Breast Milk Retinol Concentrations of Ghanaian Women: Effect of Lipid-Based Nutrient Supplements Taken During Pregnancy and the First 6 Months Postpartum, and Associations with Maternal Dietary Patterns and Sociodemographic Factors.

By

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Dedication

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Breast Milk Retinol Concentrations of Ghanaian Women: Effect of Lipid-Based Nutrient Supplements Taken During Pregnancy and the First 6 Months Postpartum, and Associations with Maternal Dietary Patterns and Sociodemographic Factors.

Abstract

Vitamin A deficiency (VAD) is associated with increased morbidity and mortality among children. Despite decades of efforts to control it, VAD remains a global public health problem. Among Ghanaian pregnant and lactating women, we examined: (i) the effect of lipid-based nutrient supplements (LNS) and multiple micronutrient supplements (MMN) intake during pregnancy and the first 6 months postpartum on breast milk retinol concentrations at 6 mo postpartum; (ii) associations between maternal background and demographic factors and breast milk retinol concentrations at 6 mo postpartum; and, (iii) relationships between maternal dietary patterns and breast milk retinol concentrations at 6 mo postpartum.

The first study assessed (i) the effect of LNS providing daily low doses of vitamin A during pregnancy and the first 6 mo postpartum on breast milk retinol concentrations at 6 mo postpartum, and (ii) interaction effect of daily low dose vitamin A and high dose postpartum vitamin A supplementation on breast milk retinol concentration at 6 mo postpartum. This study forms part of the International Lipid-Based Nutrient Supplement (iLiNS)-DYAD trial in Ghana, a randomized controlled trial to examine the effect of LNS on maternal and child outcomes. Women were enrolled at ≤ 20 wk gestation and assigned to receive LNS or MMN during pregnancy and the first 6 mo postpartum; the control group received iron and folic acid supplements (IFA) during pregnancy and a placebo during the first 6 mo postpartum. We measured the breast milk retinol concentrations at 6 mo postpartum. There were no significant

differences in mean breast milk retinol per gram of fat concentration at 6 mo postpartum among the 3 supplement groups (LNS = 58.5 ± 2.4, MMN = 55.2 ± 2.4, IFA 55.4 ± 2.4 nmol/g, p = 0.78). No significant interaction was observed between daily low dose vitamin A and high dose postpartum vitamin A supplementation on breast milk retinol concentration at 6 mo postpartum (p = 0.41).

The second study explored whether milk retinol concentration (per gram of fat) at 6 mo postpartum was associated with any of the following maternal factors: age, education, high dose postpartum vitamin A supplementation, gestational age at enrolment, baseline body mass index (BMI) and hemoglobin concentration, household assets and housing indices, household food insecurity access scores, primiparity, and season of sample collection. This was analyzed as a cohort study within the iLiNS-DYAD Ghana trial, controlling for supplement group assignment. Maternal age was positively associated with milk retinol concentration (p = 0.02), and milk fat content was negatively associated with milk retinol concentration (p < 0.0001). Sample collection during the dry season was associated with lower milk retinol concentration, but the association was no longer significant after controlling for milk fat content. Prevalence of low milk retinol concentration (≤ 28 nmol/g fat) was 17.1%; again, higher maternal age was associated with lower odds of having low milk retinol concentration (p = 0.006), and higher milk fat content was associated with increased odds of having low milk retinol concentration (p < 0.0001). High dose postpartum vitamin A supplementation and primiparity were not associated with milk retinol concentration.

