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Bioavailability of lead from various milk diets studied in a suckling rat model

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The bioavailability of lead from various milk diets was studied in 14 day old suckling rats. Human milk, infant formula, cow's milk, rat milk and deionized water labeled with ^{203}Pb were given to rat pups by gastric intubation. Animals were killed after 2 or 6 h and the radioactivity in the tissues was measured. At 2 h after administration the lead bioavailability, defined as lead uptake in the body, excluding the gastrointestinal tract, was 47% from water, 42% from human milk, 40% from infant formula, 31% from cow's milk and 11% from rat milk. After 6 h the bioavailability of lead was about 50% from water and human milk, 45% from infant formula and cow's milk, and 36% from rat milk. The blood lead levels in the pups reflected the total body uptake and were also correlated to the brain lead levels. Thus, rat pups given lead in human milk had approximately twice as high lead levels in blood and brain than pups given lead in rat milk. The intestinal absorption of lead was dependent on the milk diet given to the sucklings. In duodenum, the highest uptake of lead was found in rats given water or human milk, whereas in rats given rat or cow's milk the highest uptake of lead was found in ileum. The distribution of lead in cream, whey and casein fractions of the milk diets after *in vitro* labeling with ^{203}Pb was also studied. The casein fraction in cow's and rat milk contained 90–96% of the total amount of lead in the diet. In infant formula and human milk, 77 and 56% lead was found in the casein fraction, respectively. The higher lead bioavailability observed in the suckling rat fed human milk than in those fed rat and cow's milk may partly be explained by a lower proportion of lead bound to casein in human milk.

Keywords: casein, intestinal absorption, lead

Introduction

During infancy the central nervous system is especially susceptible to the toxic effects of lead. Several reports have shown that lead exposure during this period is connected with intellectual impairment and behavioral deficits (Davis & Svendsgaard 1987, McMichael *et al.* 1988, Mushak *et al.* 1989, Needleman & Gatsonis 1990, Needleman *et al.* 1990). The major source of lead exposure in infants and young children living in non-contaminated areas are the diet and drinking water. In addition, it is known that both absorption and retention of ingested lead is higher for children than for adults (Kostial *et al.* 1971, Ziegler *et al.* 1978). The intake of lead in the neonate is dependent on the concentration in breast milk, infant formula and drinking water (FAO 1986). Human milk generally has a low concentration of lead, about 1 ng g^{-1}, as reported by Dabeka & McKenzie (1988). However, in polluted areas the concentration is much higher, a mean of 62 ng ml^{-1} with a maximum value of 350 ng ml^{-1} being reported for women living close to a smelter in Mexico (Namihira *et al.* 1993). The levels of lead in infant formula are dependent on the water content of lead and the packaging material. Concentrations from 1.6 to 95 ng g^{-1} in infant formula were found by Dabeka (1989), with the highest levels present in evaporated milk stored in lead-soldered cans. Normally, the lead concentration in tap water is less than 10 $\mu g\, l^{-1}$, but markedly higher concentrations are found in regions where lead piping carries soft water, where the concentration can reach 5–10 times higher levels (DHSS 1980).

Dietary factors are known to influence the absorption of lead, e.g. lactose and milk diets have been shown to increase the retention of ingested lead in experimental animals (Kello & Kostial 1973, Bushnell & DeLuca 1981), probably by facilitating intestinal absorption. However, there is conflicting evidence, as other investigators have not found

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any stimulatory effect of milk on lead absorption (Garber & Wei 1974, Henning & Leeper 1984).

Most dietary studies of lead have been performed in adult animals and may thus not be relevant to the suckling period, since the sucklings have in some respects an immature gastrointestinal function. There might also be some species differences in gastrointestinal absorption. Suckling rats have been suggested as a suitable model for humans concerning the gastrointestinal absorption of zinc, as studies have shown that the bioavailability of zinc in the suckling rat was similar to the bioavailability of zinc found in humans (Sandström *et al.* 1983). They also suggested that the lower bioavailability of zinc and copper from cow's milk in contrast to human milk was influenced by the casein content of the milk (Lönnerdal *et al.* 1985, Lönnerdal 1991). It has been shown that lead associates to the casein micelles in milk to a high extent (Beach & Henning 1988), hence the bioavailability of lead might be influenced by the casein content in the milk diet. The purpose of the present study was to investigate the bioavailability of lead from various milk diets and to study the impact of the casein content in milk.

