COMPOSITION AND PHYSIOLOGICAL PROPERTIES OF HUMAN MILK

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Edited by

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FOREWORD

This volume contains the original papers and discussion remarks presented at the international workshop 'Composition and Physiological Properties of Human Milk' held from May 29th to June 1st, 1985, in Kiel, Federal Republic of Germany. The symposium focused on three topics: 'trace elements', 'lipids' and 'immune factors influencing intestinal germ growth'.

The investigation of human milk is a challenge to nutritionists, biochemists, biologists, microbiologists, immunologists and pediatricians, since with human milk, nature has produced a product uniquely designed to meet the requirements of the infant. Studies on human milk are not only performed because of pure academic interest there are also some practical aspects to be considered. Although it is accepted that breast-feeding is best for the infant, there are still many infants who are not breast-fed for various reasons. An inquiry in the community of Kiel revealed that only 60% of the infants are exclusively breast-fed during the first week after birth; then this percentage declines progressively until it reaches 33% at three months.

Infants who are not breast-fed do need a substitute which comes as close as possible to the natural product. That is the point where the results of the workshop can help to make bottle-fed infants participate in the benefits of human milk currently withheld from them. For example, I am especially thinking of the immunological properties of human milk, which up to now cannot be imitated in artificial formulas.

The international workshop was generously supported by Milupa AG, Friedrichsdorf, West Germany. It was also conducted as a contribution to the efforts to promote breast-feeding, since this is for some time after birth the biologic norm for the infant. To help assure the best possible nutrition for infants and children, Milupa AG follows the recommendations of the World Health Organization regarding breast-feeding and has taken steps to assure that its marketing practices in developing countries conform to the principles and aim of the international code of marketing of breast milk substitutes as well as to national legislation in the developed countries.

The editor gratefully acknowledges the support of the company for this workshop n recent results in human milk.

Kiel, November 1985

JÜRGEN SCHAUB

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BIOAVAILABILITY OF TRACE ELEMENTS FROM HUMAN MILK, COW'S MILK AND INFANT FORMULAS

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INTRODUCTION

A general need to supplement infant formulas with essential trace elements such as iron, zinc and copper is recognized by professional organizations such as the Committee on Nutrition of the American Academy of Pediatrics and the European Society for Pediatric Gastroenterology and Nutrition (ESPGAN). The rationale for recommending this supplemention is that infants fed unsupplemented cow's milk formula for prolonged periods are at risk of developing iron, zinc and copper deficiency (1-3). In contrast, breast-fed infants rarely develop deficiencies of these elements. Since the concentration of iron, zinc and copper in human milk is lower or equal to that of unsupplemented cow's milk formula, a higher bioavailability of these elements from human milk was implicated. However, direct measures of trace element bioavailability are scarce. There is an urgent need for methods to study trace element absorption in human infants. As a complement to such studies, other approaches can be used: a) assessment of trace element bioavailability in adult humans, b) trace element absorption studies in suckling animals and c) trace element retention studies in weanling or adult animals. The first method has the inherent disadvantage that absorption is studied in an adult with fully developed gastrointestinal function, while the results are expected to relate to infants with immature gut function. Gastric acid, bile and pancreatic secretion is not fully developed in infancy and activities of several proteolytic enzymes are lower than in adults. However, if differences in bioavailability are detected in the adult they will likely be expressed (possibly even more pronounced) in the infant. Therefore, this approach has a value as a screening method. The use of suckling animals has the advantage that a similar time period of life is studied; i.e. a period with immature digestive function. However, the problem with this approach is that there are pronounced differences among species in degree and rapidity of gastrointestinal development. Therefore, ideally the suckling animal should be chosen so that developmental stages are comparable to those of the human infant. The use of weanling or adult animals to assess bioavailability is most commonly used, although this approach suffers from the disadvantages of both approaches previously described.

This paper will present and discuss recent results on trace element absorption from infant diets in human adults and suckling animals.

TRACE ELEMENT CONCENTRATION IN INFANT DIETS

Bioavailability of trace elements can be defined as the fraction of the total amount of an element in the diet which is absorbed and retained by the body. Therefore, both the concentration and the degree of utilization will be of importance for the trace element nutrition of the infant. The concentration of trace elements varies considerably among infant diets (Table I).

TABLE I

CONCENTRATION OF TRACE ELEMENTS IN MILK AND FORMULAS[a]

	mg/L			
	Iron	Zinc	Copper	Manganese
Human milk (mature)	0.4	1.5	0.3	0.005
Cow's milk	0.3	3.5	0.1	0.05
Cow's milk formula[b]	1-2	4-5	0.5-0.6	0.03-0.2
(iron supplemented)	7-12[c]	4-5	0.5-0.6	0.03-0.2
Premature formula	1-3	5-10	0.6-1.7	0.2
Soy formula	12-13	4-5	0.5-0.6	0.2

[a]Data adapted from Refs. 4 and 5.
[b]Data for all formulas are those currently given by U.S. manufacturers.
[c]Lower value represents most European formulas, the higher value U.S. formulas.

