



## ORIGINAL ARTICLE

# Assessment of vitamin A levels in breast milk and serum of lactating mothers in Southeast Nigeria

Nkiru Nwamaka Ezeama <sup>1</sup> , Nene Okunna <sup>2\*</sup> , Onyenmechi Johnson Afonne <sup>3</sup> 1. Department of Community Medicine and Primary Health Care, Faculty of Medicine, Nnamdi Azikiwe University, Awka, Nigeria. [nn.ezeama@unizik.edu.ng](mailto:nn.ezeama@unizik.edu.ng)2. Department of Public Health Sciences, West Chester University of Pennsylvania, West Chester, Pennsylvania, USA. [nokunna@wcupa.edu](mailto:nokunna@wcupa.edu)3. Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, Nnamdi Azikiwe University, Awka, Nigeria. [o.j.afonne@unizik.edu.ng](mailto:o.j.afonne@unizik.edu.ng)

## ABSTRACT

**Background:** Vitamin A (VA) is an essential micronutrient critical to human health, infants rely on their mother's breast milk for sufficient supply. This makes the maternal diet a crucial source of VA for infant growth and development. **Aims:** This study aimed to assess maternal VA status by assessing serum and breast milk retinol concentrations as well as dietary intake in nursing mothers. **Subjects and Methods:** A clinic-based cross-sectional study, conducted in Awka, south-east Nigeria, was used to assess 127 lactating women at one to 24 months post-partum. Blood and breast milk samples were obtained from each participant in a fasted state while dietary intake was assessed via an interviewer-administered semi-structured food frequency questionnaire. Retinol concentration was analyzed by spectrophotometry. Descriptive statistics were used to summarize the data while Spearman rank correlation analysis was used to determine associations between breast milk and serum retinol concentrations and study variables. **Results:** The mean retinol concentration in the serum of the women was a borderline value of  $0.75 \pm 0.64$   $\mu\text{mol/L}$  while the mean retinol concentration in breast milk was  $0.99 \pm 0.71$   $\mu\text{mol/L}$ . Further, the mean milk fat content was  $56.18 \pm 32.32$  g/L while the mean milk retinol to fat ratio was  $0.027 \pm 0.044$   $\mu\text{mol/g}$ . Maternal diet consisted of a preference for provitamin A foods with low bioavailability. The mean retinol concentration in breast milk increased with time post-partum. There was no statistically significant relationship found between the frequency of consumption of most foods and the concentration of retinol in breast milk. **Conclusion:** The evidence of VA deficiency highlights the need for screening and nutrition education of breastfeeding mothers during ante- and post-natal visits.

**Keywords:** Human milk, serum, vitamin A, maternal diet.

## ARTICLE INFORMATION

\* **Corresponding authors:** Nene Okunna, [nokunna@wcupa.edu](mailto:nokunna@wcupa.edu), Phone (+1) 610-436-2185, Fax: (+1) 610-436-2860**Received:** May 24, 2023**Revised:** October 30, 2023**Accepted:** November 08, 2023**Published:** November 22, 2023**Article edited by:**

- Pr. Meghit Boumediene Khaled

**Article reviewed by:**

- Dr. Gözde Türköz Bakırcı

- Pr. Khaled Kahloula

Cite this article as: Ezeama, N. N., Okunna, N., & Afonne, O. J. (2023). Assessment of vitamin A levels in breast milk and serum of lactating mothers in Southeast Nigeria. *The North African Journal of Food and Nutrition Research*, 7 (16): 120 – 129. <https://doi.org/10.51745/najfnr.7.16.120-129>© 2023 The Author(s). This is an open-access article. This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third-party material in this article are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit <http://creativecommons.org/licenses/by/4.0/>.

## 1 Introduction

Vitamin A (VA) is an essential micronutrient of public health importance, critical to human health due to its role in vision, immune function, cellular communication and maintenance of function, epithelial integrity, reproduction, and red blood cell production <sup>1, 2</sup>. Breastfed infants particularly newborns rely on their mother's milk for their supply of VA. Maternal VA status is therefore critical to the health outcomes of the mother-child dyad. Although vitamin A deficiency (VAD) affects all age groups, children and women of reproductive age especially pregnant and breastfeeding women are the groups most at risk with current estimates indicating VAD in 190

million preschool children and 19 million pregnant women worldwide, corresponding to 33.3% and 15.3% of these segments of the population <sup>2-4</sup>.

