Published in final edited form as:

Am J Hum Biol. 2011 ; 23(5): 664–673. doi:10.1002/ajhb.21195.

Vitamin A Dynamics in Breastmilk and Liver Stores: A Life History Perspective

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Abstract

Objectives—Newborns are dependent on breastmilk vitamin A for building hepatic stores of vitamin A that will become critical for survival after weaning. It has been documented that vitamin A concentrations in breastmilk decline across the first year postpartum in both well-nourished and malnourished populations. The reason for this decline has been assumed to be a sign of concurrently depleting maternal hepatic stores. This study investigates this assumption to clarify why the decline occurs, drawing on life history theory.

Methods—A cross sectional survey was conducted among lactating mothers in Kenya in 2006. Data were used to examine (1) the relationship between liver vitamin A and time, (2) if the relationship between milk and liver vitamin A varies by time, and (3) by maternal parity.

Results—The relationship between liver vitamin A and time fits the quadratic pattern with marginal significance ($P = 0.071$, $n = 192$); the liver vitamin A declined during early postpartum then recovered in late postpartum time, controlling covariates. The milk-liver vitamin A relationship varied by postpartum time periods ($P = 0.03$) and by maternal parity ($P = 0.005$). Mothers in earlier postpartum or higher parity had a stronger positive relationship between milk and liver vitamin A than mothers in later postpartum or lower parity.

Conclusions—Our observations are consistent with life history tradeoffs and negate the assumption that maternal hepatic and milk vitamin A decline together. Rather, maternal liver vitamin A has a dynamic relationship with milk vitamin A, particularly depending on postpartum time and maternal parity.

Vitamin A plays a crucial role in a variety of bodily processes, notably vision, cellular differentiation, growth, reproduction, and the immune system (McLaren and Frigg, 2001). Even asymptomatic or subclinical vitamin A deficiency compromises human immune function and is associated with a 25–30% elevated risk of infectious disease and death (Micronutrient Initiative and UNICEF, 2004, Sommer and West, 1996). Worldwide, 1

million deaths of young children are attributed to vitamin A deficiency every year (Micronutrient Initiative and UNICEF, 2004). Underscoring the magnitude of vitamin A deficiency as a cause of death, globally, 16–21% of all children under 5 years of age are immunocompromised by vitamin A deficiency, suffering increased rates of death from diarrhea, measles, and malaria (Black, 2003; WHO, 2004).

Vitamin A is an essential vitamin that humans must consume either in the form of retinol (preformed vitamin A) or provitamin A carotenoid (precursor of vitamin A) because we cannot synthesize it in our body. Ingested vitamin A that is not used immediately can be stored in the body, mostly in the liver, and when necessary the liver retinol is mobilized, bound to retinol-binding protein (RBP), via serum to supply for the body's physiological needs and, for mothers, breastmilk retinol. A simplified model of flow of vitamin A in the maternal system is shown in Fig. 1. Vitamin A deficiency occurs when liver stores of retinol become depleted either through insufficient dietary intake or through increased physiological requirements, or both. Major risk factors for vitamin A deficiency include age (young children are susceptible; Sommer, 1982), premature weaning for children (Williams, 1983), pregnancy and lactation for women (Katz et al., 1995), and diets habitually low in vitamin A (McLaren and Frigg, 2001).

Newborns have meager retinol stores, and are fully dependent on breastmilk for physiological needs and for building liver stores needed after weaning (Stoltzfus and Underwood, 1995). Studies, however, report that breast-milk retinol concentrations generally decrease across the first postpartum year (Ahmed et al., 2003; Haskell and Brown, 1999; Gebre-Medhin et al., 1976; Gross et al., 1998; Newman, 1993; Roy et al., 1997; Stoltzfus et al., 1993) in both well-nourished and mild-to-moderately mal-nourished populations (Haskell and Brown, 1999; Rice et al., 1999). This decline makes mother-child retinol transfer via breastfeeding progressively restrictive over the course of the first year because average milk output also decreases slightly between 1 and 12 months in exclusively or predominantly breastfeeding women (Jelliffe and Jelliffe, 1978; Underwood, 1994). This declining postpartum trend in breastmilk retinol concentration has been assumed to be a sign of concurrently depleting maternal liver stores, presumably in direct correlation with breastmilk vitamin A concentrations (Stoltzfus and Humphrey, 2002). Some studies, however, suggest that the decline in breastmilk retinol concentration across the first postpartum year may occur regardless of the maternal vitamin A status, even in supplemented mothers with adequate retinol liver stores, a truer indicator of maternal vitamin A status than milk or serum levels (Rice et al., 2000a,b; Stoltzfus et al., 1993). Why the decline is pervasive despite potential adversity on child health merits investigation. The study presented here seeks to develop an explanatory model of the relationship between breastmilk retinol and maternal hepatic retinol stores, using a life history theoretical framework (Ellison, 1991, 2003; Rose, 1983; Stearns, 1992) and hypothesis tests. Preliminary, more descriptive analyses for this research have appeared in the form of a dissertation chapter (Fujita, 2008a) and abstracts (Fujita, 2007, 2008b, 2009; Fujita et al., 2009b).