Material and methods

Milk diets

Human milk was obtained from four healthy donors by a manual breast pump at the morning feeding and used in experiments on the same day. The donors were between 1 and 6 months after parturition. Pasteurized, homogenized cow's milk, 3% fat (Arla, Sweden) and whey-adjusted infant milk formula based on cow's milk, BabySemp 1 (Semper AB, Stockholm, Sweden), were commercially obtained. Fresh rat milk was obtained from lactating Sprague-Dawley rats (Möllegaard, Denmark) at day 14 of lactation by a milk pump operated at vacuum (Oskarsson 1987).

Isotope labeling of milk, fractionation and analysis

^{203}Pb chloride, specific activity 72 mCi μmol^{-1}, was purchased from the The Svedberg Laboratory, Uppsala University, Sweden. To each milk diet of 8 ml was added 0.1 μCi ^{203}Pb in a volume of 40 μl deionized water. The labeled diets were incubated for 1 h at 37°C on rotating tables to allow isotopic equilibrium. About 1.5 ml of milk and formulas were separated into casein, whey and cream by ultracentrifugation (105 000 *g*, 45 min, 4°C) as described by Fransson & Lönnerdal (1983). The cream layer was removed by a spatula and the whey fraction by a pipette. The radioactivity in each fraction was measured in a gamma counter (Nuclear Chicago, Model 4230) using the characteristic line of 279 keV photon emission with a counting efficiency of 60%. The activity obtained was corrected for decay using a $t_{1/2}$ value for ^{203}Pb of 52.1 h. A ^{203}Pb standard, with known content of radioactivity, was counted at each counting session to check the half-life and the counting efficiency.

The casein concentration in the milk diets was estimated as follows. The total milk protein concentration in whole milk was measured by the method of Lowry *et al.* (1951). After ultracentrifugation, the protein concentration in whey was determined. The difference between total protein and whey protein concentrations provided an estimate of the casein concentration.

The distribution of ^{203}Pb in milk labeled *in vivo* was determined in six Sprague-Dawley rats on day 14 of lactation. The rats were given an intravenous injection of 2.4 μCi ^{203}Pb diluted in deionized water (1 μl g^{-1} body weight). After 4 h about 1.5 ml of milk was collected from each rat and fractionated in the same way as described above.

Animal experiment

Sprague-Dawley rats with litters, obtained from Möllegaard, Denmark on day 7 after parturition, were housed in individual cages and fed R3 pellets (Astra Ewos AB, Södertälje, Sweden) and tap water *ad libitum*. On day 14 *post partum*, litters were separated from their dams and randomly divided into 10 groups with five pups per group. ^{203}Pb, 80 μCi (232 ng Pb) in a volume of 20 μl was mixed with 8 ml of milk and formulas for 1 h at 37°C on rotating tables to allow isotopic equilibrium. At 5 h after separation, the pups received a dose of 5 μCi ^{203}Pb (14.5 ng Pb) in 0.5 ml of human milk, cow's milk, infant formula, rat milk or water by gastric intubation. The pups were sacrificed 2 or 6 h after intubation by heart puncture under anesthesia with diethylether. Blood, liver, kidney and brain were taken from all pups, and the radioactivity was measured. The stomach and whole intestine were carefully removed, and the small intestine was cut out beginning at the pylorus and continuing to the ileocecal junction. The intestine was flushed with 20 ml of 150 mM Na-acetate, containing 5 mM EDTA. After washing, the intestine was divided into 12 equal segments and only the segments with odd numbers were used for measurement of ^{203}Pb. Segment 1 representing duodenum, segments 3 and 5 representing jeujenum, and segments 7, 9 and 11 representing ileum, were transferred to counting vials. After removal of the tissues the radioactivity in the pups' carcasses was measured in a whole body gamma counter (NaI well crystal; diameter 80 mm; depth 120 mm). The counting efficiency was 50%. The total amount of absorbed lead in the body was obtained by summarizing the lead content in the blood and tissues with the whole body content, excluding the gastrointestinal tract. The results were tested for significance between the groups with analysis of variance and LSD multiple range test (Stat Graphics; STCS Inc., Rockville, MD, USA).