Vitamin A deficiency disproportionately affects the poorest populations in low- and middle-income countries; the highest rates of VAD in children under five years of age in 2013 were 48% in sub-Saharan Africa and 44% in South Asia <sup>5</sup>. The basic underlying cause of VAD as a public health problem in developing countries is chronically insufficient dietary intake of vitamin A which depletes body stores, impairs tissue growth and function, disrupts normal metabolism as well as lowers resistance to infection <sup>2,6</sup>.

Dietary VA is provided in two main forms preformed and provitamin A. Preformed VA has good bioavailability and is found predominantly as retinyl esters, in animal source foods such as liver, milk, cheese, and eggs, fortified foods, and supplements. Provitamin A occurs in the diet as carotenoid precursors, (primarily  $\beta$ -carotene,  $\alpha$ -carotene, and  $\beta$ -cryptoxanthin) in plant-based foods such as green leafy vegetables, carrots, ripe mangoes, and other orange-yellow vegetables and fruits and needs to be converted to VA in the body <sup>2,7</sup>.

Neonates are typically born with insignificant amounts of VA stored in their livers and are thus dependent on their mothers' milk. Breast milk is therefore the first critical source of VA for infants. Exclusively breastfed infants by well-nourished mothers receive enough VA to maintain their health, grow normally, as well as develop and establish sufficient liver stores of vitamin <sup>7</sup>. The determination of breast milk VA is a recommended approach for monitoring the VA status of nursing mothers and their infants. Serum retinol is used extensively to determine populations at risk for VAD <sup>8,9</sup>. A serum retinol concentration of  $<0.70 \mu\text{mol/L}$  ( $<20 \mu\text{g/dL}$ ) is a well-recognized cut-off for VAD <sup>10</sup>. Normal values for serum retinol concentration range from 0.70 to 2.09 micromoles per liter ( $\mu\text{mol/L}$ ) or 20 to 60 micrograms per deciliter ( $\mu\text{g/dl}$ ). Further, human breast milk contains 3.8% (35 – 40g/L) fat and its fat content positively affects retinol levels in breast milk <sup>11,12</sup>.

Available literature has shown a high prevalence of VAD in Nigeria with severe deficiencies prevalent among children <sup>13</sup>. Most studies on VA deficiency have assessed pregnant women, showing prevalence rates that range from 15.8% to 65% <sup>14-16</sup>. There is a dearth of literature on VA status of lactating women in Africa general and Nigeria in particular. A previous longitudinal study that assessed factors influencing VA status of 569 lactating women in Tanzania observed less than recommended levels of VA intake by the participants <sup>17</sup>.

In order to develop strategies to address the problem of VAD among lactating mothers in Nigeria, there is need for data on their VA status and intake. Unfortunately, nationally representative data for this population sub-group is largely absent while sub-national data is mostly outdated. This study contributes towards the development of adequate and timely data that will form an evidence base for food and nutrition policy formulation and program implementation in order to address the problem of VAD in the country. Furthermore, it informs assessment of the effectiveness of existing food and nutrition programs including VA supplementation in Anambra State with the aim of attaining the second sustainable development goal (SDG 2) which aims to end all forms of hunger and malnutrition by 2030, in the State and in Nigeria in general.

## 2 Methods

### 2.1 Study design and population

This study utilized a clinic-based, cross-sectional design. Study participants were recruited from the Child Welfare Clinic (CWC) of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, a public tertiary health care facility located in Awka South Local Government Area (LGA), Anambra State, South-East Nigeria. The CWC operates three days a week providing routine immunization, growth monitoring, health education, food demonstrations, and other child preventive health services to a monthly average of 360 children under the age of five years. The study population constituted lactating mothers whose breastfed infant was at least one month old at the time of the study; this is because milk nutrient concentrations begin to stabilize at this age. Mothers who reported severe fever, diarrhea with dehydration, or any other severe illness in the 72 hours before data collection were excluded <sup>18</sup>.

The minimum sample size was calculated using the formula  $n = \left(\frac{tS}{r\bar{Y}}\right)^2$  where  $t$  is the t-value for  $\alpha$  level of 0.05 = 1.96;  $S$  is the standard deviation of mean breast milk retinol in Kenyan women = 0.03 <sup>19</sup>;  $r$  is the acceptable margin of error = 0.05 and  $\bar{Y}$  is mean breast milk retinol concentration in Kenyan women = 0.11 <sup>19</sup>. A crude sample size of 114.29 was obtained and adjusted for a non-response rate of 10% to obtain a sample size of 126.98 which was rounded up to the nearest hundred. The systematic sampling technique was used to select the study participants using a sampling interval ( $k = N/n$ ) of every 3<sup>rd</sup> lactating mother who arrived at the clinic;  $N$  was the population size derived from monthly clinic attendance and  $n$  was the sample size. Thus, approximately 130 lactating women were sampled.