HYPOTHESIS

Life-history theory provides a framework for analyzing variation in reproductive strategies adopted by different organisms as a result of natural selection (Ellison, 1991, 2003; Rose, 1983; Stearns, 1992). The theory predicts that demographic traits or life history traits in an organism's lifespan are coordinated in a coherent manner through constraining relationships of tradeoffs (Gadgil and Bossert, 1970; Hill, 1993) because limited resources must be allocated between current reproduction, somatic growth and maintenance, and future reproduction (Rose, 1983; Sibly and Calow, 1987; Stearns, 1992).

From this perspective, we hypothesize that the perplexing decline in breastmilk retinol is a result of life history tradeoffs. Since vitamin A is a scarce nutrient in the natural world, it is expected that the human maternal body has been selected for with the ability to optimally allocate vitamin A between competing needs, namely for breastmilk and for body stores depending on the length of time since birth and the number of births previously given (Williams, 1966). We define milk retinol as the maternal investment in the infant (current reproduction), and maternal liver stores as the investment in maternal survival and future reproduction. This classification does not explicitly depict the importance of maternal survival for the success of current reproduction. It is implicit, however, and this simplification is granted when we view that at any given moment retinol that is stored in the liver is what has been spared from being transferred to breastmilk (and to the serum for immediate maternal physiological needs). We hypothesize that maternal hepatic retinol stores will initially show a parallel decline with breastmilk retinol but the trends will eventually diverge as the hepatic stores recover, whereas breastmilk retinol concentrations continue to decline as time since birth advances, reflecting the priority shift from the investment of vitamin A in milk to liver within the maternal system (Fig. 2). We further hypothesize that the degree of priority shift will vary by parity. Increasing parity should be associated with a less pronounced priority shift (i.e., smaller recovery of liver retinol) as the future reproduction becomes less important as the mother has already achieved reproductive rounds.

To test the above predictions, we examine (1) the relationship between liver vitamin A and time, (2) if the relationship between milk and liver vitamin A varies by time, and (3) by maternal parity, using the following hypotheses:

1. The relationship between liver retinol and postpartum time will follow a quadratic pattern; liver retinol will decline during the early postpartum period then recover in the later postpartum period.
2. Milk retinol and liver retinol have a different relationship by postpartum time. Mothers in earlier postpartum are more likely to have a stronger positive relationship between milk retinol and liver retinol than mothers in later postpartum.
3. Milk retinol and liver retinol have a different relationship by parity. High parity mothers are more likely to have a stronger positive relationship between milk retinol and liver retinol than low parity mothers.

These hypothesized relationships are assumed to be a universal human trait with links to individual-, household-, and community-level factors which influence food consumption and breastfeeding patterns. Thus, we expect to see the same pattern in both vitamin A deficient and replete women. The study population, which includes women of various vitamin A statuses, will allow us to investigate this (see below).

MATERIALS AND METHODS

Ariaal community

To test the above hypotheses, data were collected in Kenya. The study population is Ariaal women in Marsabit District. Research procedures were approved by the Institutional Review Boards of the University of Washington (UW) and the Kenya Medical Research Institute (KEMRI). The Ariaal have traditionally practiced pastoralism, subsisting on the milk of camel and cattle (Fratkin, 1998; Sobania, 1988), but today about one half of ~ 10,000 Ariaal are permanently settled, subsisting as farmers, livestock, and dairy traders, or wage earners (Fratkin, 1998, 1991). Biological concomitants of sedentism for the Ariaal include reduced growth velocity of children (Fratkin et al., 1999; Nathan et al., 1996; Shell-Duncan and Obiero, 2000), likely attributable to children and their mothers' low dietary intake of herd's

milk, which provides essential nutrients for human growth (Fujita et al., 2005; Shell-Duncan and Obiero, 2000).

Ariaal women were chosen for this study for three reasons. First, Ariaal women have on average over six children during the course of their reproductive span, and thus, transfer considerable amounts of energy and nutrients to their offspring through pregnancy and lactation. (Roth, 1994, 1999). Second, health practitioners in the area consider vitamin A research a community priority because vitamin A deficiency is common among children and pregnant women in the area. Our previous dietary surveys found low vitamin A intake estimate in the Korr location (Shell-Duncan and Yung, 2004). Third, our long-term relationship with Arrial people spanning for over 10 years facilitated the acceptance of this study despite the invasiveness of the blood collection needed for liver vitamin A reserve estimates.

The vitamin A situation has since somewhat improved in this region with the start of vitamin A intervention around 2005. For example, in August 2005 Food for the Hungry International of Marsabit began distributing oral dose vitamin A (200,000 IU) to all accessible mothers within 6 weeks postpartum in Karare area, with the coverage rate of approximately 50%. Our 2005 pilot project has suggested the presence of mothers with varied vitamin A status in the study area, spanning from vitamin A replete, subclinical deficiency, to clinical deficiency, as well as high-dose vitamin A capsule supplemented mothers within a single population (Fujita, 2006).

