CRITICAL REVIEW

Effects of Maternal Dietary Intake on Human Milk Composition

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ABSTRACT The composition of human milk can be affected by the diet consumed by the lactating woman. The influence of the maternal diet on milk composition varies in magnitude between nutrients; for some nutrients no effect at all has yet been documented. Concentrations of fatty acids, fat-soluble and water-soluble vitamins in milk are, in general, significantly affected by the levels of these nutrients in the diet. Protein concentration may be affected under some conditions, although the magnitude of this change appears relatively limited. Lactose, mineral, trace element and electrolyte concentrations seem comparatively resistant to varying maternal intakes. Although there has been significant progress in this research area in the past decade, many early studies are difficult to interpret due to limitations in the study design and analytical methods. This review demonstrates a distinct need for carefully controlled studies on the effects of both nutrient deficiencies and supplements on milk composition. Interactions among nutrients, homeostatic mechanisms and energy balance (weight loss) are factors that need to be studied further. Information from such research will suggest strategies for nutrition intervention in areas of poor nutrition and provide dietary guidelines for lactating women. J. Nutr. *116*: 499–513, 1986.

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The composition of breast milk changes significantly during the lactation period as a result of normal physiological events in the mother; an important question, however, is whether the quality of breast milk is affected by external factors. Some environmental pollutants such as pesticides and heavy metals can be detected in breast milk in concentrations reflecting those in the environment, and some drugs are known to transfer into milk. One area that is difficult to evaluate is the effect of maternal diet on breast milk composition. A primary concern, of course, is malnutrition; in no situation is the value of breast-feeding higher than in developing countries where food supply is limited, infections are common due to poor hygiene, and economic realities exclude the choice of properly used infant formulas. The effect of malnutrition on breast milk composition has been the subject of several reviews (1–5); however, there are many limitations both in our knowledge and in the possibilities of properly investigating this problem.

The state of malnutrition is complex and varies significantly among both individuals and groups. Dehydration of varying degrees can significantly affect water fluxes in the body and may consequently affect the volume of milk produced. Therefore, measurement of concentrations of nutrients will be of limited value as long as volume is not

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determined. Milk volume is also affected by psychological factors. Even if milk volume is determined, the mere fact that the woman is under observation, her baby is weighed, milk samples are taken, and she has come to an unfamiliar and potentially intimidating atmosphere, can have pronounced effects on milk volume. Methodology to measure milk volume accurately has been very limited; however, improved methods intended for field use will probably be available soon.

Another problem is that single nutrient deficiencies are rare; usually many nutrients are lacking in the diet of the malnourished mother, and the set of nutrients in low supply may be quite different in different areas. Therefore, nutrient interactions may occur. For example, an effect on protein content of milk may be caused by lack of protein, of energy, or of vitamin B-6 (required for normal protein metabolism). Thus, identification of the effect of a deficiency of any one nutrient on milk composition becomes difficult. The intention of this review is to evaluate the data available in this research area with the hope of stimulating further studies.

PROTEIN

In areas where protein–calorie malnutrition is common, there has been an understandable concern about the protein content of breast milk from undernourished women (1). However, it is particularly difficult to evaluate the effects on protein concentration, since a wide range of analytical methods have been used. In addition, data from developing countries have often been compared to published data from developed countries that are now known to be overestimations. The earlier overestimation of the protein concentration of human milk occurred because human milk contains an unusually large proportion of nonprotein nitrogen, 20–25% (6). Since protein was often analyzed by the Kjeldahl method and multiplied with a conversion factor (6.25 or 6.38), protein values were often assumed to be 1.1–1.2% instead of the more correct value of 0.8–0.9%. Some of the commonly used protein assays also overestimate the protein content of human milk (7). Therefore, published values for protein concentration must be regarded with caution.

In general, it appears that malnutrition has little if any effect on protein concentration of human milk (3). For the reasons mentioned above, the effects on protein reported in the classical dietary studies by Hoobler (8) and Deem (9) may have been due to methodological problems. More recent studies in India (10), Brazil (11), Pakistan (12), Ethiopia (13), Nigeria (14), Kenya (15), and the Gambia (16) did not find lower milk protein concentration in malnourished than in well-nourished women. Milk protein levels in a study of malnourished women in New Guinea were claimed to be low (17); however, protein was analyzed by Kjeldahl nitrogen, and it is possible that the effect on nitrogen was mainly on the nonprotein nitrogen fraction of milk. Other studies, such as those of Karmarkar and Ramakrishnan (18) and Hanafy et al. (19) may, however, indicate that under some circumstances of maternal malnutrition, milk protein concentration is affected.