The final study examined whether milk retinol concentration at 6 mo postpartum was associated with maternal: (i) intakes of vitamin A-rich foods at 6 mo postpartum; (ii) intake of study supplements containing vitamin A (LNS and MMN) during the first 6 mo postpartum; or (iii)

intake of non-study supplements containing vitamin A, iron or zinc during the first 6 mo postpartum Maternal intake of vitamin A-rich foods (colored roots and tubers, dark green leafy vegetables, colored fruits, organ meats including liver, eggs, dairy products and red palm oil) was not associated with milk retinol concentration at 6 mo postpartum. Adherence to LNS or MMN intake during the first 6 mo postpartum was not associated with milk retinol concentration, nor was intake of non-study supplements was not associated with milk retinol concentration at 6 mo postpartum. The lack of associations between dietary and supplemental vitamin A intake and breast milk retinol concentrations can be due to an adequate dietary vitamin A intake and normal vitamin A status among participants.

Chapter 1

Literature Review

1.1 Introduction

Vitamin A deficiency is a major public health problem in many developing countries. It is the most important cause of childhood blindness, and results in morbidity and mortality from infections, particularly among children and pregnant women. Night blindness affects 5.2 million preschool children and 9.8 million pregnant women globally; and low serum retinol concentration (< 0.70 μmol/L) affects 190 million preschool children and 19.1 million women globally (1). In Africa, 2.55 million preschool children are affected by night blindness, corresponding to almost half of the children affected globally; over 3 million pregnant women are affected by night blindness in Africa, which corresponds to a third of the global prevalence (1). Africa has correspondingly high biochemical vitamin A deficiency, with 56.4 million preschool children (44.4 % of global prevalence) and 4.18 million pregnant women (13.5 % of global prevalence) having low serum retinol concentrations, respectively (1).

Among children in developing countries, vitamin A deficiency is mainly caused by maternal vitamin A deficiency that results in low breast milk vitamin A levels, inadequate dietary intake of vitamin A during and after weaning, and repeated episodes of infectious illnesses, which further decrease vitamin A levels (2).

The major types of community interventions designed to help control vitamin A deficiency are dietary diversification to improve the availability and intake of vitamin A-rich foods; fortification of staple foods or condiments with vitamin A; and the periodic supplementation of preschool children with high-dose vitamin A supplements (3). In a recent review of 43 randomized trials with vitamin A supplementation of children aged 6 months to 5 years of age,

17 trials reported a 24% reduction in all-cause mortality, and 7 trials reported a 28% reduction in mortality associated with diarrhea (4).

Women are an ideal target population for vitamin A interventions because they are at risk of vitamin A deficiency, and improving their vitamin A status will improve that of their breastfed infants (5). In 1997, the World Health Organization (WHO) recommended the supplementation of mothers at risk for low vitamin A status with 200,000 IU of vitamin A within 6 weeks after delivery (3) to enrich their breast milk vitamin A content and hence the infant's vitamin A status. Studies to assess the impact of this recommendation (and higher doses of 300,000 and 400,000 IU of vitamin A) on maternal and infant vitamin A status showed some improvements in maternal and infant vitamin A status, but most of the studies reported no sustained impact throughout the duration of lactation. Based on current evidence, the WHO does not recommend maternal postpartum vitamin A supplementation as a public health strategy for the prevention of maternal and infant morbidity and mortality (6).

Studies in rats suggest that sustained chylomicron delivery may be the most important means by which vitamin A is delivered to mammary tissue (7). Further research in rats indicates that regular, higher intake of vitamin A throughout lactation maintains increased concentrations of vitamin A in milk and improves vitamin A status of the young (8). It is therefore likely that long-term, low-dose vitamin A supplementation of lactating women at risk of vitamin A deficiency would provide an optimal strategy for improving vitamin A status of infants and young children. Further research with regular, low doses of vitamin A to women during pregnancy and lactation is needed to throw more light on this issue.

1.2 Functions of vitamin A

Vitamin A is needed for a wide variety of functions in the body, including the normal functioning of the visual system, maintenance of cell function for growth, epithelial integrity, red blood cell production, immunity and reproduction (1, 9-13). In particular, vitamin A is very important for successful gestation and proper offspring development (14, 15). It is also essential for the development and proper functioning of the immune system (16, 17).

