when milk consumption was expressed on a body-weight basis, 17 d Fe-deficient pups ingested 46·9% more milk than Fe-sufficient controls ($P < 0.001$). While control pups consumed significantly more energy (kJ/d) (Table 4) than Fe-deficient pups ($P < 0.001$) when energy intake was expressed on a body-weight basis, only at day 7 did Fe+ pups receive more energy than Fe− pups. Fe intakes, expressed

Table 4. *Energy intakes, iron intakes and feed efficiency of Fe-sufficient and Fe-deficient rat pups at 7, 12 and 17 d of age*

(Mean values with their standard errors expressed per pup were obtained from seven pooled litter measurements (seven pups/litter))

	Dietary Fe (mg/kg)	Age of pup (d)					
		7		12		17	
		Mean	SEM	Mean	SEM	Mean	SEM
Energy intake[a,b] (kJ/pup per d)	250	60·5	2·3	96·6	6·7	127·0	7·0
	8	40·8**	1·9	63·6***	3·5	76·3***	5·1
Energy intake[a,b] (kJ/kg)	250	4200	200	3700	200	2900	200
	8	3600*	200	3200	100	3400	300
Feed efficiency[a,b] (g gain/kJ intake)	250	0·030	0·003	0·026	0·004	0·024	0·003
	8	0·027	0·005	0·021	0·003	−0·005	0·003
Fe intake[a,b] (μg/d per pup)	250	41·9	1·6	67·0	0·6	88·0	4·9
	8	14·4***	0·7	22·4***	1·2	26·8***	1·8
Fe intake[a,b] (mg/kg)	250	2·9	0·1	2·5	0·1	2·0	0·1
	8	1·3***	0·1	1·1***	0·0	1·2***	0·1

Analysis of variance (2 × 3 factorial): [a] significant ($P < 0·05$) time effect, [b] significant ($P < 0·05$) Fe × time interaction. Comparisons between treatments were made using least significant difference tests. Differences between dietary treatments at each time were significant. * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

either as μg/d or mg/kg per d, were significantly less among Fe− pups than control pups ($P < 0·001$).

DISCUSSION

Results from the present study provide evidence that maternal Fe deficiency affects the quality of milk ingested by the neonatal rat. Milk secreted by Fe-deficient dams contained less fat and Fe and was less energy-dense than milk from control dams. However, Fe-deficient pups attempted to compensate for poor milk quality be increasing milk intake in late lactation. Despite obvious signs of morbidity, 17-d-old Fe− pups consumed significantly more milk than control pups when quantity ingested was expressed on the basis of body-weight (363 *v.* 248 ml/kg per d).

Experimental evidence indicates that animals will eat to meet their energy and protein needs (Donaldson *et al.* 1956; Renner, 1964). Although milk samples from both control and experimental dams contained similar amounts of protein and constituent amino acids, samples from Fe− dams with less fat were less energy-dense. The observed increase in milk intake on day 17 by Fe− pups was likely in response to the reduced energy density of milk provided by Fe− dams. This observation is consistent with reports that, after 2 weeks of age, pups demonstrate the capacity to respond to a period of fasting or an energy deficit by increasing milk intake (Henning *et al.* 1979; Henning, 1981). Pups show their awareness of energy deprivation following a fasting period by quickly attaching to the teats of their mother on return to the nest and, when milk let-down is chemically blocked, by rapidly shifting among nipples. A similar transition period to adult feeding behaviour occurs in human infancy. Fomon *et al.* (1969, 1975) found that before 41 d of age, bottle-fed infants do not regulate their intake based on the energy density of formula provided. Regardless of the energy density of the formula fed, the volume of milk consumed during the first 41 d of life is relatively constant. After adult feeding behaviour is established, human

infants suck to meet their energy needs and milk intake becomes inversely proportional to the energy density of the formula fed.

Except in early lactation (day 7), Fe− pups consumed an amount of energy per kg body-weight that was similar to that of control animals (Table 4). In late lactation (day 17), however, Fe− pups showed negative energy efficiency (g gain/kJ intake), indicating that energy consumed was not being utilized for growth and that body stores were being mobilized. While Fe− pups appeared to compensate for reduced milk energy by increasing milk intake, absolute energy requirements of Fe− pups may be greater than control pups (Dallman, 1986). If this were the case, energy requirements were not met by Fe− pups even though milk consumption was increased (46%) to a level which achieved energy intakes similar to those of control pups.

Alternatively, changes in milk quality other than energy density may have been responsible for impaired growth of Fe− pups. In the present study, Fe− pups received less Fe than control pups throughout lactation despite the increased milk intake in late lactation. Results from animal studies show that Fe deficiency alone causes growth retardation (Beard *et al.* 1984; Martinez-Torres *et al.* 1984). Furthermore, Lanzkowsky *et al.* (1981) found that pups born to Fe-sufficient dams but nursed by Fe-deficient dams develop Fe deficiency as evidenced by depressed values for Hb and serum Fe on day 21 of lactation. We previously reported that sucking offspring develop signs of folate deficiency when nursed by Fe− dams and that Fe− dams secrete milk with decreased milk folate content (O'Connor *et al.* 1987*a*, *b*). Cellular depletion of folate likewise results in impaired growth (Baumslag *et al.* 1970; Iyenar & Rajalakshmi, 1975; Rolschau *et al.* 1979). Results from this investigation do not permit an evaluation of the relative impact on the neonate of various changes in milk composition during maternal Fe deficiency; however, they do stress the importance of maintaining milk quality and the vulnerability of the sucking neonate.

Observations from the present and previous studies (Kochanowski *et al.* 1983; O'Connor, 1987*a*) suggest that Fe may serve an important function in the regulation of nutrient utilization by the mammary secretory cell. In the present study, we observed that milk from Fe-deficient dams has not only a reduced Fe content but a reduced lipid content. Maternal Fe deficiency also blocks preferential partitioning of folate to the mammary gland for secretion (O'Connor *et al.* 1987*a*). In the absence of Fe deficiency, regulatory mechanisms exist to maintain the nutrient composition of milk even at the expense of maternal reserves. For example, maternal metabolism may be depressed in undernourished lactating women (Prentice *et al.* 1981) and in lactating dairy cattle (Bauman & Currie, 1980) allowing for increased nutrient partitioning to the mammary gland for milk synthesis and secretion.

In summary, results from the present study showed that Fe deficiency caused a decrease in the lipid, Fe and energy contents of milk. While increased milk intake by Fe− pups appeared to compensate for the reduced fat and energy contents of milk, it did not compensate for reduced milk Fe. In the light of present findings, the possible influence of maternal Fe deficiency on the nutrient composition of milk from human beings merits investigation.

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