Protein supplementation of malnourished women does not appear to have a pronounced effect on milk protein concentration. Several studies (14, 20) reported no significant effect, but Prentice et al. (16) found a modest 7% increase in protein concentration when mothers received protein supplements. The latter research group cautioned that the effect could have been on nonprotein nitrogen. The interplay between milk volume, concentration of a nutrient in milk and total nutrient intake by the infant should be reemphasized; e.g., Gopalan and Belavady (10) found lower milk protein concentration when mothers were supplemented with protein but higher milk volumes, resulting in a similar net output of milk protein. Edozien et al. (14) also found an increase in milk volume with protein supplementation. In a study of well-nourished Swedish women, Forsum and Lönnerdal (21) found a significantly lower milk protein concentration when women consumed a diet low in protein (8% of total energy) than when the same women consumed a "control" diet higher in protein (14% of total energy). A significant increase in milk protein concentration was observed when the mothers received a high protein diet (20% of total energy). The energy intake was kept constant during the study period; however, since each diet was consumed for only 4 d, it is possible that the effects were only transitory. It should be noted that nonprotein nitrogen was also affected by the level of dietary protein.

Recently, some studies have focused on the effects of maternal malnutrition on individual protein components of physiological significance for the infant. Miranda et al. (22) found significantly lower levels of immunoglobulins IgG and IgA, complement factor C4 and serum albumin in colostrum from malnourished Colombian women than in control women. However, since no differences were found in mature milk, it is possible that the lower levels in colostrum merely reflected lower maternal serum levels, since the paracellular pathway of milk secretion is more predominant in early lactation, allowing for a relatively larger proportion of serum proteins to transfer into milk (23). Prentice et al. (24) did not find any effect of maternal dietary supplementation of Gambian mothers on any of the antimicrobial proteins in human milk (immunoglobulins, complement components, lactoferrin or lysozyme). Compared to British women, however, Gambian women produced milk with a different pattern of milk proteins; concentrations of some proteins were lower while those of others were higher in Gambian mothers. Further studies are needed on specific milk proteins and how they are affected by maternal nutrition.

LIPIDS

The lipid compartment of human milk is to a very large extent (> 98%) comprised of triglycerides. However, physiologically important compounds such as phospholipids, sterols and fat-soluble vitamins are also part of this class. It was early recognized that the fatty acid composition of the triglycerides can be affected by maternal diet and considerable attention has been devoted to this subject (25). The effects of diets with different lipid composition as well as the effects of modified lipid composition within the same type of diet have been investigated.

In a carefully controlled study, Insull et al. (26) fed a lactating woman in a metabolic ward diets differing in caloric content, proportion of calories from fat, and fatty acid composition. From delivery until milk production was established (11 d postpartum), a regular hospital diet was consumed; subsequently, a liquid formula diet was the sole source of nutrients. The experimental diets consisted of a "maintenance diet" of 2800–2900 kcal/d based on lard or corn oil (40% of total calories), the same energy intake but with a higher proportion of fat (70% of total calories as corn oil), and a high energy diet of 3750 kcal/d with either no fat (~0%) or corn oil (70% of total calories). Each experimental diet was fed for at least 4 d. These diets were chosen to investigate the following hypotheses: *1*) at a low caloric intake (without fat) the body is in negative energy balance and fatty acids in serum and milk will reflect depot fat composition, *2*) at a caloric intake at maintenance level, the fat–carbohydrate ratio will affect milk fatty acids and *3*) at a high caloric intake, mobilization from endogenous sources (depot fat) will be minimal, and differences in milk can be related to differences in dietary fat. Neither milk volume nor total milk fat was significantly affected by the dietary changes. When the high energy diet containing no fat was fed, the breast milk contained significantly more 12:0 and 14:0 and less 18:0, 18:1 and the sum of 18:2 and 18:3 than when the maintenance (lard) diet was fed. Thus, when fatty acids were synthesized from carbohydrate, the proportion of saturated fatty acids of intermediate chain length was higher. Therefore, interestingly, dietary changes can affect the synthesis or rate of transfer of these two intermediate chain length fatty acids. When the low calorie diet (without fat) was fed, the fatty acid composition of milk resembled that of the maintenance diet (lard), the ad libitum (starting) diet and, importantly, that reported for human depot fat. When corn oil was the dietary fat source, milk levels of 18:2 + 18:3 were significantly higher than when lard or butter fat were the fat sources. Although linoleic acid was not directly analyzed, spectrophotometric diene analysis indicated that by far the major component of this increase was linoleic acid. Many of the findings in this pioneering work by Insull et al. (26) have subsequently been confirmed, although dietary conditions rarely have been as controlled. Although some diets used in this study were unphysiological, the work brought important insights to our understanding of milk fat synthesis.

Many investigations have attempted to correlate poor maternal nutrition with the total concentration of milk fat (19, 27–28). In several studies, low fat concentrations were found (12, 17, 27) in milk from poorly nourished women compared to published values from Western societies, but methodological differences cannot be excluded. In only a few studies has an effect been observed in the same group during periods of different energy intake (29, 30). The general lack of an effect of diet on total milk fat could, of course, arise from the use of depot fat to synthesize milk fat. However, there is also a high degree of variation in milk fat among individuals, during each nursing (in the same individual), during the day, and between days as reviewed by Lammi-Keefe and Jensen (25). These large variations make it difficult to detect significant differences. Carefully controlled field studies of mothers with varying nutritional status would increase our knowledge in this area.