1.3 Vitamin A and its metabolism

Vitamin A is a generic term for a variety of substances including retinol, retinal, retinoic acid, retinyl esters as well as provitamin A carotenoids such as β-carotene, β-cryptoxanthin, α-carotene and ɣ-carotene. Retinol and its derivatives are only found in animal tissues whereas β-carotene and other pro-vitamin A carotenoids are principally found in plants (9-11). Vitamin A exists in several isomeric forms; the major naturally occurring form of vitamin A is all-trans-retinol, which has the highest biological activity (18).

Dietary vitamin A is usually in the form of retinyl esters or provitamin A carotenoids. Dietary fat is required to form micelles in the intestine to solubilize and transport the retinyl esters and carotenoids. Diets that are very low in fat (below 5-10g per day) can impede efficient absorption of retinol and carotenoids (19). Retinyl esters in the diet are hydrolyzed to retinol in the lumen of the intestines through the action of pancreatic triglyceride lipase or brush border retinal ester hydrolase (20). Retinol is taken up by the epithelial cells and reesterified with long-chain fatty acids through the action of lecithin: retinol acyltransferase (LRAT) which catalyzes about 90% of retinyl ester formation, and intestinal acyl-CoA: retinol acyltransferase which catalyzes the remaining retinyl ester formation (21). The retinyl esters, together with dietary fat and

cholesterol, are packed into chylomicrons and enter the blood circulation via the lymphatic system (21, 22). A significant amount of vitamin A is also absorbed as unesterified retinol or retinoic acid into the portal circulation (20).

Dietary provitamin A carotenoids are taken up from the intestinal lumen into the epithelial cells through a process that involves scavenger receptor class B, type 1 (SR-B1) (21). Beta-carotene can be acted upon by 2 different enzymes within the epithelial cells. The major step involves the symmetric cleavage of β-carotene by the enzyme β-carotene 15-15' mono-oxygenase (BCMI), yielding 2 molecules of retinaldehyde (23). Beta-carotene is also acted upon by a second enzyme, β-carotene 9', 10' mono-oxygenase (BCMII), which generates apocarotenals which then yield 1 molecule of retinaldehyde (23). The retinaldehyde generated is either reduced to retinol which can be reesterified and packaged into chylomicrons, or oxidized to retinoic acid (21). Between 25-45% of the β-carotene is absorbed intact (23, 24) and incorporated into the chylomicrons (9, 25, 26).

Retinal and retinol are highly insoluble in water; within the aqueous environment of the intestinal cells, they are found bound to cellular retinol-binding protein type 2 (CRBP-II) (21). In adults, CRBP-II is reported to be expressed solely in the intestinal mucosa, where it has been proposed to facilitate optimal absorption of retinol from the diet (27), as well as metabolically channel retinol to LRAT for the synthesis of retinyl esters (28).

Some of the circulating chylomicrons are degraded partially by lipoprotein lipase and, depending on tissue needs and vitamin A status, part of the retinyl esters and carotenoids released may be taken up by peripheral tissues such as the skeletal muscle, bone marrow, adipose tissue, peripheral blood cells, kidney and spleen (29). The retinyl esters may be hydrolysed and utilized

in the form of retinol, or reesterified and stored in these tissues. The retinyl esters that remain in the chylomicron remnants are taken up by the liver hepatocytes where they are hydrolyzed and re-esterified for storage in the stellate cells (25, 30). They can then be converted into retinol which is secreted into the circulation bound to its transport protein, retinol binding protein (RBP) (9, 25, 31). Part of the β-carotene entering the liver is cleaved into retinal and reduced to retinol which then enters the retinoid metabolism. The other part is secreted into the circulation after incorporation into very low-density lipoprotein (VLDL) (25).