### 2.2 Sample collection and preparation

The study protocol was reviewed and approved by the Nnamdi Azikiwe University Teaching Hospital Ethics Committee (reference number: NAUTH/CS/66/VOL. 15/VER. 3/323/2021/084). Each participant was interviewed on their demographic, socioeconomic, reproductive, and household characteristics using a semi-structured questionnaire. Dietary intake was assessed using a validated food frequency questionnaire (FFQ) <sup>20</sup>, adapted to the local context of the participants. Anthropometric measurements viz body weight, and height were collected on each woman using standard procedures <sup>21</sup>. Written informed consent was obtained freely and without coercion from the respondents and respect for the confidentiality of the data obtained from them was ensured. The objectives of the study were

thoroughly explained to them, and they were assured that they were free to opt out of the study at any time.

A 5 mL venous blood sample was obtained from each participant on the morning following an overnight fast and stored in the dark at 4 °C. The blood samples were centrifuged, and the serum obtained was stored at -20 °C for subsequent analysis<sup>8</sup>. Casual, random 10 mL samples of mature breast milk ( $\geq 1$  month postpartum) were collected in the morning from each participant by manual expression. Breast milk was collected from one breast which has not been used to feed the infant for at least one hour. The mother was first allowed to feed her infant from the selected breast for exactly 30 seconds following which she expressed 10ml of milk from the breast<sup>18</sup>. Each milk sample was then transferred to an opaque container and transported within 1–2 hours to the laboratory in ice and shielded from light in a cold box<sup>22</sup>.

### 2.3 Sample analysis

Retinol concentrations in serum and breast milk were determined by adapting the manual spectrophotometric method<sup>23</sup>. The absorbance of the extracted retinol was read at 620nm in a Genesys 10UV spectrophotometer. All laboratory analyses were conducted at Docchy Analytical Laboratories and Environment Services Limited, Awka.

### 2.4 Statistical analysis

Dietary sources of VA were listed and further categorized into five groups: (1) animal sources, (2) tubers and roots, (3) cereals and legumes, (4) dark green leafy vegetables, and (5) yellow and red colored fruits. Serum retinol concentration was the biochemical indicator used to determine an individual's VA status. Cut-offs of below 0.70  $\mu\text{mol/l}$  (20.06  $\mu\text{g/dl}$ ) and 1.05  $\mu\text{mol/l}$  (30.09  $\mu\text{g/dl}$ ) were set for concentrations in maternal serum and breast milk respectively<sup>8,10</sup>.

The data was cleaned by checking for any data collection or coding errors. Data entry and analysis were carried out with the aid of Microsoft Excel 2016 and Statistical Package for the Social Sciences (IBM-SPSS) version 23.0. The normality of continuous variables was assessed using the Kolmogorov-Smirnov test whereby a p-value of  $< 0.05$  indicated that the variable was not normally distributed. Frequency distributions of all relevant variables were developed. Means  $\pm$  standard deviation and medians (interquartile range) for continuous variables and proportions for categorical variables were calculated. Associations between variables were tested using chi-square, Student t-test, and analysis of variance (ANOVA) test. Spearman rank correlation analysis was used to determine associations between breast milk and serum retinol concentrations and continuous and ordinal variables. Statistical significance was set at a p-value  $< 0.05$ .

## 3 Results

A total of 130 questionnaires were administered. Three questionnaires were discarded due to errors and incomplete data. As a result, 127 questionnaires were analyzed giving a response rate of 97.7%.

### 3.1 Characteristics of the study participants

The mean (SD) age of the participants was 30.6 (5.5) years old (range, 18–49 years). Women aged 30–34 years old comprised the highest proportion (33.9%) followed by those aged 25 – 29 years old (28.3%). All the respondents except for one were married. The majority (96.8%) had completed secondary education. Those who were self-employed comprised the highest proportion of 44.1% and the average monthly household income at the time of data collection was 142.9 US dollars. More than half of the respondents had large household sizes of five members or more (Table 1).

A large proportion (72.8%) of the women were overweight (45.5%) or obese (27.3%); the mean parity was  $2.5 \pm 1.5$  and the majority of the women (96.9%) had a gestational age of 37 weeks or higher at delivery. The mean time post-partum was 2.9 months and 88.8% of the women had been breastfeeding for one to six months. The majority of the women (89.0%) were not using any family planning method at the time of data collection (Table 1).