Karmarkar et al. (30) supplemented Indian women of poor nutritional status with varying levels of dietary fat and protein. The supplements were increased from a low level (5 g/d) to a high one (45 g/d), with each level being fed for 1 mo. Fat content of milk increased from 3.8 to 4.7–5.0% with a plateau at 35 g fat per day. The possible effect of increasing dietary energy was ruled out since protein or glucose supplementation did not affect milk fat. It was recognized that the prestudy daily fat intake of this group was only 18 g; similar effects may not be observed in mothers of better nutritional status. Also, although significant increases in milk fat were observed, all levels were comparatively high. This may be partly the result of the methods used for fat determination.

Harzer et al. (31) studied three women who were fed different proportions of calories as fat and carbohydrate at the same level of energy intake (2500 kcal/d) in a crossover design. A high fat (50% of energy), low carbohydrate (35% of energy) diet was fed for 1 wk and then a low fat (15% of energy), high carbohydrate (65% of energy) diet for the next week, or vice versa. When the high fat diet was fed, milk triglyceride levels were lower than when the low fat diet was fed. Plasma triglycerides followed the same pattern; cholesterol and phospholipid levels correlated closely to milk triglyceride levels. Fatty acid patterns were highly affected by the fat intake; as the high fat diet contained vegetable oils, a high concentration of linoleic acid and consequently a high polyunsaturated fat–saturated fat (P/S) ratio was found in milk from mothers who consumed it. However, the medium-chain fatty acids lauric and myristic acid (12:0 and 14:0) were not affected by diet; this is consistent with the previous study (26) indicating that these fatty acids are synthesized by the mammary gland. Lower levels of homo-γ-linoleic (20:3ω6) and arachadonic acids (20:4ω6) were found in milk from mothers fed the high fat diet. This probably reflects the inhibition of the desaturase that synthesizes longer-chain fatty acids as happens at high levels of linoleic acid (32). This was not found in the study by Mellies et al. (33); however, although the diet had a defined P/S ratio, dietary intake of linoleic acid was not given.

Recently there has been interest in the long-chain fatty acid 22:6ω3 (docosahexaenoic acid; DHA), which is abundant in the brain's structural lipids. Deficiency of this fatty acid during gestation has been shown to cause visual impairment in the offspring of Rhesus monkeys (34). Most infant formulas do not contain any significant quantities of DHA, while human milk does (35). DHA can be formed from linoleic acid, but it has been shown that high intakes of linoleic acid can inhibit this transformation. Therefore, preformed DHA in the diet may be a more efficient way of assuring an adequate supply to the infant. This fatty acid is provided by fish oil, and Harris et al. (35) have shown that fish oil supplementation to mothers at a modest level (5–10 g/d) increased milk concentration of DHA from a control level of 0.1 to 0.5–0.8 and to 4.8% at a high level of supplementation (47 g/d). Sanders et al. (36) showed that DHA levels were higher in vegans than in omnivore controls. Finley et al. (37) have shown a higher level of DHA in milk from women who were frequent fish-eaters than in milk from those who were not.

There has been some concern about the transfer of *trans* fatty acids to human milk. These fatty acids are produced in hydrogenation reactions and margarine and fried foods are known to contain them. Aitchison et al. (38) showed that the dietary intake of *trans* fatty acids correlated well with their concentrations in human milk. Finley et al. (37) showed that mothers with a preference for margarine had significantly higher milk levels of *trans* fatty acids than those who preferred butter. Chappell et al. (39) elegantly showed by a crossover design that the level of elaidic acid (18:1 *trans*) in margarine was reflected in the concentration of this fatty acid in milk from women consuming these margarines. A low level of 2% (wt/wt) and a high level of 6% (wt/wt) were found. These investigators also made the important observation that the rate of postpregnancy weight loss significantly affects the level of elaidic acid in milk and that this may have a larger influence on milk *trans* fatty acid level than maternal diet. Mothers losing 0–2 kg by 5 wk postpartum had a milk elaidic acid concentration of 1.5–2.0% (wt/wt), while mothers losing 4–7 kg of 5 wk had a concentration of 2.8–3.5% (wt/wt). The higher levels of elaidic acid in milk from mothers losing weight more rapidly can be explained by mobilization of fatty acids from adipose tissue. This mobilization of previously stored *trans* fatty acids could also release fat-soluble environmental pollutants. The authors cautioned against rapid weight loss during lactation.

Several studies have shown that the dietary habits of different population groups affect milk fatty acid composition (37, 40–43). In general, linoleic acid concentration and P/S ratio in the diet and in milk are well correlated.