In August 2006, a total of 241 lactating Ariaal mothers were sampled, through stratified random sampling (Kalton, 1983) in which five postpartum age range strata—0–3, 3–6, 6–9, 9–12, and 12–20 postpartum months—were equally represented. These women were recruited as participants/subjects for coupled quantitative and semi-quantitative surveys, including the collection of biological data. The inclusion criteria for women were: (1) 20 months or less postpartum; (2) currently lactating; and (3) not taking oral contraceptives. For those who satisfy these criteria, exclusion criteria were (1) clinical or reported symptoms of liver and/or kidney diseases or acute infections, and (2) subclinical indications of inflammation or infection (acute phase reaction), since these conditions could bias the assessment of serum and liver retinol.

Data collection and preparation

Quantitative and semiquantitative surveys—Survey questions relevant for this study included those regarding demographic information, reproductive history, socioeconomic status, breastfeeding frequency, 24-hour dietary recalls, and whether or not respondents received a high-dose vitamin A capsule postpartum (PPVA capsule). Biological data collection featured anthropometric measurements, and blood and milk samples.

Blood samples were used to determine hemoglobin concentrations, serum C-reactive protein (CRP) concentrations, and liver retinol stores based on the relative-dose response (RDR) test of serum retinol. The RDR test is based on the percentage change in serum retinol concentrations between two blood samples collected 5 hours apart, between which a specific dose of vitamin A (commonly retinyl palmitate) is administered orally. Larger responses represent lower liver stores where RDR ≥ 20 are considered inadequate stores (WHO, 1996). This test is based on the observation that RBP that has not bound to retinol accumulates in the liver in vitamin A deficiency, and that a dose of vitamin A facilitates this relative excess of RBP to bind to vitamin A and enter the serum circulation in a rapid and sustained manner (Fujita et al., 2009a; WHO, 1996). The RDR test is a categorical indicator of vitamin A status (sufficient/deficient), and the utilization of RDR values as a quantitative measure to assess individuals' vitamin A status is not recommended (Tanumihardjo, 2005). We adopted

RDR as a semiquantitative variable to explore the population-level liver vitamin A trends otherwise difficult to estimate in a nonclinical setting. Hemoglobin was used as a covariate for modeling liver retinol; it has been reported that serum retinol and hemoglobin are closely associated in lactating women (Ettyang, 2003). Similarly, CRP, a biomarker of subclinical infection/inflammation or acute phase reaction, was used to correct for potential bias on the serum or liver retinol due to the acute phase reaction which transiently decreases serum vitamin A concentration (Thurnham et al., 2003) through a reduction in hepatic RBP synthesis and secretion of the retinol-RBP complex (Rosales et al., 1996). Milk samples were used to determine milk fat levels (based on creatinocrit as explained below) and milk retinol concentrations. Being a fat soluble vitamin, milk retinol is associated with milk fat.

Dietary intake data—Typical patterns of maternal diet were estimated by a 24-hour dietary recall interview. A single 24-hour recall for a small population tends to suffer low validity due to day-to-day variation in diets (Gibson, 1990). Repeated recalls are superior for assessing food consumption patterns (Gibson, 1990). Because of the logistical limitation, however, we compromised by using a single recall. The relatively monotonous diets consumed by adult women previously reported from the study area (Fujita et al., 2004) suggests a relatively small day-to-day or weekend effects. The mothers were asked to report all foods that they consumed the previous day, to estimate the amount, in common household units, such as cups and spoons, and to assess if it represented her typical diet. Only the recall data for women who stated that yesterday's diet represented their typical daily diet were used for the analysis. Portion sizes were quantified using food models and by filling local dishes and utensils with dried rice to the level specified by a participant. After listing all specific foods, information on recipes and mixed dishes was collected. The final volume of the recipe was then estimated from the size of the pot in which it was prepared and the level of the contents after the dish was cooked. The amount of each ingredient consumed by the mother was estimated from her portion as a fraction of the amount prepared. The resulting recall list of foods and portion sizes in grams was then used to compute the total daily energy, fat, and vitamin A intake estimates, using the SIGHT AND LIFE Vitamin A Intake Calculator (Erhardt, 2003). These nutritional intake data were used for descriptive statistics, as well as for inferential statistical modeling if the variable is at least marginally correlated with liver or milk retinol.