Results

Distribution of lead in the milk diets

The distribution of ^{203}Pb in the diets is shown in Table 1. In all the milk diets the highest content of lead was found in the casein fraction. Human milk had the lowest content of lead in the casein fraction, 56%. On the other hand, the

Table 1. Distribution of ^{203}Pb in various milk diets, labeled *in vitro*; all samples were analyzed in duplicate or triplate [the distribution is expressed as percentage of total ^{203}Pb in the diet (mean ± SD)]

Milk	Fat (%)	Whey (%)	Casein (%)	Casein (mg ml^{-1})
Human milk ($n=4$)	9.3 ± 1.1	34.4 ± 13.0	56.0 ± 12.9	3.2 ± 1.1
Infant formula ($n=3$)	16.2 ± 5.5	6.7 ± 2.8	77.2 ± 7.6	7.8 ± 3.7
Cow's milk ($n=4$)	4.8 ± 1.3	4.5 ± 1.9	90.6 ± 2.3	30.0 ± 4.0**
Rat milk ($n=5$)	2.7 ± 1.4	3.9 ± 1.8	93.5 ± 3.0	71.1 ± 5.9**[a]
Rat milk, *in vivo* ($n=6$)	2.0 ± 0.7	1.6 ± 0.5	96.5 ± 0.8	

[a] The casein content in rat milk was determined in five control rats.

** Significantly different ($P<0.01$) from human milk and infant formula.

Table 2. Concentration of lead in blood, brain, liver and kidney in 14 day old pups at 6 h after gastric intubation of ^{203}Pb (14.5 ng Pb) in water and various milk diets [values are expressed in ng g^{-1} wet weight tissue (mean ± SD)]

Milk diet	Blood	Brain	Liver	Kidney
Water	0.73 ± 0.06**[b]	0.028 ± 0.003	1.49 ± 0.11	0.81 ± 0.12
Human milk	0.84 ± 0.07	0.032 ± 0.004	1.44 ± 0.12	0.94 ± 0.06
Infant formula	0.60 ± 0.05**[a,b]	0.021 ± 0.002**[a,b,d]	1.16 ± 0.08**[a,b]	0.79 ± 0.06
Cow's milk	0.65 ± 0.05**[b]	0.028 ± 0.002	1.27 ± 0.13**[a]	0.81 ± 0.05
Rat milk	0.39 ± 0.05**[a,b,c,d]	0.017 ± 0.003**[a,b,d]	0.85 ± 0.12**[a,b,c,d]	0.73 ± 0.11**[b]

** Significantly lower ($P<0.01$) from [a] water, [b] human milk, [c] infant formula and [d] cow's milk.

lead content in the whey fraction of human milk was higher than in the other diets. Cow's and rat milk showed a similar distribution, with about 90% or more of the total lead in the casein fraction and minor amounts of lead in the whey and fat fractions. Rat milk labeled with ^{203}Pb *in vivo* showed an almost identical distribution of lead as in the *in vitro* labeled rat milk. Although not tested due to practical reasons, this is suggested to be true also for the other milk diets. The casein concentration in human milk was estimated to be 3.2 ± 1.1 mg ml^{-1}, which was about 10 and 20 times lower than in cow's and rat milk, respectively.

Bioavailability of lead

The total body absorption of ^{203}Pb at 2 and 6 h after gastric intubation of water or milk diets is shown in Figure 1(a and b). At 2 h after administration, the absorption of lead decreased in the order: water ~ human milk > infant formula > cow's milk > rat milk with 47% absorption from water and 11% from rat milk. At 6 h after administration the absorption of lead from rat milk had increased to 36%, while the absorption from water remained at approximately the same level as at 2 h. Lead absorption from the other milk diets also increased after 6 h, but not as much as from rat milk.

Table 2 shows the lead concentrations in blood, liver, kidney and brain at 6 h after administration. The blood lead levels were lowest in pups given lead in rat milk and highest in pups given lead in human milk and water. Infant formula and cow's milk resulted in intermediate and similar blood lead levels. In brain, rat milk as well as infant formula gave