Mellies et al. (33) studied the effects of feeding a diet high in saturated fats (P/S = 0.12) and cholesterol (520 mg/d) compared to a diet with a high P/S ratio (1.8) and lower in cholesterol (190 mg/d). A crossover design was used in which each diet was fed for 4 wk. Linoleic acid concentration doubled when the polyunsaturated-rich diet was consumed, while levels of palmitoleic, stearic, palmitic and myristic acid decreased. When the diet high in saturated fats was fed, the fatty acid pattern of milk was similar to that from the ad libitum feeding period prior to the feeding of experimental diets.

Cholesterol levels of milk appear to be unaffected by maternal cholesterol intake (190–520 mg/d) and plasma level (44, 45). Phytosterol concentration in the diet, however, significantly affected both maternal plasma and milk phytosterol levels; milk levels were 0.17 and 2.2 mg/g with maternal diets containing 50 and 1200 mg/d, respectively (44). The difference in effects observed for cholesterol and phytosterol may be explained at least in part by the synthesis of cholesterol in the mammary gland, while there is no endogenous synthesis of phytosterol.

LACTOSE

Many reports have shown that maternal malnutrition, or, conversely, energy supplementation, has little effect on lactose concentration in milk, and it has been stated that of all nutrients in human milk, lactose is the nutrient least likely to be affected by maternal nutrition (1). A single report of low lactose levels in milk from strictly vegetarian mothers (46) is difficult to evaluate, since the method used for lactose analysis was not given, no control women were studied, and the values were about 40–50% of those in all other studies. Harzer et al. (31) reported that lactose in milk increased when the diet was changed from low fat, high carbohydrate to high fat, low carbohydrate. When the order of the diets was reversed, a decrease in lactose was observed. Only a few women participated in this study, and it is not known whether the effects were transitory.

FAT-SOLUBLE VITAMINS

It is well known that fat-soluble compounds in general are transported into milk fat. However, there are few systematic studies of the effect of diet on the fat-soluble vitamins in human milk. The task is complicated by the storage of vitamins A and D; therefore, long-term assessment of vitamin stores and circulating levels may be needed.

Vitamin A. Vitamin A content of human milk is significantly affected by maternal nutrition. Gebre-Medhin et al. (47) showed that Ethiopian women of low socioeconomic status had 281–331 μg/L of milk vitamin A and 239–256 μg/L of β-carotene, while in milk of Ethiopian mothers of high socioeconomic status the corresponding values were 362–364 and 262–281 μg/L, respectively. In milk from Swedish women, the values were 400–531 and 163–208 μg/L, respectively. Vitamin A status as assessed by plasma retinol-binding protein (RBP) values was normal in Swedish mothers, lower in privileged Ethiopian women, and lowest in nonprivileged Ethiopian women. Although low plasma RBP values can be affected by protein status, there were no indications that the Ethiopian women were protein malnourished. The proportion of retinyl esters in the milk vitamin A was low in the nonprivileged women, possibly due to inadequate esterification in the mammary gland. The nonprivileged women were found to have a low intake of vitamin A-containing foods. High intake of dietary vitamin A appeared to be reflected in elevated milk levels (48–50). In contrast, others (51, 52) have reported that in mothers with poor vitamin A status, milk levels of the vitamin were not increased by supplementation. It was suggested that milk levels would not change until a certain level of vitamin A stores is repleted. High dietary levels of β-carotene do not appear to affect milk concentration of vitamin A or β-carotene.

Vitamin D. The analysis of vitamin D and its metabolites in human milk has not been possible until recently (53). Hollis et al. (54) showed that low vitamin D status results in a low level of vitamin D in milk, while supplemental vitamin D given to mothers with adequate vitamin D status results in significantly higher levels. Milk from mothers deficient in vitamin D had nondetectable levels of vitamin D, while mothers receiving 0, 500 and 2500 IU ergocalciferol had milk vitamin D levels of 39, 218 and 3040 pg/ml. Concentration of 25-hydroxyvitamin D was not affected to the same extent by maternal supplementation. Nevertheless, these studies were performed in winter when maternal vitamin D status is comparatively low. There had been an indication of such an effect in an earlier report (55) in which massive vitamin D supplementation of the mother increased milk vitamin D levels. Recently, Greer et al. (56) showed that pharmacological doses of ergocalciferol taken by a woman with hypoparathyroidism resulted in a high concentration of this vitamin in the milk. It should be noted that the increased milk levels of vitamin D may be of minor physiological importance to the infant since their major contribution of antirachitic sterols appears to come from sunlight and not from milk (57).

Vitamin E. Few studies are available on the influence of maternal vitamin E intake on milk levels. Kramer et al. (58) reported that when sunflower oil (rich in vitamin E) was substituted for lard in the diet, a 50% rise in milk vitamin E level was observed.

WATER-SOLUBLE VITAMINS

A more direct effect on milk concentrations of dietary intake of water-soluble than of fat-soluble vitamins might be expected, as they are not stored to any large extent.