The concentration of iron is comparatively low in human and cow's milk, while most formulas are supplemented to high levels (7-12 mg/L). Due to contamination during the processing of infant formulas, the iron concentration of unsupplemented formulas will be higher than that of human or cow's milk. The concentration of zinc is higher in cow's milk and most formulas than in human milk. Copper concentration of cow's milk is lower than in human milk and that in both milks lower than in most formulas. Cow's milk and all formulas are considerably higher in manganase concentration than human milk. It should be noted that the trace element levels given for infant formulas represent recent label claims. It has been shown previously that the actual levels when analyzed can differ considerably from these values (6).

TRACE ELEMENT BINDING COMPOUNDS IN MILK AND FORMULAS

The trace elements iron, zinc, copper and manganese rarely occur free in solution; due to their positive charge, they form complexes with various ligands. Recently, there has been considerable effort to isolate and characterize the ligands in milk that bind trace elements. This is a necessary prerequisite for studying the fate of the complexes between ligands and trace elements in the gastrointestinal tract. It has been hypothesized that the nature of the complexes to which trace elements are bound in milk (molecular localization) will affect their bioavailability (4). While considerable re-distribution of trace elements may occur during digestion in the adult, this may be limited in the infant with immature gastrointestinal function. Gastric acid, bile acid and pancreatic enzyme secretion is not fully developed in the newborn infant (7) and consequently proteolysis may be incomplete. It has been shown that intact casein (8) and individual proteins such as lactoferrin and secretory IgA (9) can pass the gastrointestinal tract undigested even until the age of 4 months. Therefore, identification of trace element binding compounds in infant diets has considerable relevance.

Iron binding compounds

A unique iron-binding protein, lactoferrin, was found to be present in high concentration in human milk but in low concentration in cow's milk (10). This protein binds two ferric ions together with two bicarbonate ions. Although the binding constant for iron is very high ($K_d \sim 10^{30}$), only one-third of the total iron in human milk is bound to lactoferrin (11). As a consequence, lactoferrin in human milk is present in a very unsaturated form, only 3-5% of its total iron-binding capacity. The remainder of iron in human milk is bound to milk fat (~30%) low molecular weight ligands (~30%) and casein (Table II). It has been shown that the iron in human milk fat is localized to the outer milk fat globule membrane, most likely as a part of xanthine oxidase (11).

Zinc binding compounds

A large fraction (32%) of zinc in human milk is bound to the low molecular weight fraction. The major ligand in this fraction has been identified as citrate (13-15). Zinc in human milk fat is bound to the outer milk fat globule membrane, most likely as part of alkaline phosphatase (11). In whey, zinc has been shown to bind to serum albumin (16). Reports of zinc bound to lactoferrin (15,17) are most likely reflecting non-specific binding of zinc and/or re-distribution during separation processes (16). When lactoferrin incubated with ^{65}Zn was passed through an anti-lactoferrin immunosorbent, lactoferrin, but not ^{65}Zn, bound to the column, showing that lactoferrin in human milk does not bind zinc (18). As can be noted, only a small fraction of zinc in human milk

(8%) is bound to casein, while cow's milk casein binds a large fraction of total zinc (84%).

TABLE II

DISTRIBUTION OF TRACE ELEMENTS AMONG MAJOR MILK FRACTIONS[a]

	Iron	Zinc	Copper	Manganese
Casein (%)				
Human milk	9	8	7	11
Cow's milk	24	84	44	67
Whey proteins (%)				
Human milk	26	51	56	67
Cow's milk	29	13	8	14
Fat (%)				
Human milk	33	18	15	18
Cow's milk	14	1	2	1
LMW[b] (%)				
Human milk	32	19	21	4
Cow's milk	32	2	47	18

[a]Data adapted from Refs. 5 and 18.
[b]LMW = low molecular weight fraction (<10,000 MW).

Copper and manganese binding compounds

The distribution of copper in human and cow's milk is similar to that found for zinc; serum albumin, casein and citrate have also been shown to bind copper (14,16). Manganese in human milk, on the other hand, is predominately bound to lactoferrin in the whey, while in cow's milk casein binds most of the manganese.

Information on trace element binding in infant formulas is limited. Since many formulas are based on bovine casein and whey, binding compounds in these formulas can be expected to be similar to those of bovine casein and whey. In soy formulas, phytate can be expected to bind trace elements (19).