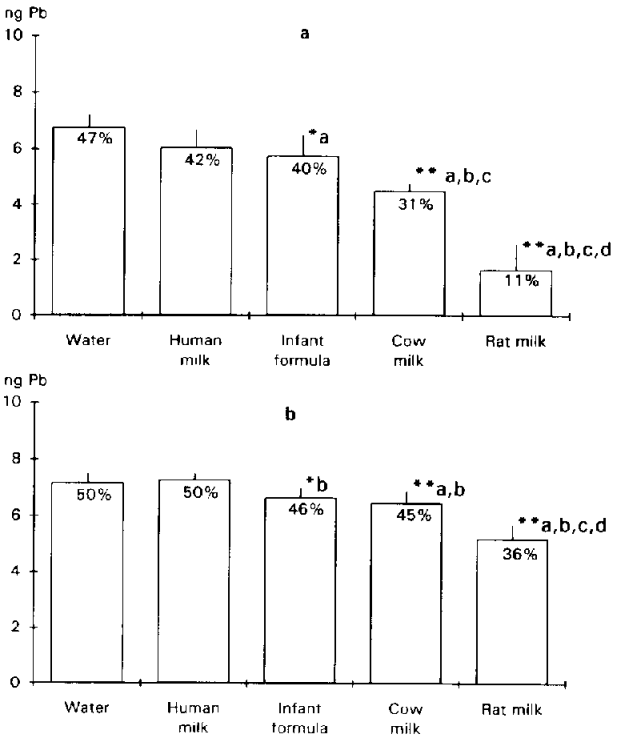

Figure 1. Lead absorption from various milk diets and water in 14 day old rat pups 2 h (**a**) and 6 h (**b**) after gastric intubation. Mean values (±SD; $n=5$) of total lead content in the rat pup and percentage absorption of total dose given. * $P<0.05$, ** $P<0.01$ compared with [a] water, [b] human milk, [c] infant formula and [d] cow's milk.

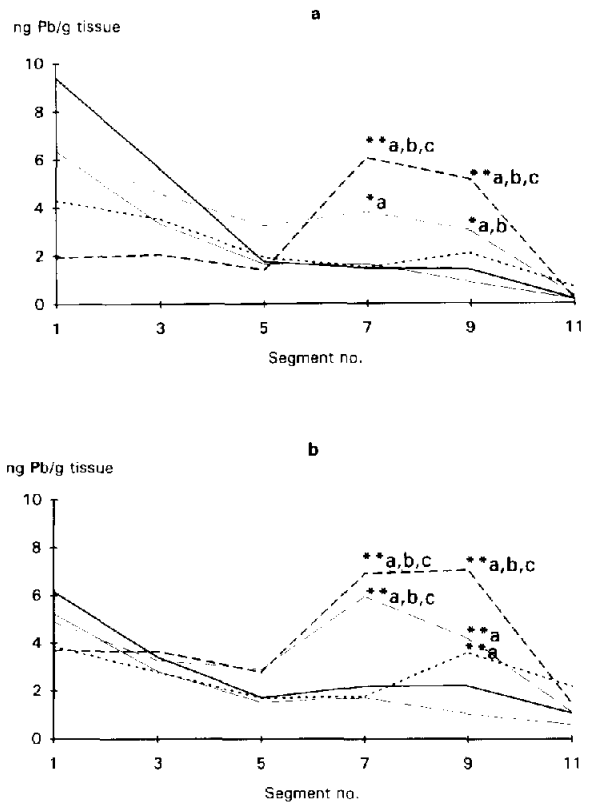

Figure 2. Lead uptake from various milk diets and water in different segments of the small intestine of 14 days old rat pups 2 h (**a**) and 6 h (**b**) after gastric intubation. Water ( ), human milk (——), infant formula (·····), cow's milk (— — —) and rat milk (– – –). * $P<0.05$ and ** $P<0.01$ compared with [a]water, [b]human milk, [c]infant formula and [d]cow's milk

the lowest lead levels, and the other diet groups had about the same lead levels in brain. In liver, the lowest lead levels were found in pups given lead in rat milk, while the highest lead levels were found when lead was given in human milk or water. There were significantly lower lead levels in the kidney in pups given lead in rat milk than in pups given lead in human milk.

The uptake of lead in different segments of the small intestine at 2 and 6 h post-feeding is shown in Figure 2(a and b). At 2 h after administration, the highest uptake of lead in duodenum (segment 1) was observed in pups given human milk, 9.4 ng g^{-1} wet weight tissue. The lowest uptake of lead in this segment, 1.9 ng g^{-1} tissue, was observed in pups given rat milk. In contrast, these pups had the highest uptake of lead in segments 7 and 9 (ileum), i.e. 6.1 and 5.1 ng g^{-1} tissue, respectively. At 6 h after administration, the uptake of lead in segment 1 was still highest in pups given lead in human milk and lowest in pups given lead in rat milk. The high uptake of lead in segments 7 and 9 of pups given lead in rat milk was even more pronounced after 6 h, when there also was a high lead uptake in pups that received lead in cow's milk.