Thiamin. Early studies indicated that maternal thiamin deficiency could lead to low breast milk levels (10, 59) and that maternal supplementation does not increase milk levels beyond a certain limit (60). There is a pronounced lack of recent studies in this area. Short-term (6 wk) maternal supplementation with thiamin from 1.3 to 3.4 mg/d did not increase milk thiamin levels in adequately nourished women in the U.S. (61, 62). Since urinary excretion of thiamin was significantly higher in supplemented than in nonsupplemented women, there appears to be a limitation in the amount of this vitamin that can be transferred into milk. However, in a study of malnourished Indian women, Deodhar et al. (63) found that maternal thiamin supplementation increased milk thiamin levels.

Riboflavin. Low maternal intake of riboflavin has been shown to produce low breast milk concentrations (10, 16, 63, 64). In one of these studies, low maternal riboflavin status was documented by a high activation constant for maternal erythrocyte glutathione reductase (EGR) (64). Modest supplementation with riboflavin (2 mg/d) increased milk riboflavin levels concomitant with a decrease in the activation constant for EGR and disappearance of maternal deficiency signs (64). Since the diet provided 0.5 mg riboflavin/day, a maternal daily intake of 2.5 mg was assessed as being sufficient to maintain riboflavin status during lactation. Riboflavin supplementation from 2.6 mg/d to a level of 4.7 mg/d increased levels of riboflavin in early milk significantly (61); while a longer period of supplementation (6 mo) did not result in any difference in milk riboflavin.

Niacin. Niacin content of milk has received comparatively little attention. Pratt et al. (59) showed in 1951 that the milk niacin concentration of U.S. women with a niacin intake of 15–23 mg/d was 1.96 mg/L and that it could be increased to 3.9 mg/L by supplementation (120 mg/d) for 6–14 d. Indian women of low nutritional status with a niacin intake of 2.4 mg/d had a milk concentration of 1.0 mg/L; it was increased to 2.75 mg/L with a supplement of 60 mg niacin/day (63). In this study, the lack of dietary niacin most likely was compounded by a lack of dietary tryptophan (protein). Similar conditions may explain the low levels of niacin in milk from Gambian women; during the wet season (low dietary supply) the concentration was 1.13 mg/L while during the dry season it was 1.49 mg/L. Supplementation with niacin (18.8 mg/d) during the wet season increased milk levels to 1.62 mg/L (16).

Pantothenic acid. Pantothenic acid concentration of human milk appears to vary with maternal intake. Johnston et al. (65) found that milk pantothenic acid level correlated strongly with maternal intake of the vitamin the preceding day. At a pantothenic acid intake of 7.6 mg/d, milk concentration was 6.7 μg/ml; while at intakes of 15–25 mg/d, milk concentration was 9–12 μg/ml. Since the authors suggest that pantothenic acid is stored in the body, it is uncertain to what extent the observed differences reflected long-term or more acute effects of pantothenic acid intake. Prentice (29) found that pantothenic acid levels of Gambian women were 2.0–2.7 μg/ml; no significant effects were observed after maternal supplementation (16). Deodhar et al. (63) reported low pantothenic acid concentrations (1.0 μg/ml) in milk of Indian women, which was increased by supplementation during lactation; previous dietary intake of this vitamin was significantly lower in this group (2.2 mg/d) than in the U.S. group. It should be recognized, however, that low pantothenic acid levels in milk may be explained in part by methodological problems. Not until recently (65) was total pantothenic acid analyzed by treating samples with phosphatase to release bound pantothenic acid.

Biotin. The concentration of biotin in milk from U.S. women was reported to be 5–12 μg/L in 1951 (59). Supplementation with high levels of other B vitamins did not appear to affect milk biotin levels. Biotin concentration in milk of Indian women with low biotin intake (30 μg/d) was reported to be 1.5 μg/L (63); supplementation with up to 250 μg biotin/day increased milk biotin levels to 5.0 μg/L. Recent studies in Gambia (29) showed a biotin level in milk of 9–10 μg/L, which was not increased by supplementation (16).

Vitamin B-6. Vitamin B-6 concentration of human milk has been shown to be low in mothers with low vitamin B-6 intake (63, 66–68). Concentration of this vitamin in milk from women with intakes of vitamin B-6 at the RDA level (2.5 mg/d) appears to be around 210 μg/L (67). Vitamin B-6 levels in milk from undernourished Indian women with an intake of 0.35 μg vitamin B-6/day was 80 μg/L (63) and in milk from U.S. women with low socioeconomic status and low vitamin B-6 intake it was 120 μg/L (68). In the latter group, low maternal vitamin B-6 intake (1.4 mg/d) was confirmed by a high EGPT (erythrocyte glutamic-pyruvic transaminase) stimulation index. Supplementation with vitamin B-6 to a level higher than the RDA (5.3 mg/d) did not affect milk concentration of the vitamin (69). Studies of New Guinean (17) and Gambian mothers (29) also demonstrated low levels of vitamin B-6 in milk, 120 and 90–115 μg/L, respectively. Milk levels of vitamin B-6 in the Gambian women were not increased by maternal supplementation (16). Recently, Styslinger and Kirksey (70) showed that mothers with an intake of 1.8 mg vitamin B-6/day had a milk concentration of 93 μg/L. A 3-d supplementation with 2.5, 10 or 20 mg vitamin B-6 was paralleled by increases in milk vitamin B-6 concentrations of 192, 247 and 413 μg/L, respectively. It should be noted that supplementation of lactating women with high levels of vitamin B-6 should be avoided, since it has been documented that this vitamin can suppress lactation (71, 72).