The liver is the major site for RBP synthesis (32). RBP has a single binding site for a single molecule of all-trans retinol (21). The secretion of RBP by the hepatocyte is tightly regulated by the vitamin A status of the animal, such that RBP secretion is blocked in times of dietary vitamin A deficiency and restored upon retinol repletion (33). In the blood, RBP is bound in a 1:1 ratio with another protein, transthyretin (TTR) (32). The retinol-RBP-TTR complex is the predominant mechanism for transport of retinol to the peripheral tissues. Association of RBP to TTR helps prevent the filtration of the relatively small RBP molecule through the glomeruli of the kidney (34). Circulating retinol is taken up by extra-hepatic tissues such as eye, placenta, adipose tissue or mammary gland by way of a membrane-bound RBP receptor (25, 35-37). It is further transformed into retinyl esters or converted into retinal or retinoic acid (25, 35).

The active metabolites of vitamin A are synthesized within the target cells, and all-trans retinol from plasma is the main source for this synthesis (38). The major active cellular metabolite of vitamin A, all-trans retinoic acid, is synthesized in a 2-step reaction. The first step involves the reversible oxidation of all-trans retinol to all-trans retinal, through the actions of alcohol dehydrogenases (ADH-I, -III and IV); the second step involves oxidation of all-trans retinal to all-trans retinoic acid, through the action of retinal dehydrogenases (RALDH-I, -II and –III) (38).

Retinoic acid is catabolized into polar metabolites (4-hydroxy retinoic acid, 4-oxo retinoic acid, 18-hydroxy retinoic acid, 5,6-epoxy retinoic acid, and 5,8-epoxy retinoic acid) through the action of cytochrome P450 enzymes (CYP26A1, CYP26B1 and CYP26C1) (39-43). The kidneys are the main excretory path for RBP and retinol excretion from the body, mainly through renal catabolism and glomerular filtration (44).

Retinal is involved in the visual cycle, where it is required for the formation of rhodopsin, a molecule that is stimulated by light energy adsorbed into the eyes results in the transmission of neural signals to the optic center of the brain (45). Retinoic acid, the biologically active form of vitamin A, functions as a ligand for specific nuclear receptors (retinoic acid receptor or retinoid X receptor) that regulate the expression of many genes (46-48) that control many processes in the body, including embryonic development (49).

1.4 Metabolism and transfer of vitamin A during lactation

Vitamin A in milk is derived from 2 sources, chylomicrons and holo-retinol-binding protein (holo-RBP) (50).

Serum vitamin A is mainly transported as retinol bound to its transport protein, the RBP-transthyretin complex (22). The levels decrease at the end of gestation, suggesting that receptors involved in the uptake of RBP-retinol may be markedly increased in the mammary gland around parturition, allowing higher vitamin A uptake and transfer into colostrum (51).

Some of the retinyl esters packed in chylomicrons are taken up by the mammary gland through the action of lipoprotein lipase (52, 53). The uptake by the mammary gland of vitamin derived from the maternal diet may explain why the concentrations of vitamin A in milk vary even when plasma retinol levels are unchanged (25, 53, 54).

Rat studies indicate that chylomicrons play an important role in the delivery of vitamin A to the lactating mammary gland and thus to milk (53). The recovery of labeled vitamin A in mammary tissue after administration of chylomicrons containing labeled vitamin A peaks shortly after dosing, and concentrations decrease over time, suggesting that the uptake of vitamin A increases as chylomicron triglycerides are hydrolyzed by mammary tissue lipoprotein lipase. A recent kinetic study indicated that the contribution of chylomicron vitamin A versus holo-RBP to milk increased as a function of dietary vitamin A intake during lactation (7). This and other evidence suggest that increasing concentrations of vitamin A in milk are a direct result of dietary vitamin A intake and not likely due to holo-RBP because plasma retinol concentrations remain relatively constant during lactation in these studies.