**Table 1.** Characteristics of the study participants

Characteristic	Frequency	Percentage
<b>Age (years)</b>		
- Mean $\pm$ SD	30.6 $\pm$ 5.5	
- $\leq 24$	19	15.0
- 25-29	36	28.3
- 30-34	43	33.9
- $\geq 35$	29	22.8
<b>Marital status</b>		
- Single	1	0.8
- Married	126	99.2
<b>Level of education</b>		
- Primary	4	3.1
- Secondary	39	30.7
- Tertiary	84	66.1
<b>Occupation</b>		
- Student	9	7.1
- Professional	9	7.1
- Civil servant	13	10.2
- Public servant	24	18.9
- Self-employed	56	44.1
- Housewife	16	12.6
<b>Average monthly household income (in Nigerian naira)</b>		
- US dollar equivalent	142.9 USD	
<b>Household size</b>		
- $< 5$	59	46.8
- $\geq 5$	67	53.2

BMI, kg/m <sup>2</sup>		
- Underweight	1	0.9
- Normal	29	26.4
- Overweight	50	45.5
- Obese	30	27.3
Parity		
- Primiparous	39	30.7
- Multiparous	88	69.3
Gestational age at birth of breastfeeding child		
- Preterm	4	3.1
- Term	123	96.9
Time post-partum (in months)		
- Mean (SD)	2.9 (3.2)	
- Median (IQR)	2.0 (1.0 – 3.0)	
- 1 – 6	111	88.8
- 7 – 12	12	9.6
- 13 – 24	2	1.6
Currently using a family planning method		
- Yes	13	11.0
- No	114	89.0

### 3.2 Serum and breast milk retinol concentrations and breast milk fat

The mean retinol concentration in the serum of the women was a borderline value of  $0.75 \pm 0.64 \mu\text{mol/L}$  (25<sup>th</sup> percentile 0.45, median 0.55, 75<sup>th</sup> percentile 0.70, range 0.15–3.75; Table 2). The mean retinol concentration in breast milk was  $0.99 \pm 0.71 \mu\text{mol/L}$  (25<sup>th</sup> percentile 0.51, median 0.77, 75<sup>th</sup> percentile 1.30, range 0.07–4.17; Table 3), a value less than the recommended cut-off of  $1.05 \mu\text{mol/L}$ <sup>10</sup>. The mean milk fat content was  $56.18 \pm 32.32 \text{ g/L}$  (25<sup>th</sup> percentile 33.77, median 51.98, 75<sup>th</sup> percentile 68.21, range 3.59–172.54; Table 2) while the mean milk retinol to fat ratio was  $0.027 \pm 0.044 \mu\text{mol/g}$ .

Based on the  $0.70 \mu\text{mol/L}$  cut-off for serum retinol concentration, the majority of the women (72.4%) in the present study were found to have VAD. Furthermore, 63.7% of the women had low levels of retinol in their breast milk.

**Table 2.** Distribution of concentrations of breast milk fat and retinol in breast milk and serum of 127 Nigerian lactating mothers

Variable	Mean $\pm$ SD	Median	25 <sup>th</sup> percentile	75 <sup>th</sup> percentile	Min.	Max.
Breast milk retinol ( $\mu\text{mol/L}$ )	$0.99 \pm 0.71$	0.77	0.51	1.30	0.07	4.17
Serum retinol ( $\mu\text{mol/L}$ )	$0.75 \pm 0.64$	0.55	0.45	0.70	0.15	3.75
Milk fat (g/L)	$56.18 \pm 32.32$	51.98	33.77	68.21	3.59	172.54
Breast milk retinol: Fat ( $\mu\text{mol/g}$ )	$0.027 \pm 0.044$	0.016	0.009	0.026	0.001	0.422

SD, standard deviation, Min, minimum, Max, maximum

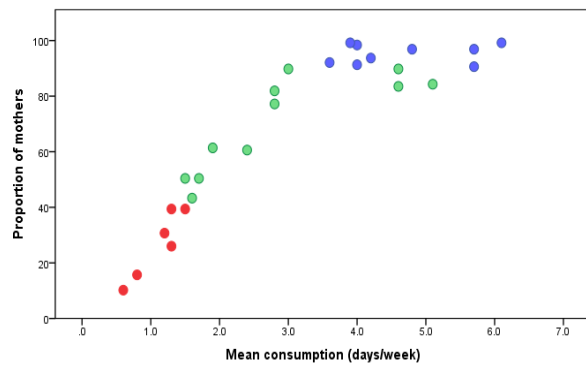
### 3.3 Dietary intake of vitamin A

Figure 1 summarizes the pattern of dietary intake of some vitamin A-rich food sources. High frequencies of intake in high proportions of women (> 90%) were observed for red palm oil and fluted pumpkin leaf (*ugu*) among others. Intermediate frequencies of intake (40% to 90%) were seen for beef/red meat, carrot, amaranth, chicken, and eggs with yolk. Low frequencies of intake (<40%) were observed for pumpkin, cod liver oil, butter, papaya, and talinum.