Folate. Reported values for folate concentration of milk vary widely and should be considered with caution. The following are reasons for the high variability: whether free folate or total folate (after conjugase treatment) was assayed, methodological problems (protein interference with turbidimetric assays), varying stage of lactation and maternal folate status (68, 73, 74). Tamura et al. (73) reported that plasma and red cell folate levels increased by maternal supplementation with 1 mg pteroylmonoglutamate daily, while milk folate levels remained unchanged (141 μg/L). Cooperman et al. (74) showed that the folate concentration of human milk increases with lactation time; concentrations ranged from 15–20 μg/L in early lactation to 40–70 μg/L in mature milk. In this study, milk was treated with rennin and ascorbic acid to avoid protein interference. It is likely that the protein precipitation method used resulted in low total folate values due to precipitation of folate bound to its binding protein. Ek (75) found that some folate was released with conjugase treatment, although the free (monoglutamate) form was the major part of total folate. These results are supported by Selhub et al. (76) who reported a significant part of total folate bound as polyglutamates to the folate-binding protein(s) in human milk. These authors found total folate values in mature milk to vary between 70 and 135 nmol/L. The study of Ek (75) suggested that there is a mechanism for the regulation of adequate milk folate levels, because it appeared that milk folate levels had priority over red cell folate levels.

It has been shown that supplementation of well-nourished U.S. women with 0.8 mg folate/day did not affect milk folate concentration (69). However, U.S. women with low socioeconomic status and low folate intake (60% of RDA) had low milk folate levels that were increased by supplementation (68). In Gambian women (29), milk folate levels (38 μg/L) were lower during the wet season when nutrient intake was low than in the dry season (54 μg/L). Supplementation during the wet season increased milk folate values to 47 μg/L. Indian (63) and Nigerian (14) women also had low milk folate levels that were increased by maternal supplementation; however, folate was significantly lower in these studies than in all other studies, indicating methodological differences. Although maternal folate intake and/or status most likely was very low in these studies, a substantial difference in folate status is not expected between Gambia and Nigeria.

Vitamin B-12. Low intakes of cyanocobalamin (vitamin B-12) appear to be reflected in milk concentration of this vitamin (29, 63, 67, 68). Indian women had levels of 0.78 ng/ml (63) while breast milk of Gambian women contained 0.16–0.29 ng/ml (29). In comparison, mean vitamin B-12 levels of milk from well-nourished U.S. women were 0.97 (77) and 1.10 ng/ml (69), and of women of low socioeconomic status, 0.55 ng/ml (68). Since this latter level was increased only to 0.79 ng/ml by maternal supplementation for 40 d, it is possible that long-term impaired maternal vitamin B-12 status was not completely alleviated by this time. Analytical problems cannot be ruled out in some of these studies (67–69), since a competitive protein-binding assay was used and vitamin B-12 in human milk is not free but is protein bound.

Daily vitamin B-12 intake of women without supplementation was 5–7 μg; this level was increased to 12–13 μg with additional vitamin B-12. That vitamin B-12 levels can be low in milk of U.S. women is evidenced by the report of Higginbottom et al. (78) in which the infant of a vegetarian mother developed vitamin B-12 deficiency. These levels should be compared to intake data from the Indian and the Gambian women, which was 0.26 μg/d (29, 63) in both studies. Supplementation of well-nourished lactating women with vitamin B-12 does not appear to result in increased milk concentration of the vitamin (69, 77).

Vitamin C. Vitamin C concentration of human milk appears to be affected by low maternal vitamin C intakes (52, 63, 79). Milk vitamin C levels of 23 mg/L were found in Indian women with a dietary intake of 1.5 mg/d (63); when supplemented with 200 mg/d, milk levels increased to 61 mg/L. The latter level of vitamin C in milk is similar to that found in U.S. women with a dietary intake of 200 mg/d (67). Bates et al. (79) found that the level of ascorbic acid in milk from Gambian women varied with the season, as did their intake and plasma levels. During the dry season, milk levels were 45–60 mg/L while levels of 20–40 mg/L were observed during the rainy season. Moderate supplementation with vitamin C (35 mg/d) failed to increase low maternal plasma vitamin C levels, indicating that tissue stores were repleted prior to any effects on plasma and milk. In U.S. women neither short-term supplementation with high levels (10 × RDA) of vitamin C (80) nor long-term (6 mo) supplementation of well-nourished women with vitamin C above RDA level significantly affected milk levels of this vitamin (69). Therefore, it is possible that there is a level above which further supplementation of vitamin C may not affect milk vitamin C levels.