1.5 Recommendations for vitamin A supplementation to women postpartum

Marginal vitamin A status was found to be common among women of reproductive age (5, 55, 56), prompting the World Health Organization (WHO) to recommend that 200,000 IU of vitamin A be given within 1 month after delivery to lactating women in high risk areas (57). However, some studies among populations where subclinical vitamin A deficiency is prevalent indicated that a single dose of 200,000 IU did not completely correct subclinical vitamin A deficiency in women. The dose neither maintained adequate maternal vitamin A status for more than a few months nor increased breast milk vitamin A concentrations to levels needed for their breastfed infants to accumulate adequate liver vitamin A stores, suggesting that the current recommendation should be reviewed and a higher dosage considered. The International Vitamin A Consultative Group (IVACG) subsequently recommended that 400,000 IU of vitamin A be administered to women in two doses at least 24 hours apart within 6 weeks postpartum (58).

Because studies showed no sustained beneficial effects of high dose postpartum vitamin A supplementation, the WHO revised their recommendations in 2011 and published a new set of guidelines for vitamin A supplementation of women and children. Among the major recommendations are: (i) maternal vitamin A supplementation is not recommended for the prevention of maternal and infant morbidity and mortality, but in populations where vitamin A deficiency is a severe public health problem, vitamin A supplementation is recommended in pregnancy for the prevention of maternal night blindness, up to a dosage of 10,000 IU daily or 25,000 IU weekly for at least 12 weeks; and (ii) vitamin A supplementation of new-borns and infants 1-5 months old is not recommended for the prevention of infant morbidity and mortality (6, 59).

1.6 Impact of dietary intake on vitamin A status of women of reproductive age

Several studies have documented associations between dietary intake of vitamin A and vitamin A status among women of reproductive age.

Among Indonesian women of child-bearing age, expenditures on grain foods were associated with increased odds of night blindness whereas expenditures on plant-based foods (fruits and vegetables), animal-based foods and eggs were associated with reduced odds of night blindness (60). Spanish women whose intake of vitamin A from foods and supplements was less than the RDA during pregnancy had significantly lower mean serum and breast milk retinol concentrations than women who had higher intakes of vitamin A (61). Other studies (12, 56, 62, 63) showed that the vitamin A content of breast milk is affected by women's diets during both pregnancy and lactation, but among Gambian women no association was found between reported intakes of vitamin A and its concentration in breast milk (64).

In Bangladeshi women supplemented with fat from middle- and late- pregnancy to 6 months postpartum, breast milk retinol levels were significantly higher at 3 months postpartum compared to the control group, suggesting that fat supplementation among pregnant and lactating women with low dietary fat intake has beneficial effects on maternal vitamin A status postpartum (65). Among pregnant and lactating Tanzanian women, red palm oil increased the α- and β-carotene levels of breast milk and serum; and red palm oil and sunflower oil also seemed to prevent the decrease in breast milk retinol concentrations that occurred in the control group from 1-3 months postpartum (66). Lactating women in Cameroon who reported consuming red palm oil in the previous week had higher breast milk vitamin A compared to women who did not consume any red palm oil in the previous week (67). This is in agreement with an earlier study among Bangladeshi women that showed that β-carotene supplementation conserved breast-milk retinol up to 6 months postpartum and increased it thereafter (68). A study to evaluate the impact of short-term red palm oil supplementation on the vitamin A status of the mother-infant pair in Honduras found that red palm oil in the maternal diet increases pro-vitamin A carotenoids in breast milk and serum of the mother-infant pair; there were however no increases in maternal serum or milk retinol. In agreement with these results, pregnant women in rural Tanzania supplemented with 4 tablespoons red palm oil daily for three months had significant increases in α-carotene and β-carotene but not retinol in serum (69). Conversely, Indonesian women who received an enriched wafer containing 3.5 mg β-carotene/day for 12 weeks showed significant increases in both serum and milk retinol (70). In Vietnam, daily intake of both plant and animal vitamin A-rich foods by breastfeeding women for 10 weeks resulted in increased serum and milk retinol concentrations (71).