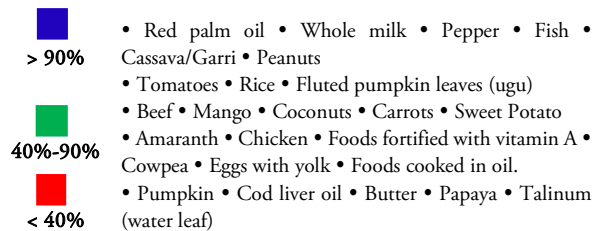
### 3.4 Factors associated with breast milk retinol content

The mean frequencies of consumption of the items included in the FFQ and the proportions of women consuming them at least once a week are shown in Table 3. Regarding animal sources of VA, whole milk was consumed most frequently (5.7 days/week), followed by fish (4.8 days/week) and beef/red meat (4.6 days/week) with 96.9%, 96.9%, and 83.5% of the women consuming these items at least once a week respectively. The least consumed was cod liver oil (0.8 days/week) with only 15.7% of the women consuming it at least once per week.

Among the plant sources, red palm oil was most frequently consumed (6.1 days/week) with 99.2% of the women consuming it at least once per week. Hot peppers (5.7 days/week), mango (5.1 days/week), and tomatoes (4.0 days/week) were next, consumed by 90.6%, 84.3%, and 98.4% of the women respectively. Fluted pumpkin leaves were consumed on average, 3.6 days/week by 92.1% of the women. In comparison, lower frequencies of consumption and lower proportions of women consuming them were seen for the other dark green leafy vegetables: amaranth (1.9 days/week, 61.4%) and talinum (1.5 days/week, 38.4%). Carrots and papaya were also poorly consumed, 1.5 days/week (50.4%) and 1.2 days/week (30.7%) respectively.



**Figure 1.** Pattern of consumption of food items included in the food frequency questionnaire



Except for coconuts, there was no statistically significant relationship found between the frequency of consumption of any other type of food and the concentration of retinol in the breast milk of the participants ( $p > 0.05$ ). Consumption of coconuts was positively associated with breastmilk retinol concentration ( $r = 0.19$ ,  $p = 0.03$ ). Inverse relationships were observed for several plant source foods except for carrots ( $r = 0.15$ ,  $p = 0.09$ ), fluted pumpkin leaves ( $r = 0.08$ ,  $p = 0.37$ ), green amaranth ( $r = 0.12$ ,  $p = 0.18$ ) and pumpkin ( $r = 0.03$ ,  $p = 0.74$ ). In contrast, the consumption of beef/red meat ( $r = 0.09$ ,  $p = 0.34$ ), fish ( $r = 0.12$ ,  $p = 0.18$ ), chicken ( $r = 0.03$ ,  $p = 0.77$ ), and eggs ( $r = 0.04$ ,  $p = 0.64$ ) were all positively correlated with breastmilk retinol concentration (Table 3).

**Table 3.** Semi-quantitative assessment of consumption of food items included in the food frequency questionnaire

Food item	Mean frequency of consumption (days/week)	Mothers consuming the item at least once per week (%)	Spearman correlation coefficient $r$ ( $P$ -value)
Red palm oil	6.1	99.2	-0.07 (0.44)
Whole Milk	5.7	96.9	0.01 (0.86)
Hot peppers	5.7	90.6	-0.05 (0.58)
Mango	5.1	84.3	-0.02 (0.79)
Fish	4.8	96.9	0.12 (0.18)
Foods cooked in oil	4.6	89.8	-0.08 (0.41)
Beef/Red meat	4.6	83.5	0.09 (0.34)
Cassava	4.2	93.7	-0.11 (0.20)
Tomatoes	4.0	98.4	-0.06 (0.50)