Anthropometry

Anthropometric measurements were taken as per the methods described by Frisancho (1990). Women were weighed using a scale to the nearest 100 grams. Height was measured with an anthropometer to the nearest millimeter. Mid-arm circumference (MAC) was measured with an insertion tape to the nearest millimeters, and triceps skinfold thickness (TSF) was measured with a caliper to the nearest millimeters. Measurement of TSF was taken three times, and the mean value was recorded. Resulting measurements were used to calculate derived indices of body mass index (BMI), upper arm muscle area (UMA), and upper arm fat area (UFA). BMI was used for descriptive purpose and derived with the equation $BMI = \text{weight (kg)} / (\text{height (m)})^2$. UFA represents the subcutaneous fat contribution to the upper arm. UMA represents the volume of the arm exclusive of the contribution of subcutaneous fat. UFA was derived with the equation $UFA = TUA - UMA$, where $TUA \text{ (cm}^2\text{)} = (MAC^2) / 4 * \pi$ and $UMA \text{ (cm}^2\text{)} = (MAC - (TSF * \pi))^2 / 4 * \pi$ (Frisancho, 1990). These anthropometric indices were used for descriptive statistics and inferential statistics if the variable was at least marginally correlated with liver or milk retinol.

Acute phase reaction determination

Serum CRP was assessed using a microtiter plate-based sandwich enzyme immunoassay (Brindle et al., 2010). 3.0 ml of fasting venous blood was collected from each consenting

mother using a disposable sterile needle and syringe. The whole blood was centrifuged in the field station to obtain serum specimens. The serum specimen was stored frozen in liquid nitrogen for 13–42 days, and then transported to the Centre for Public Health Research laboratory of KEMRI in Nairobi. All samples were then shipped via air express on dry ice to the UW and stored at -20°C until CRP assays at the Biodemography Laboratory 251–553 days after serum collection.

The CRP assay method has been described elsewhere (Brindle et al., 2010). Capture (C5 anti-CRP MAb, cat. No. M86005M) and detection (clone C6 anti-CRP MAb, cat.no. M862484M) antibodies were purchased from BIODESIGN International/OEM Concepts (Saco, Maine). The detection antibody was then conjugated to biotin in Biodemography Laboratory. Calibrators purchased from Fitzgerald Industries International (Concord MA; cat. No. 30-AC10) were used for an 8-point (0, 0.316, 0.631, 1.262, 2.524, 5.048, 10.1, 20.2 mg/L) calibration curve. In the assay, standards, controls, and unknown serum samples were incubated in microtiter plate wells, which were coated with capture antibody overnight at 4°C . The wells were then blocked with assay buffer and incubated for 30 min. After washing, wells were incubated with biotinylated detection antibody in assay buffer for 2 hours at room temperature. Wells were then washed and incubated with horseradish peroxidase-conjugated streptavidin in assay buffer for one hour. Finally, the wells were washed and incubated with ABTS substrate solution, covered on a plate shaker for 45–60 min at room temperature. The resulting turnover in substrate was read by dual wavelength absorbance measurement at 405 nm and 507 nm. Low, medium, and high serum controls for 11 assay plates diluted independently for 3 batches gave mean values of 3.25, 5.25, and 8.86 mg/L, with intra-assay CVs of, 6.66, 7.39, and 5.46%, and interassay CVs of 20.2, 20.1, and 8.0%, respectively.

Data for individuals with CRP values above the cut-off for acute phase response ($>10\text{ mg/L}$) were excluded from the statistical analysis, as per the exclusion criterion. CRP was also used as a covariate in statistical modeling of liver retinol for the remaining individuals to adjust for the effect of CRP variations below the exclusion cut-off.

Hemoglobin determination

To assess hemoglobin concentrations, immediately after the blood collection 50 μl of whole blood was applied to the portable HemoCue B-Hemoglobin system (HemoCue, Mission Viejo, CA), calibrated daily in the field station. Hemo-Cue readings (g/L) were recorded.

Liver Vitamin A determination

To estimate maternal vitamin A liver stores using the RDR test, two blood samples were required from each mother before and after a small vitamin A dose. To achieve this, immediately after the fasting blood collection, an oral dose of 1,000 μg of retinyl palmitate was administered, followed by meals and beverages with no vitamin A content. A second blood collection after 5 hours of the oral dose (post-dose blood; WHO, 1996) was done followed by another meal. The afternoon serum specimens were stored and transported in the same manner as the morning specimens. Using thawed samples, retinol concentrations were assessed in the Clinical Nutrition Research Unit Laboratory of the Harbor Medical Center via reverse-phase HPLC (Waters, Milford, MA; Bieri et al., 1979), run in 11 batches 177–342 days after collection. The predose (A0) and postdose (A5) specimens from each mother were run in the same batch to avoid interassay variation. The retinol values were then used to calculate the RDR values for estimating vitamin A liver stores by multiplying the RDR value by -1 where $\text{RDR} = \{(A5-A0)/A5\} * 100$.

The HPLC protocol was as follows: Retinol was isolated from serum by lipid extraction and fractionated using a C18 reverse-phase column (Waters Bondapak) developed by isocratic elution with methanol, and detected at 313 nm. Retinol acetate (Sigma-Aldrich R4632) was added to each specimen as an internal standard; standard curves were verified using standard reference material from the National Institute of Standards and Technology. Assay controls were within the laboratory's acceptable range.