MINERALS

Calcium. The concentration of calcium in human milk appears to be affected under some conditions. Bailey (17) found a concentration of 154 mg/L in milk from New Guinea mothers with poor nutrient intake, compared to values of 260–340 in other published studies. However, Lindblad and Rahimtoola (12) found a level of 284 mg/L in milk from malnourished Pakistani women, which is within the range of recently published values (81). Similar low values had been found previously in another province of New Guinea (82). This observation was tentatively explained by exceptionally low intakes of calcium in this region, a theory which is not commensurate with current knowledge of calcium metabolism. The findings of low milk calcium did agree with findings of poor skeletal calcification of infants in this area; however, methodological problems are quite likely, as milk magnesium levels were very low (13.6 mg/L) compared to normal levels [30–40 mg/L (81)] in spite of a high magnesium intake. Greer et al. (83) have found a correlation between maternal calcium intake and milk calcium; however, others have not (84). Maternal serum calcium levels did not correlate with milk calcium; however, no attempt to correlate ionized calcium in maternal serum to milk was made.

Magnesium. Maternal magnesium intake within a normal range does not appear to affect milk magnesium (83, 84). However, mothers treated for preeclampsia with pharmacological doses of magnesium sulfate had colostrum magnesium levels that were significantly higher than that of control women (85), although these higher levels were still within the normal range published for a milk magnesium. This effect was only transitory; 24 h after termination of the treatment, milk magnesium levels were not significantly different between treated women and controls. No effect of magnesium treatment on milk calcium was observed. The authors of this study caution that fore milk was obtained from treated mothers while hind milk was obtained from control women. It is known that the large difference in fat content during a feeding can lead to differences in concentrations of water-soluble compounds; however, the fat content of colostrum is usually quite low and no differences have been found for magnesium in milk during one feeding (86). As mentioned earlier, the magnesium concentration of New Guinean milk samples was found to be low in spite of high dietary magnesium intakes. The reason for this is not known, but it might be speculated that the very low calcium intakes had an effect on whole-body and serum ratios of calcium/phosphorus and that a secondary effect on magnesium in serum and milk was obtained. It is apparent that more information is needed on the interrelationship between calcium, magnesium and phosphorus and its effect on milk levels of these nutrients.

Sodium and potassium. Maternal dietary sodium intake appears to have no significant effect on milk sodium concentration (87, 88). When a low sodium diet was given to lactating women, no effect on milk sodium or potassium was observed. Since a correlation was found between urinary and milk potassium, but not sodium, these authors (87) suggested that an effect of dietary potassium on milk potassium may be found. However, their study did not address this issue.

Chlorine. It is generally believed that the chlorine level of breast milk is not affected by maternal diet. In a rare case of chlorine deficiency in a breast fed infant (89), maternal serum levels and dietary intake of chlorine were normal.

Iodine and fluorine. Iodine deficiency is still a problem in some parts of the world. However, in the U.S. there is growing concern about excessive iodine intake. High intakes of iodine can be caused by iodized salt, and by bread (dough conditioners) and milk products (cleansing chemicals for equipment and udders). Another concern is that iodine, which is concentrated in breast milk (higher levels than in serum), can be absorbed from medical preparations of iodine (90). It needs to be resolved whether or not the concentration of iodine in milk can be elevated by high maternal iodine intakes. It appears that milk iodine concentrations are higher today (91) than in the 1930s. However, this observation could be explained by a lack of studies on breast milk iodine during the past decades and/or by lack of accurate analytical methods for analyzing iodine at that time.

Recent reports on the effect of maternal dietary iodine intake on breast milk iodine are conflicting (91, 92). This may be explained by differences in the types of dietary sources ingested by the mother, e.g., in one study iodized salt was found to affect breast milk iodine but intake of cow milk, bread or seafood was not correlated to breast milk iodine (91).

Fluorine intake during the neonatal period has also been considered to be too low or too high. Esala et al. (93) recently found that human milk from areas with low water fluorine concentration contained 7 μg of fluorine/liter (range 4–14 $\mu g/L$) while milk from high fluorine areas contained 11 $\mu g/L$ (range 4–51 μg of fluorine/liter). However, Backer-Dirks et al. (94) did not find a significant effect of drinking water fluorine on breast milk fluorine. This may be explained by the larger differences between the water fluorine concentrations in the Finnish study (93) (0.2 and 1.7 mg/L) than in that of Backer-Dirks et al. (94) (0.1 and 1.0 mg/L). Ekstrand et al. (95) studied the acute effects of maternal fluorine intake on breast milk fluorine; maternal plasma fluorine peaked after 2 h concomitant with an increase in milk fluorine. However, the elevation in milk fluorine was small compared to that of plasma.