Peanuts	4.0	91.3	0.01 (0.88)
Rice	3.9	99.2	-0.14 (0.12)
Dark green leafy vegetables (DGLV) (fluted pumpkin leaf/Ugu)	3.6	92.1	0.08 (0.37)
Eggs with yolk	3.0	89.8	0.04 (0.64)
Cowpea	2.8	81.9	-0.01 (0.92)
Food fortified with vitamin A	2.8	77.2	-0.13 (0.15)
Chicken	2.4	60.6	0.03 (0.77)
DGLV (amaranth)	1.9	61.4	0.12 (0.18)
Yellow/Orange sweet potato	1.7	50.4	-0.08 (0.39)
Coconuts	1.6	43.3	0.19 (0.03)
Carrots	1.5	50.4	0.15 (0.09)
DGLV (talinum/water leaf)	1.5	39.4	-0.06 (0.53)
Liver	1.3	39.4	-0.09 (0.32)
Butter	1.3	26.0	-0.03 (0.71)
Papaya	1.2	30.7	0.12 (0.18)
Cod liver oil	0.8	15.7	-0.10 (0.24)
Pumpkin (Anyu/Ugboguru)	0.6	10.2	0.03 (0.74)

The relationship between various other maternal factors and breast milk retinol concentration is shown in Table 4. Serum retinol was negatively correlated with breast milk retinol, but this was not statistically significant ( $r = -0.173$ ,  $p = 0.059$ ). Maternal age was also weakly negatively correlated with breastmilk retinol concentration ( $r = -0.055$ ,  $p = 0.541$ ) while anthropometric status (BMI) was weakly positively correlated ( $r = 0.139$ ,  $p = 0.936$ ). The mean breast milk retinol concentration in primiparous women ( $1.06 \mu\text{mol/L}$ ) was higher than that of multiparous women ( $0.96 \mu\text{mol/L}$ ), this difference was not statistically significant ( $p = 0.475$ ; Table 4).

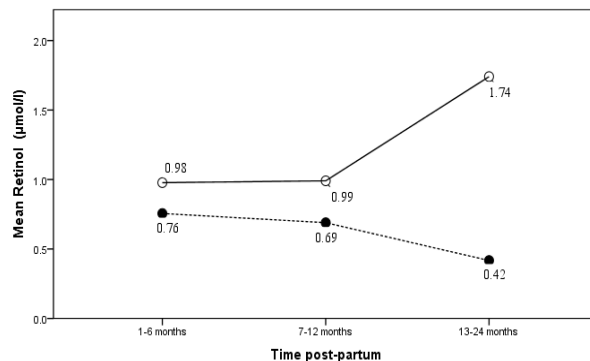
**Table 4.** Association between selected factors and concentration of retinol in breast milk 127 Nigerian lactating mothers

Factor	Test statistic	P value
Serum retinol <sup>a</sup>	-0.173	0.059
Milk fat content <sup>a</sup>	0.099	0.276
Maternal age <sup>a</sup>	-0.055	0.541
Educational status <sup>b</sup>	0.615	0.543
Household size <sup>c</sup>	0.310	0.757
Body mass index <sup>b</sup>	0.139	0.936
Parity <sup>c</sup>	0.716	0.475
Gestational age at birth <sup>c</sup>	0.030	0.976
Duration of breastfeeding <sup>b</sup>	1.133	0.326
Family planning <sup>c</sup>	1.063	0.290

<sup>a</sup> Spearman rho correlation; <sup>b</sup> ANOVA; <sup>c</sup> Independent samples t-test



The mean retinol concentration in breast milk increased the longer the time post-partum. The mean breast milk retinol concentration for women who were 1 – 6 months post-partum was 0.98  $\mu\text{mol/L}$ , that for women 7 – 12 months post-partum was 0.99  $\mu\text{mol/L}$  and for those 13 – 24 months post-partum, the mean was 1.74  $\mu\text{mol/L}$ . The reverse was observed for serum retinol; concentrations declined from 0.76  $\mu\text{mol/L}$  at 1 – 6 months post-partum, to 0.69  $\mu\text{mol/L}$  at 7 – 12 months post-partum to 0.42  $\mu\text{mol/L}$  at 13 – 24-month post-partum (Figure 2).



**Figure 2.** Mean concentrations of retinol in maternal serum and breast milk categorized by time post-partum

- Concentration of retinol in breast milk
- Concentration of retinol in serum

## 4 Discussion

This study assessed serum and breast retinol levels in lactating mothers in Southeast Nigeria. The mean retinol concentration in the serum of the women was a borderline value of  $0.75 \pm 0.64$   $\mu\text{mol/L}$  (25<sup>th</sup> percentile 0.45, median 0.55, 75<sup>th</sup> percentile 0.70, range 0.15 – 3.75). According to the WHO, a serum retinol concentration of less than 0.70  $\mu\text{mol/L}$  is indicative of vitamin A deficiency in an individual [10,24]. Based on the WHO cut-off for serum retinol concentration, the majority of the women (72.4%) in the present study were found to have VAD. Similar studies that assess VA levels in serum retinol found deficiency rates of 40% in Zimbabwe <sup>25</sup>, 13.3% in Bangladesh <sup>26</sup>, and 9.3% in Brazil <sup>8</sup>.