Breastmilk fat (Creatocrit) determination

Breastmilk samples of 10 ml were collected in the morning by manually expressing from whichever breast was not nursed overnight. Samples were transferred to poly-propylene tubes, covered from light, stored in liquid nitrogen then in a -20°C freezer at the Centre for Public Health Research laboratory in Nairobi. Samples were then express shipped on dry ice to the UW and stored at -20°C . Two sets of sample aliquots were made. One set was used for milk fat determination and the other set was used for retinol determination.

The milk fat level of each breastmilk sample was measured in triplicate by the primary author at the Biodemography Lab, using the creatocrit method (Lucas et al., 1978 explained in detail by Wang et al., 1999) 427–451 days after specimen collection. In short, a creatocrit measure is the % of a cream column length measurement out of a total milk column length measurement of a thawed and mixed milk sample, drawn in and centrifuged in a standard capillary tube with one end sealed with clay (3000 rpm, 15 min). The averaged values of triplicate measures (creatocrit %) were recorded.

Breastmilk Vitamin A determination

The second set of milk aliquots was express shipped on dry ice to the Mayo Medical Laboratories in Rochester, Minnesota where breastmilk retinol concentrations per volume ($\mu\text{g/L}$) of milk was assessed by HPLC (Mayo Medical Lab Unit Code 8766) 633–662 days after specimen collection. Because of the accidental breakage of milk vials during transportation in Kenya, about 20% of milk samples were lost, contributing to the smaller sample size for milk vitamin A than liver vitamin A analysis. Sufficient volume of milk samples and other necessary variables of statistical modeling were available from 155 mothers.

Of these 155 milk samples, 55 had milk retinol levels below the limit of the HPLC assay range ($<20 \mu\text{g/L}$ or $<0.07 \mu\text{mol/L}$). This high occurrence of subrange results may be due to the limitation of HPLC with previously frozen milk at Mayo (Mayo Medical Laboratories, email correspondence) and/or the effect of long storage ($>600 \text{ d}$). There were, however, some observations indicative that these subrange values reflect biological variations rather than laboratory or storage effects. For example, the subrange specimens were significantly associated with low breastfeeding frequency ($P = 0.02$) and lower maternal age ($P = 0.04$). To avoid potential systematic biases that may arise from excluding sub-range specimens that may be an important segment of the sample population, we assigned the value $19 \mu\text{g/L}$ to all of them and used for analysis rather than excluding them.

General characteristics of mothers for liver retinol ($n = 192$) and milk retinol ($n = 155$) analysis are summarized in Table 1. The mean maternal age ($\pm\text{SD}$) was 28 ± 7 years old, and the mean parity was 3.5 ± 2 . We define parity as the number of live births given to the offspring who have survived at least 1 year.

Statistical methods

For the first hypothesis, we used the regression model (**Model A**) with liver retinol as the dependent variable and postpartum time as the independent variable. For this, postpartum

time was modeled as a quadratic to test the fit to the hypothesized positive quadratic pattern. The following covariates were included in the statistical model: postpartum time (day), age (years), PPVA capsule (yes = 1/no = 0), land size (1 2 3 ...acres), livestock (yes = 1/no = 0), fat intake (g/day), UFA, hemoglobin (g/L), CRP (mg/L) and parity (1 2 3 ...births).

To clarify how liver retinol predicts breastmilk retinol, and how this prediction dynamically change over time and across parity, we used the model that tests milk vitamin A as a function of liver retinol, time postpartum, maternal parity, and the interactions, controlling for covariates, using a subsample of mothers ($n = 155$), for whom both milk and liver retinol data were available. The following covariates were included: age, PPVA capsule, land size, livestock, fat intake, UFA, breastfeeding frequency (1 2 3 ...times/day), and creatatocrit (%). The quadratic term of postpartum time applied in this model was not significant. A diagnostic plot of the residuals vs. predicted values based on the above regression model with the continuous variable of time postpartum indicated that the linearity assumption was violated (see Appendices A1 and A2 for the model and plot). To solve this problem, an alternative model (**Model B**) was fitted using dummy variables of early postpartum (0 if ≥ 180 d/1 if <180 d) and late postpartum (0 if ≥ 360 d/1 if <360 d) instead of the continuous variable of time postpartum to test the differences by time periods and by parity.

To test hypotheses 1 regarding liver vitamin A, we used **Model A**: Liver retinol = $\beta_0 + \beta_1 \text{ time} + \beta_2 \text{ time}^2 + \beta_3 \text{ parity} + \beta_4 \text{ age} + \beta_5 \text{ PPVA} + \beta_6 \text{ land size} + \beta_7 \text{ livestock} + \beta_8 \text{ fat intake} + \beta_9 \text{ arm fat} + \beta_{10} \text{ hemoglobin} + \beta_{11} \text{ CRP}$.