1.7 Vitamin A requirements in pregnancy and lactation

1.7.1 Pregnancy

During pregnancy, metabolic demands are increased as a result of physiological and hormonal changes in the mother and the growth of the fetus (72). Vitamin A requirements are increased in pregnancy, compared to the non-pregnant state. These extra requirements are small and mostly confined to the last trimester of pregnancy (12). The RDA for pregnancy is set at 750 μg RAE/day of vitamin A for women aged 14–18 years and 770 μg RAE/day for women aged 19-50 years, compared to an RDA of 700 μg RAE/day for non-pregnant, non-lactating women (73). The FAO/WHO recommends a safe level of intake of 800 μg RAE/day for pregnant women (74). In areas where intake of animal source foods is low, particularly in developing countries, pregnant women are at an increased risk of vitamin A deficiency.

1.7.2 Lactation

During lactation, there is a daily loss of maternal vitamin A through breast milk consumed by the infant. Therefore, requirements are higher in lactation than in pregnancy (3). The RDA for lactation is set at 1,200 μg RAE/day of vitamin A for women aged 14–18 years, and 1,300 μg RAE/day of vitamin A for women aged 19-50 years (73). The FAO/WHO recommends a safe level of intake of 850 μg RAE/day for lactating women (74).

1.8 Consequences of vitamin A deficiency

Even though vitamin A supply to the developing fetus is highly regulated, the placenta and fetus are affected when maternal dietary intake is very low (12, 75). Vitamin A deficiency may induce fetal resorption, stillbirth and malformation (11, 15, 76).

Vitamin A deficiency in pregnant women, particularly during the third trimester, results in night blindness (77) and also compromises the integrity of the epithelial layer of the gastric and respiratory tracts, resulting in increased risk of morbidity and mortality due to infectious diseases (78). Vitamin A deficiency is associated with adverse maternal, fetal and neonatal outcomes and may be a cause of anemia (79).

The potential adverse effect of poor vitamin A status on pregnancy outcome was shown in an intervention study in a region in Nepal with endemic vitamin A deficiency, where weekly supplementation of pregnant women with approximately their recommended daily intake of vitamin A reduced maternal mortality by 40% (80); the apparent cause of the reduced mortality risk was less susceptibility to infection. An additional advantage of vitamin A supplementation of pregnant women is that it can increase hemoglobin concentrations, by about 10 g/L in marginally deficient populations (81).

Vitamin A status during pregnancy has been linked to breast milk vitamin A levels. Spanish women with serum retinol concentrations < 1.05 μmol/L during the third trimester of pregnancy had lower mean breast milk retinol concentrations, when compared to women with higher serum retinol concentrations (61).

Deficiency of vitamin A adversely affects children because of the growth and rapid cellular differentiation that occur in early childhood (82). Vitamin A affects iron status because it is involved in mobilization of hepatic iron stores, and it may enhance hematopoiesis in children with vitamin A deficiency (83); in Indonesian pregnant women with marginal iron deficiency, vitamin A supplementation increased hemoglobin concentration by about 10 g/L (81). Vitamin A

deficiency also increases susceptibility to respiratory infections and diarrhea (84), and is associated with increased morbidity and mortality among children (17, 85).

1.9 Consequences of vitamin A excess

Animal studies indicate that excessive intake of vitamin A in early pregnancy, when organogenesis is occurring rapidly, increases the risks of teratogenesis (86). Thus, high-dose vitamin A supplementation to women of reproductive age should be avoided.

A major strategy for improving the vitamin A status of young infants is to supplement them directly, either at birth or during other contacts with the health-care system. In Bangladesh, infants 6-17 weeks old who received 3 doses of 25, 000-50, 000 IU vitamin A at monthly intervals with immunizations had increased serum retinol concentrations (87, 88); however, 10–15% of the infants in these studies developed bulging fontanel shortly after dosing (89, 90). In a safety study conducted in Indonesia, a 50,000 IU dose of vitamin A given to infants at birth was associated with a 2% excess rate of bulging fontanel (91).