The mean retinol concentration in breast milk was  $0.99 \pm 0.71$   $\mu\text{mol/L}$  (25<sup>th</sup> percentile 0.51, median 0.77, 75<sup>th</sup> percentile 1.30, range 0.07–4.17; Table 3). This value is less than the recommended cut-off of 1.05  $\mu\text{mol/L}$  and is less than those found in previous studies <sup>24,27-30</sup>. Breast milk retinol concentration has been identified as a superior indicator for VAD in lactating women <sup>27, 28</sup>. Based on the recommended cut-off of 1.05  $\mu\text{mol/L}$ , 63.7% of the women had deficient levels of retinol in their breast milk. These findings raise public

health concerns given the increased need for VA during lactation, estimated to be 60 times more than the quantity required by the fetus during pregnancy <sup>28</sup>. The VA content of human milk is significantly affected by maternal nutrition during lactation. There is a need to educate nursing mother to increase their daily intake of vitamin A through their diet.

Research shows that factors such as milk fat content, maternal serum retinol, postpartum age, gestational age at delivery, maternal parity, use of oral contraceptives, and body mass index have been reported to influence the concentration of retinol in breast milk <sup>31</sup>. The standard cut-off for retinol to fat ratio is 0.028  $\mu\text{mol/g}$  <sup>11,32</sup>. The mean milk retinol to fat ratio in this present study was  $0.027 \pm 0.044$   $\mu\text{mol/g}$  and is lower than the mean ratio of  $0.038 \pm 0.016$   $\mu\text{mol/g}$  obtained in previous research <sup>11</sup>. A large proportion of the women (72.8%) however were either overweight or obese. Previous research has found associations between VAD and greater body adiposity <sup>33,34</sup>.

The present study found that breast milk retinol concentrations declined with increasing maternal age contrasting with results from previous studies <sup>18,31</sup>. For example, maternal age had a positive association with breast milk vitamin A among breast milk donors in Brazil <sup>31</sup>, but no relationship was observed among lactating women in Cameroon <sup>18</sup>. The assessment of relationships between various other maternal factors and breast milk retinol concentration of our study sample showed that serum retinol levels were negatively correlated with breast milk retinol levels, but this was not statistically significant. Also, maternal age and anthropometric status (BMI) were not associated with breast milk retinol concentration, agreeing with similar results observed by Tokuşoğlu et al. <sup>30</sup>, as well as those found in a study among lactating mothers in rural Chiang Mai, Thailand <sup>9</sup>. Although the mean breast milk retinol concentration in primiparous women (1.06  $\mu\text{mol/L}$ ) was higher than that of multiparous women (0.96  $\mu\text{mol/L}$ ), this difference was not statistically significant ( $p = 0.475$ ). Panpanich et al. <sup>9</sup> also found no association between parity and breast milk retinol concentration.

The present study found a frequent consumption of plant sources of VA such as red palm oil. These findings are similar to a previous study in Tanzania which found consumption of fortified edible oil including palm oil and sunflower oil <sup>17</sup>. Similar findings were observed in Arsi, a rural community in Ethiopia where the main source of dietary VA intake was fortified cooking oil while animal foods contributed only 19% VA RNI <sup>35</sup>. Dietary vitamin A obtained as precursor carotenoids derived from plant-based sources have lower bioavailability and efficiency for conversion into retinol and lactating women in developing countries who subsist on such diets are considered at risk of VAD <sup>36</sup>. There was no

statistically significant relationship found between the frequency of consumption of any type of food and the concentration of retinol in the breast milk of the participants ( $p > 0.05$ ), similar to findings observed by Tokuşoğlu et al. <sup>30</sup>.

The mean serum retinol concentration and the high proportion of vitamin A deficiency observed in this present study may be due to inadequate intake of vitamin A by the mothers as well as the increased demand by their infants for their breast milk supply of vitamin A. This suggests that the intake of VA was not due to inadequate consumption of food but the low VA content of foods that were preferentially consumed by the women <sup>24</sup>. A similar preference for low VA-rich foods was also found in a comparative study of lactating and non-lactating women in Zambia <sup>37</sup>. These findings highlight the need for nutrition education for lactating mothers on the importance of consuming VA-rich foods to maintain the requisite VA concentrations in breast milk retinol. This is important as the WHO no longer recommends high-dose VA supplementation for lactating mothers <sup>38</sup>.