To test hypotheses 2 and 3 regarding milk vitamin A, we used **Models B**: Milk retinol = $\beta_0 + \beta_1 \text{ early postpartum} + \beta_2 \text{ late postpartum} + \beta_3 \text{ liver retinol} + \beta_4 \text{ parity} + \beta_5 \text{ age} + \beta_6 \text{ PPVA} + \beta_7 \text{ land size} + \beta_8 \text{ livestock} + \beta_9 \text{ fat intake} + \beta_{10} \text{ arm fat} + \beta_{11} \text{ creatatocrit} + \beta_{12} \text{ breast-feeding} + \beta_{13} \text{ liver retinol} * \text{early postpartum} + \beta_{14} \text{ liver retinol} * \text{late postpartum} + \beta_{15} \text{ liver retinol} * \text{parity}$.

To aid the interpretation of the results, fitted lines and data distributions for liver vitamin A postpartum were examined in graph forms for the overall trend. The shape of the predicted liver retinol across the postpartum period was visually assessed. Line graphs were drawn to examine how the relationships between milk retinol and liver retinol may vary by postpartum time or by parity. Statistical tests and graphs were prepared using STATA version 9.2 (StataCorp, 2004–2007) and Microsoft Office Excel 2003.

RESULTS

Hypothesis 1: Liver retinol will decline during the early postpartum period then recover in the later postpartum period

The relationship between liver retinol and postpartum time fits the quadratic pattern with marginal significance ($\beta_1 = -0.001$, $P = 0.059$; $\beta_2 = 1.1\text{e-}06$, $P = 0.071$; see Table 2 and Fig. 3) in which the liver vitamin A decline during the early postpartum period then recover in later postpartum period, after controlling for covariates.

Hypothesis 2: Milk retinol and liver retinol have a different relationship by postpartum time. Mothers in earlier postpartum are more likely to have a stronger positive relationship between milk retinol and liver retinol than mothers in later postpartum

The interaction between liver retinol and late postpartum was significant ($\beta_{14} = -24.579$, $P = 0.03$; Table 3). The nature of the interaction is depicted in the differing slopes for the model predictions between late postpartum time and else (Fig. 4a). This regression model predicts that mothers in late postpartum have a negative relationship between milk and liver retinol while others do not, as depicted in the negative slope for late time in contrast to the

positive slope for others. This suggests that mothers in earlier postpartum tend to have a stronger positive relationship between milk retinol and liver retinol than mothers in later postpartum.

Hypothesis 3: Milk retinol and liver retinol have a different relationship by parity. High parity mothers are more likely to have a stronger positive relationship between milk retinol and liver retinol than low parity mothers

The liver retinol-parity interaction was also significant ($\beta_{15} = 6.514$, $P = 0.005$; Table 3). The nature of the interaction is depicted in the differing positive slopes for the model predictions between low and high parity (Fig. 4b). Mothers in high parity tend to have a stronger positive relationship between milk retinol and liver retinol than mothers in low parity.

DISCUSSION AND CONCLUSIONS

Our investigation of the paired trends in breastmilk and maternal hepatic retinol based on life history models using cross-sectional data from northern Kenya revealed that maternal liver vitamin A is in a dynamic relationship with milk vitamin A postpartum, and depending on time since birth and maternal parity in a tradeoff relationship. For example, liver retinol did not track declining breastmilk retinol, but instead recovered during the late postpartum period. Further, mothers in late postpartum had a negative relationship between milk and liver retinol. This observation negates the assumption that milk and liver vitamin A are in direct correlation, and suggests that the vitamin A allocation within the maternal system between liver and milk may be based on the principle of tradeoffs late postpartum. The parity-related variations in milk and liver retinol also support the prediction from life history tradeoffs. High parity mothers were more likely to have a stronger positive relationship between milk and liver retinol than low parity mothers. Combined, our data support the idea that the allocation priority is on the recovery of liver stores in lower parity and this priority will shift toward milk retinol in higher parity. These observations are consistent with the idea that maternal body has evolved to optimally allocate vitamin A between competing physiological domains.

It is possible that the observed tradeoff relationship between liver and milk retinol may arise from variations in infant feeding patterns or maternal dietary intake of vitamin A. For example, the reduction in breastfeeding intensity/frequency across the postpartum months may result in similar patterns (i.e., milk vitamin A decline while liver vitamin A recovers) through the reduction in vitamin A expenditure over time, rather than the biological priority shift. If this were the case, the tradeoffs would be a reflection of behavioral ecology rather than the genetically evolved biology. The current study attempted to account for the effects of infant feeding and maternal dietary behavior, by including such variables as breastfeeding frequency and maternal fat intake in statistical modeling (vitamin A intake was not significantly associated with milk/liver retinol and was not included in the models). We suggest that a further study investigate the role of infant feeding more thoroughly, including not only breastfeeding but also nonmilk complementary feeding practices in order to clarify the pathways through which the tradeoffs may occur. Regardless of the relative contribution between biology and behavior, our observation of the systematic variations in the concurrent milk and liver vitamin A trends by a key reproductive status marker of parity provides an important empirical example of human life history tradeoffs.