A causal association between the bulging fontanel in infants and the intake of 50,000 IU of vitamin A at monthly intervals in the Bangladeshi trial (88) was deemed most likely because: (a) the intervention was evaluated in a randomized double blind trial, (b) it is biologically plausible because raised intracranial pressure is a known manifestation of hypervitaminosis A, (c) bulging fontanel occurred after the 2nd or 3rd dose indicating a dose effect, and (d) similar transient bulging fontanel was recently reported in disease free young infants given the same course of vitamin A supplementation (90). Maternal supplementation programs to indirectly deliver vitamin A in breast milk to infants less than 6 months of age in developing countries would prevent this potential problem, as well as potentially benefit mothers. Two recent trials among

neonates in Guinea Bissau showed that neonatal vitamin A supplementation (25, 000-50, 000 IU) did not result in significant reductions in mortality; the data suggest an increased risk of mortality among girls but not in boys (mortality ratio of 1.39-1.42 in girls and 0.74-0.84 in boys, respectively) (92, 93). In contrast, a study in Bangladesh in which infants were supplemented immediately after birth with 50, 000 IU vitamin A resulted in a 15% reduction in all-cause mortality (94).

A recent review raised new concerns about the safety of vitamin A intake from observations in Western countries, suggesting an association between preformed vitamin A (retinol) intakes of ≥1500 µg/d and increased risk of bone fracture (95). Other manifestations of chronic hypervitaminosis A include central nervous system effects, skin disorders, conjunctivitis, nausea, vomiting, and hepatotoxicity in adults (73, 96, 97).

1.10 Breast milk vitamin A as an indicator for assessing interventions to improve vitamin A status

The vitamin A content of breast milk is a relatively sensitive indicator of vitamin A status of a woman and her breast-fed infant, especially in monitoring and intervention studies (98). In a study to compare the ability of different indicators to detect a response to high dose postpartum vitamin A supplementation (200, 000 IU) among Bangladeshi women, breast milk vitamin A was found to be a responsive indicator of vitamin A status, especially among women with mild vitamin A deficiency (99).

Milk vitamin A has been shown to be responsive to low dose interventions as well. Milk vitamin A concentration was used to successfully evaluate a program to fortify sugar with vitamin A (100) and in a trial of vitamin A-fortified monosodium glutamate (101). Recently, a randomized

controlled trial providing multiple micronutrients containing vitamin A to mildly deficient Indonesian women also concluded that milk vitamin A is a good indicator for monitoring the effects of vitamin A interventions in women (102).

The adoption of maternal milk sampling to assess the vitamin A status of populations is a good option because it is less invasive than blood collection and predicts the vitamin A status of both the mother and the infant (103).

Retinol is esterified to fatty acids in milk, and it is present in the fat globules in milk (99). Milk fat content varies in relation to the time since the child was last breastfed, lactation stage and other factors (104). It has been noted that the fuller the breast, the lower the fat content; hence, taking milk samples from a breast that has recently been used to feed a child will result in higher fat and vitamin A levels compared to samples taken from a breast that has not been used to feed the child recently (105). To standardize breast milk collection for vitamin A analysis, 2 main techniques have been used: the full or casual collection methods (99). The full collection method involves the use of a breast pump to collect the entire contents of one breast that has not been used to feed a child for at least 2 hours; for casual collection, the mother manually expresses a small volume of breast milk (about 5 mL) into a container, without controlling for the time since the last breastfeeding episode (99).

A study among Guatemalan mothers found less variability in milk fat content when milk samples were collected from fasting mothers between 6am to 8am, with greater variability in milk samples collected from 12noon to 2pm and from 4pm to 6pm (104). It is important to standardize milk collection procedures and to measure milk fat, and to express milk vitamin A concentration per unit volume and per gram of fat (105). The ratio of vitamin A to milk fat may be a better