TRACE ELEMENTS

General effects. In general, diet appears to have little influence on trace element concentration of breast milk (84, 96–98). It should be noted, however, that few controlled studies have been carried out. In addition, there is currently a popular belief in, and accessibility of, trace element supplements, which can lead to dietary intakes that can be considered excessive. The effects of supplements on the concentration of trace elements in milk need to be determined as well as the effects of high intakes of one element on the absorption and transfer of other elements sharing absorptive pathways. For example, zinc and copper, iron and zinc, and iron and manganese can interact at the level of absorption (99); thus, beneficially high intake of one essential element may compromise the intake and status of another. Thus far, most supplementation studies have focused on one element and have not considered the effect on others.

Iron. Iron deficiency during pregnancy and lactation is common in many areas of the world, leading to low levels of circulating iron. In contrast, iron supplementation up to comparatively high levels is common in many affluent countries during pregnancy and is often continued into the lactation period. It is surprising that so few studies have been carried out on the effects of these two contrasting levels of maternal iron status.

Lack of an effect of low maternal iron status on milk iron concentration has been reported in some studies (18, 100). Loh and Sinnathury (101) described differences in breast milk iron among women of different ethnic origin; however, there did not appear to be any correlation between hematological indices and milk iron concentration. Murray et al. (100) found no reduction in iron concentration of milk from mothers with low hemoglobin values. Similarly, Celada et al. (102) were unable to correlate maternal hemoglobin to milk iron level. However, hemoglobin values in these studies were not severely reduced. Fransson et al. (103) found that severely anemic Indian mothers (Hb $\leq$ 8 g/dl) had milk iron levels that were significantly higher than those of mothers with higher or normal hemoglobin values. Concentration of the major iron-binding protein of human milk, lactoferrin, was also high in the anemic mothers, possibly suggesting altered mechanisms of milk iron accumulation in this condition. It is evident that further studies on iron in human milk and its relation to maternal iron metabolism are needed.

Dietary iron intake appears to have little effect on human milk iron concentration (18, 84, 96, 98, 104–107).

Zinc. Most studies have failed to find any correlation between maternal dietary zinc intake and milk zinc concentration (84, 96, 98, 105, 107). There are some recent reports on low concentrations of zinc in human milk; however, maternal serum zinc values were not low or not reported and in one case (108), zinc supplementation did not increase milk zinc levels. It is possible, however, that other zinc pools may be repleted before an effect on breast milk zinc can occur.

Several studies have reported that maternal zinc supplementation does not affect milk zinc concentration (105, 107, 109). In one study, however, a slight increasing effect on milk zinc was found in a few subjects in the late part of lactation when mothers received 13 mg of supplemental zinc/day (110). Therefore, if there is an effect of maternal zinc supplements it is very weak and is apparent only in mothers nursing for long periods.

Copper. There appears to be no effect of dietary copper on the copper concentration of human milk (84, 96, 98, 105, 107). Not even when copper was given intravenously was an effect noted (111). Copper deficiency in the adult human is rare, and there are no reports of copper deficiency in lactating women.

Manganese. The concentration of manganese in human milk is very low; about 4–8 μg/L (96). Therefore, very sensitive techniques have to be used for milk manganese analysis and few studies have been performed. In the study by Vuori et al. (96), a correlation between maternal dietary manganese intake and milk manganese concentration was found. Vaughan et al. (84) have also indicated an effect of manganese intake on its concentration in milk. However, it is difficult to evaluate these findings, since little is known about the interrelationships between dietary manganese, absorbed manganese and manganese status in the human.

Selenium. This element has recently received considerable attention with regard to its nutritional and toxicological role. It is well known that several areas in the world have very low levels of selenium in the soil and that these affect dietary intake. Selenium status as assessed by serum selenium concentration appears to reflect dietary intake. It has also been shown that the level of selenium in soil affects its concentration in breast milk (112). Kumpulainen et al. (113) found low selenium levels in milk from mothers in Finland, an area low in selenium. Supplementation with selenium (100 μg/d) as selenite or in yeast increased milk selenium significantly from 9–11 μg/L to 13–14 μg/L when yeast was used. Selenite also increased milk selenium, but to a much lesser extent. Similar effects were observed in maternal serum. Possibly an organic form of selenium (yeast) is better absorbed than an inorganic salt (selenite), or possibly selenomethionine from yeast is incorporated into milk proteins to a larger extent than selenite. This should be considered when selenium supplementation programs are instituted.

CONCLUDING REMARKS

It is evident that further studies on the effects of maternal diet on human milk composition are needed. Carefully controlled studies are required in which the plane of maternal nutrition is considered, the quantity of milk produced (consumed by the infant) is assessed, and the short-term and long-term effects on concentrations in milk are evaluated. Such information is needed to determine optimal means of providing maternal supplementation or diet counseling, and to provide further insight into the mechanisms controlling nutrient transfer into milk. Finally, since human milk is frequently used as a norm on which the nutrient requirements of infants are based, it is essential to provide more information on milk composition and its variation in different nutritional conditions.

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