As a study of human life history traits, our study is significant in two ways: (1) the use of a single currency (i.e., vitamin A) and (2) the use of the trend rather than the absolute values or counts for evaluating the tradeoffs between current and future reproduction. Previous attempts at measuring reproductive effort relied on the analysis of “those isolated

characteristics most obviously associated with reproduction” (Lombardi, 1998). For example, the breastfeeding practice/maternal energy balance has been used for measuring current reproduction and the resumption of postpartum fecundity for future reproduction (e.g., Vallegia and Ellison, 2004). This approach is practical, yet the cascade of events that lay between these characteristics as well as “many complex and energetically costly interactions” of these events that together consist of an overall reproductive effort (Lombardi, 1998: 359) pose a major challenge against empirical studies of the human reproductive effort (Lombardi, 1998: 359). Although this critique is valid for Life-historians, actually measuring the complex overall reproductive effort in humans is impossible. Instead, we refined the measures for reproductive effort by narrowly focusing on a single micronutrient vital for human reproduction (Fujita, 2008a). By defining breastmilk vitamin A as the investment for current reproduction and liver vitamin A for future reproduction, our measures of reproductive efforts no longer depend on “isolated characteristics most obviously associated with reproduction” but on closely linked traits of vitamin A levels, without which human reproduction is impossible (Fujita, 2008a).

Our study suffers some limitations. First, our breast-milk retinol data had very low concentrations across the board, all falling below the cutoffs for vitamin A deficiency (<0.7 $\mu\text{mol/L}$), and a substantial proportion of milk samples fell below the assay range. The bivariate characteristics of our milk vitamin A data we discussed in the earlier section support our belief that the data are adequate for delineating the within-population postpartum trends. Yet, we cannot deny the likelihood that the data underestimate the variance in milk retinol at the lower range.

Second, we compromised with a single 24-hour dietary recall per participant instead of repeating multiple recalls. This compromise may be a reason why our results show a lack of association between milk/liver retinol status and vitamin A intake.

The third limitation is the cross sectional study design (Fujita, 2006). Our findings were consistent with the idea of a tradeoff relationship, but this needs to be addressed prospectively. Cross sectional surveys consider prevalence rather than incidence. As such, the data reflects determinants of survival as well as outcome (Kelsey et al., 1986). Because vitamin A is crucial for human immune function and reproduction, women in the lowest levels of vitamin A would be vulnerable to infection, miscarriage or early child loss and unlikely to be surveyed. This could introduce bias if their breastmilk or liver vitamin A levels were distinct from the sampled population. Further, the use of cross sectional data to describe trends across time assumes relatively small influences of extraneous factors. If unexplained variability was large for the statistical power, our findings might be compromised. The logistic constraints dictated our choice of current study design. Many previous studies have also adopted cross sectional designs to examine the relationship between serum or breastmilk vitamin A and the time since birth (Boediman et al., 1979; Ettyang et al., 2003; Gebre-Medhin et al., 1976; Gross et al., 1998; Panpanich et al., 2002).

We plan to improve upon these limitations in our future investigation. Insights from life history theory on vitamin A transfer will have public health implications. The current recommendation is to give a single high-dose vitamin A supplement in the early postpartum period (WHO/UNI-CEF/IVACG Task Force, 1988) with a belief that this would benefit both the mother and the infant (Stoltzfus and Humphrey, 2002; WHO, 1998). The benefits for infants, however, have been less than expected (e.g., Rice et al., 1999) or potentially adverse in HIV positive mother-offspring dyads (e.g., Fawzi et al., 2002). Perspectives from life history theory would suggest that the transfer rates via breastmilk may be set before the birth of the infant. Further research is warranted for a more comprehensive understanding of mother-offspring vitamin A transfer.

Acknowledgments

Contract grant sponsor: NSF Dissertation Improvement; Contract grant number: 0622358; Contract grant sponsor: Wenner-Gren Foundation Research; Contract grant number: 7460; Contract grant sponsor: Micronutrient Initiative (Ottawa).

The authors thank two anonymous reviewers for their feedback which greatly enhanced this manuscript. Also, we are grateful to the following parties for their participation in or assistance for the development of this study: Ariaal mothers and research assistants of Marsabit District, Kenya; Mrs. Lydia Kaduka, Mr. Saidi Kisiwa, and Dr. David Mwaniki of KEMRI; Anita Rocha, Jane Shofer, and Dr. Jonathan Gorstein of UW; Dr. Thomas McDade of Northwestern University; Dr. Elizabeth Ngugi of the University of Nairobi, Kenya; and Dr. Eric Roth of the University of Victoria, Canada. Retinyl palmitate was provided by the Task Force SIGHT AND LIFE through the DSM Nutritional Products, Inc.