IRON ABSORPTION FROM INFANT DIETS

It has been known for a long time that a breast-fed infant is less likely to become iron-deficient than an infant fed cow's milk or unsupplemented cow's milk formula (20,21). Saarinen et al. (22) showed in human infants that iron absorption from human milk is very high (~50%), while the absorption from cow's milk or cow's milk formula is considerably lower (19 and 9-12%, respectively). Similar results were obtained in human adults by McMillan et al. (23); these

investigators extrapolated the values obtained from adults to infants and obtained estimated absorption values of 50% and 34% for human and cow's milk, respectively. The smaller difference in iron absorption between these two fluids found by McMillan et al. may be explained by some of the assumptions made in their extrapolations. For example, iron may be less available from cow's milk casein in infants because of their limited digestive capacity. Casein has been shown to bind iron strongly to the negatively charged phosphoserine groups located in its subunits (24).

Since the lactoferrin concentration of human milk and cow's milk is very different, a specific role for lactoferrin in iron absorption could be implicated. However, initial studies on this putative role of lactoferrin were not supportive. Using everted duodenal sacs in guinea pigs and rats, DeLaey et al. (25) showed that apo-lactoferrin inhibited mucosal iron uptake, while iron-saturated lactoferrin had no effect. DeVet and van Gool (26) reported a negative correlation between the duodenal lactoferrin concentration and iron absorption in human adults; these authors then hypothesized that lactoferrin protects the intestinal mucosa from absorbing excessive amounts of iron. Brock (27) has subsequently suggested that the role of lactoferrin in iron absorption may change during infancy; in early life, lactoferrin would protect against iron uptake, while later, lactoferrin would be digested and deliver its iron for absorption. McMillan et al. (28) showed that lactoferrin added to infant formula had a negative effect on iron absorption in adults. However, several criticisms of this experiment can be raised (29); the formula containing lactoferrin had an iron concentration three times higher than the unsupplemented formula, neither was it described how the iron was added to lactoferrin nor how much time that elapsed between isotope addition and formula consumption. Iron uptake into lactoferrin can be very slow and is dependent on factors such as pH, presence of bicarbonate and chelators. The association of iron to lactoferrin is so slow that saturation of lactoferrin with iron as an inorganic salt is not complete in 24 hrs. Therefore, if iron saturation of lactoferrin is not analyzed, it is possible that iron is present in an inorganic form and may in fact become complexed to other compounds in the formula (such as casein). Consequently, a role for lactoferrin in iron absorption in infants cannot be ruled out by this experiment.

Other studies in experimental animals do support a role for lactoferrin in iron absorption. Fransson et al. (30) used suckling piglets and found a significantly faster uptake of iron from a formula with lactoferrin-iron than from a formula with ferrous sulfate. In another study of weanling mice (31), it was found that the addition of apo-lactoferrin to a milk-based diet did not reduce iron retention, speaking against the previously suggested inhibitory role

of lactoferrin in iron absorption. Recently, we have been able to demonstrate a receptor for lactoferrin in brush border membrane vesicles from young Rhesus monkeys (32,33). The receptor was found to be specific for lactoferrin and the binding constant (K_d=3.6 μM) was similar to that found for transferrin binding to its hepatocyte receptor. Both monkey lactoferrin and human lactoferrin could bind to the monkey brush border receptor and a close homology between the two proteins was demonstrated by amino acid and carbohydrate analysis; antibodies against monkey and human lactoferrin cross-reacted with the antigen from the other species. Support for receptor-mediated iron uptake in the small intestine can be gained by the finding of significant amounts of intact lactoferrin in feces of breast-fed infants (9).

Dietary factors other than lactoferrin and casein will also affect iron absorption. Soy protein has been shown to have a negative effect on iron absorption (34,35). However, the inhibitory effect of soy formula can largely be overcome by addition of ascorbic acid at a high concentration (35). Since most formulas are supplemented with ascorbic acid for other reasons, the negative effect of soy formula may become minimal. The use of lactose in infant formulas may also have a positive effect on iron absorption; it has been shown in human adults that iron absorption is higher from diets based on lactose than from the same diet with starch (36). Iron provided in the form of fortification compounds such as ferric pyrophosphate or orthophosphate and reduced (elemental) iron has a very low bioavailability (37). Iron absorption from soluble iron salts is considerably higher than from these insoluble compounds; however, the soluble iron salts frequently produce unwanted side effects such as off-color and rancidity. It is therefore evident that there is a need for other sources of iron to be used in fortification of formulas.