Discussion

The present results show that milk composition has an impact on the bioavailability of lead in the suckling rat. Casein was found to be the major component binding lead in all the tested milk diets. The reason for this is probably the presence of phosphoserine groups on the casein molecule, which have been shown to bind cations like iron and calcium (Greenberg *et al.* 1976, McMahon & Brown 1984) and most likely also lead, as lead and calcium interact in many biological systems (Simons 1986). It is possible that the absorption of lead in milk is limited by the casein binding of lead. In rat and cow's milk with a casein concentration of 71 and 30 mg ml^{-1}, respectively, about 90% of total lead in these diets was bound in the casein fraction. These diets resulted in the lowest absorption of lead in the suckling rats both at 2 and 6 h after administration. Human milk resulted in the highest absorption of lead. In human milk, the casein concentration was only 3 mg ml^{-1} and a much smaller proportion of total lead, 56%, was bound to casein. Similar casein concentrations, 2–6 mg ml^{-1} in human milk, have been reported by others (Stebler & Guentert 1990, Lönnerdal & Forsum 1985). Besides the low casein concentration, there are other possible explanations of the high bioavailability of lead. About 30% of the lead in human milk was found in the whey fraction. The whey in human milk contains serum albumin and lactoferrin, which have been shown to bind trace metals like iron, zinc and copper, and thereby may facilitate absorption (Fransson *et al.* 1983, Lönnerdal 1991). Lead binding to these components in human whey has not yet been investigated.

Henning & Leeper (1984) and Henning & Cooper (1988) studied the uptake of lead in the intestine of suckling rats. They found two sites of lead uptake in the small intestine, i.e. the duodenum and ileum. They suggested that the duodenum is the site of absorption of lead into the circulatory system. However, lead levels in other tissues or in blood were not reported. In the present study, we also found these two sites of retention in the small intestine and also that the retention of lead in the intestine was dependent on the milk diet. Pups administered lead in milk with a low casein content, such as human milk, showed the dominant uptake of lead in the duodenum, whereas pups given milk with high casein content, such as cow's and rat milk, had the highest concentration of lead in the ileum both at 2 and 6 h after administration. The total body absorption of lead measured without the gastrointestinal tract was most rapid and highest in the groups that received lead in milk with a low casein content, i.e. human milk. In this case the absorption of lead seemed to occur mainly in the duodenum, which also is supposed to be the site of absorption of lead in adult animals (Mushak 1991). It has been shown that ileal epithelium in suckling rats and mice has a high capacity for non-specific pinocytosis, which is the principal route of absorption of dietary proteins at this age (Jones 1978, Keller & Doherty 1980). In the present study, the total body absorption of lead increased after 6 h in rat pups given lead in milk with high casein content. It is suggested that the

ileal uptake represents a site of absorption of lead associated to casein.

It is not known if human infants absorb proteins with a pinocytotic activity in the ileum in the same way as the rat and mouse pup. It has been shown that pepsin digestion of human infants is poor (Mason 1962) and that intact casein and individual proteins are able to pass through the gastrointestinal tract undigested until the age of about 4 months (Fomon 1974). In addition, infants fed formulas based on cow's milk are more likely to suffer from deficits in trace elements, such as iron, copper and zinc, compared with breast-fed infants (Lönnerdal 1985). This may partly be explained by the binding of trace elements to casein in the diet together with a limited digestive capacity of the infant (Harzer & Kauer 1982, Fransson & Lönnerdal 1983, Lönnerdal 1985). In contrast, human milk will result in a high bioavailability of trace elements, which may be explained by low levels of inhibitory factors such as casein in human milk but also the presence of some stimulatory factors may be responsible for this high bioavailability.

The concentration of lead in blood is often used as an indicator of lead exposure. It was shown in the present study that blood lead levels gave a good reflection of the total lead uptake in the body after administration of lead in various milk diets. In this model, blood lead levels were also very well correlated to brain lead levels. Brain is the main target organ for lead toxicity in newborns and the present results indicate that lead levels in blood can be used as a predictive measure of lead levels in brain. In rat pups given human milk, the lead levels in blood and brain were about twice as high as in pups given rat milk.

The post-natal exposure of lead is dependent on the lead concentration in breast milk, infant formula and drinking water. The present results indicate that the casein content in milk and infant formula is of importance for the bioavailability of lead. This should be taken into consideration when preparing the composition of infant formula and for the risk assessment of lead in infants.

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