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APPENDIX A

TABLE A1

Regression model for milk retinol, modeled with continuous time in days

Predictor	Model B ^a β
Constant	7.728
Time	0.005
Liver retinol	-4.173
Parity	-0.293
Age	0.299 ^b
PPVA	-0.385
Land size	0.156
Livestock	2.585 ^b
Fat intake	0.029
Arm Fat	0.079
Creamatocrit	0.918 ^c
Breastfeeding	0.266
Liver Time	-0.047
Liver Parity	5.739 ^d

^a $R^2 = 0.20$, Adjusted $R^2 = 0.13$, $P = 0.0016$, $n = 155$.

^b $P < 0.1$.

^c $P < 0.01$.

^d $P < 0.05$.

APPENDIX B

Fig. A1.
The residuals vs. predicted values for milk vitamin A, modeled with continuous time in Days.

Fig. 1.

Flow of vitamin A in maternal system. Ingested vitamin A that is not used immediately can be stored in the liver, and when necessary the liver retinol is mobilized, bound to retinol-binding protein (RBP), via serum to supply for the body's physiological needs and breastmilk retinol.

Fig. 2.

(a) Conceptual framework for retinol allocation priority due to maternal reproductive strategy (b) Hypothesized trends between breastmilk and maternal liver retinol levels across the 1st year postpartum. On the basis of this Life History model, maternal hepatic retinol stores are expected to initially show a parallel decline with breastmilk retinol but the trends will eventually diverge as the hepatic stores decelerate the decline, whereas breastmilk retinol concentrations continue to decline, reflecting the priority shift from the investment in milk to liver within the maternal system.

Fig. 3.
Liver vitamin A across postpartum time.

Fig. 4. Milk-liver relationships by (a) postpartum time and (b) parity.

TABLE 1

Characteristics of mothers for milk and liver retinol analysis

	Milk retinol <i>n</i> = 155	Liver retinol <i>n</i> = 192
Maternal age (years $\pm$ SD)	28.2 $\pm$ 7.09	28.0 $\pm$ 6.92
Reproductive status		
Days since childbirth	227.8 $\pm$ 7.10	231.9 $\pm$ 134
Parity	3.6 $\pm$ 2.11	3.5 $\pm$ 2
% Primipares	20%	18%
Socioeconomic status		
Family land size (acres)	2.1 $\pm$ 3.26	2.0 $\pm$ 3.08
% Livestock	65%	64%
Anthropometric profile		
Height (cm)	160.5 $\pm$ 6.56	160.5 $\pm$ 6.63
Weight (kg)	51.0 $\pm$ 6.95	50.5 $\pm$ 7.02
Body Mass Index (kg/m ²)	19.8 $\pm$ 2.67	19.6 $\pm$ 2.63
Dietary profile (24-hour recall)		
Energy (Kcal)	1735.4 $\pm$ 612.65	1681.0 $\pm$ 584.49
Fat (g)	21.6 $\pm$ 11.99	20.7 $\pm$ 11.25
Total vitamin A (μ g)	123.9 $\pm$ 83.19	119.8 $\pm$ 77.60
Biochemical profile		
Serum retinol (μ mol/L)	1.5 $\pm$ 0.42	1.5 $\pm$ 0.43
Serum C-reactive protein (μ g/L)	1.9 $\pm$ 2.02	1.8 $\pm$ 1.99
% Postpartum vitamin A supplementation	15%	19%
Breastfeeding Frequency Per Day	9.3 $\pm$ 4.08	9.2 $\pm$ 4.14

TABLE 2

Regression model for liver retinol^a

Predictor	Model A (<i>n</i> = 192) β
Constant	0.046
Postpartum time	-0.001 ^b
Postpartum time ²	1.17e-06 ^b
Parity	0.011
Age	-0.001
PPVA	0.007
Land size	-0.005
Livestock	0.007
Fat intake	-0.002 ^b
Arm Fat Mass	-0.004 ^c
Hemoglobin	0.010
C-reactive Protein	0.008

^a $R^2 = 0.1$, Adjusted $R^2 = 0.05$, $P = 0.0328$, $n = 192$.^b $P < 0.1$.^c $P < 0.05$.

TABLE 3

Regression model for milk retinol, modeled with categorical time periods

Predictor	Model B ^a β
Constant	10.415 ^b
Early postpartum	-1.975
Late postpartum	0.602
Liver retinol	-11.267
Parity	-0.361
Age	0.299 ^c
PPVA	-0.253
Land size	0.179
Livestock	2.248
Fat intake	0.021
Arm fat	0.057
Creamatocrit	0.868 ^d
Breastfeeding	0.298 ^c
Liver early	-1.938
Liver Late	-24.579 ^a
Liver Parity	6.514 ^d

^a $R^2 = 0.23$, Adjusted $R^2 = 0.15$, $P = 0.0009$, $n = 155$.^b $P < 0.05$.^c $P < 0.1$.^d $P < 0.01$.