ZINC ABSORPTION FROM INFANT DIETS

A higher bioavailability of zinc from human milk than from cow's milk was suggested by Eckhert et al. (38). These authors postulated that the beneficial effect of human milk in the treatment of acrodermatitis enteropathica, a genetic disorder of zinc metabolism (39), was due to the high bioavailability of zinc from human milk. Since the zinc concentration of human milk is lower than that of cow's milk, the noted difference in zinc binding observed in these fluids was suggested to cause the postulated difference in bioavailability. Studies on breast-fed and formula-fed infants (40) also supported a high bioavailability of zinc from human milk; plasma zinc values for breast-fed infants were significantly higher than those of formula-fed infants at 6 months of age. Supplementation of formulas to a level 3 times higher than originally used increased

plasma levels significantly. In human adults plasma zinc uptake from various infant diets in human adults also indicated a higher bioavailability of zinc from human milk (41). Recently, we have used a radioisotope of zinc (^{65}Zn) and whole body counting in human adults to study zinc bioavailability from infant diets (42). Zinc absorption from human milk was found to be very high, 41%, while the absorption from cow's milk was 28% (Table III). Absorption of zinc from a "humanized" cow's milk formula was found to be similar to that from cow's milk, while absorption from soy formula (14%) was significantly lower than all other diets studied.

TABLE III

ZINC BIOAVAILABILITY FROM DIFFERENT INFANT DIETS[a]

	% zinc absorbed
Human milk	41
Cow's milk	28
Cow's milk formula:whey predominant	32
Cow's milk formula:casein predominant	21
Soy formula	14
Soy formula with lactose	15
Cow's milk formula with phytate	16
Cow's milk formula without Fe-supplement (2.2 mg Fe/L)	24
Cow's milk formula with Fe-supplement (19 mg Fe/L)	18

[a]Data adapted from Refs. 42 and 45.

In our initial report on the identification of citrate as the major low molecular weight zinc binding ligand in human milk (13), we noted that the concentration of citrate was similar in human and cow's milk although a larger fraction of total zinc in human milk compared to cow's milk was bound to citrate. We therefore hypothesized that the difference in zinc bioavailability between these milks is caused by a) a larger accessible pool of zinc bound to citrate in human milk and b) a larger unavailable pool of zinc bound to casein in cow's milk. Similar suggestions were subsequently presented by other groups (43,44). We have recently demonstrated in human adults (45) that zinc absorption from casein predominant formula (21%) is significantly lower than from whey predominant ("humanized") formula (32%). A direct inhibitory effect of cow's milk casein on zinc absorption was shown in a suckling rat pup model

(46). Zinc uptake from human casein, human whey and cow's whey was similarly high, while the uptake from cow's casein was significantly lower. It has also been shown in a long term study that zinc retention was higher in weanling rats fed cow's whey than in those fed cow's casein (47).

The low bioavailability of zinc from soy products has been suggested to be due to the presence of phytate in soy. In our study we added phytate to cow's milk formula at a concentration similar to that found in soy formula (Table III). Zinc absorption decreased significantly from 31 to 16%, strongly suggesting that phytate in soy formula inhibits zinc absorption. The type of carbohydrate used in soy formula did not seem to affect zinc absorption; similar results were obtained when lactose was used to substitute for the conventionally used starch-dextrin combination.

An inhibitory effect of high iron concentrations on zinc absorption was suggested by the studies of Solomons and Jacob (48). In one study on human adults, zinc absorption was lower from iron supplemented formula than from the same formula without iron supplement (Table III) although the difference was not significant. We have recently shown that the inhibitory effect of iron on zinc absorption is not observed when the elements are given in a meal (50). Prospective studies on infants given various levels of iron are needed to resolve some of these differences.

COPPER ABSORPTION FROM INFANT DIETS

Copper deficiency during infancy is believed to occur almost exclusively in infants prematurely born or in malnourished infants (2,3). The premature infant is born with low copper stores (51) and is often in negative copper balance for some time after birth (52). Although prolonged use of cow's milk has led to copper deficiency in a few cases (53), it is believed that the cause of this deficiency is low copper concentration of cow's milk rather than a poor bioavailability. Our knowledge regarding copper bioavailability from infant diets is very limited. We have assessed copper uptake and retention from infant diets using a suckling rat pup model (54). Liver copper retention from cow's milk was 18% while that from human milk and cow's milk formula was 25 and 23%, respectively. Copper absorption from soy formula was 10% and from a mixed cereal-cow's milk formula 17%. We have also shown that, similar to zinc, cow's casein has a negative effect on copper absorption (54).

A negative effect of zinc on copper absorption has been shown in some animal studies (55,56). However, it has recently been shown that there must be a high ratio of zinc/copper to have an effect on copper absorption in humans (57,58). We found no effect on copper uptake and retention in the suckling rat model when the zinc level of formula was increased to ten times normal (54). Therefore,