This study found a difference in the evolution of the retinol concentrations in serum and breast milk among the study participants i.e., as lactation progressed, the mean breast milk retinol concentration increased with a corresponding decrease in the mean serum retinol level. Research shows that breast milk vitamin A content is derived from both circulating and dietary retinol and is normally not affected by maternal vitamin A status and/or maternal diet unless the maternal reserves are depleted <sup>39</sup>. The decreasing serum retinol concentration observed in the study sample is likely a result of inadequate intake in the diet. Indeed, a large proportion of the study was demonstrated to have VAD. Thus, to compensate for inadequate dietary intake, the body draws upon maternal liver reserves and preferentially allocates retinol to the breast milk <sup>39</sup> thereby maintaining relatively higher levels of retinol in the breast milk despite dwindling serum retinol levels, as observed in the study sample. Nevertheless, the low breast milk retinol concentrations observed suggests that the study participants have both insufficient liver reserves as well as inadequate dietary intake <sup>39</sup>.

This study has a number of limitations that may affect the interpretations of the findings. The generalizability of study findings is impacted by the cross-sectional data collected as well as the small study size and sampling methodology. The study participants were derived from the southeastern part of Nigeria, thus maternal diet and VA concentrations may not reflect those of the entire country. However, these findings may provide a basis for extrapolation to the general population <sup>40</sup>. This study used cut-off levels of  $\leq 0.70 \mu\text{mol/L}$  to estimate VA deficiency. Recent research has suggested a cut-off set at  $< 0.1 \text{ mmol/g}$  for total liver vitamin A reserves (TLR) <sup>28,40,41</sup>. While this present study did not assess TLR, total body vitamin A reserves (TBS) is positively correlated with TLR as more than 90% of total body vitamin A is stored in the

liver <sup>42</sup>. Further, breast milk retinol concentrations have been identified as an indicator for TBS and TLR <sup>27</sup>. Recent research re-evaluating the recommended daily allowances of VA suggests that TLR of  $0.07 \mu\text{mol/g}$  liver may overestimate VA needs <sup>40,43</sup>. Nevertheless, based on current cut-off levels, the findings from this study provide evidence of VAD among lactating women highlighting the need for continued surveillance and screening within this population.

## 5 Conclusion

This study shows a high prevalence of VAD among lactating mothers in south-eastern Nigeria. VAD in the serum and breastmilk of majority of the study sample was linked to a preference for diets consisting mostly of foods that provide vitamin A in the form of carotenoid precursors. The study findings highlight the need for screening and nutrition education of breastfeeding mothers during ante- and post-natal visits.

**Funding:** The author received no financial support for the research, authorship, and/or publication of this article.

**Acknowledgments:** The authors would like to thank each participant for participating in the study. We would also like to express our gratitude to the research assistants, Child Welfare Clinic of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, and Docchy Analytical Laboratories and Environment Services Limited, Awka, Nigeria.

**Previous submissions:** This work has not been submitted.

**Authors' Contribution:** NNE conceived the study and obtained permission to conduct the research, recruited health centers and participants, and managed the research assistants. NNE, NO, and OJA conducted the statistical analysis and contributed to interpreting the output of the statistical analysis. NNE and NO drafted the manuscript and all authors contributed substantially to its writing and revision. All authors approved the final version submitted for publication.

**Conflicts of Interest:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## References

- [1] Office of Dietary Supplements - *Vitamin A and Carotenoids*. Nih.gov. Retrieved 2022, June 15 from <https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/#en1>
- [2] World Health Organization. (2009). *Global prevalence of vitamin A deficiency in populations at risk 1995-2005: WHO global database on vitamin A deficiency*. World Health Organization. <http://apps.who.int/iris/handle/10665/44110>
- [3] Bastos Maia, S., Costa Caminha, M. D. F., Lins da Silva, S., Rolland Souza, A. S., Carvalho dos Santos, C., & Batista Filho, M. (2018). The prevalence of vitamin A deficiency and associated factors in

- pregnant women receiving prenatal care at a reference maternity hospital in Northeastern Brazil. *Nutrients*, *10*(9), 1271. <https://doi.org/10.3390/nu10091271>
- [4] Wirth, J., Petry, N., Tanumihardjo, S., Rogers, L., McLean, E., Greig, A., Garrett, G., Klemm, R., & Rohner, F. (2017). Vitamin A supplementation programs and country-level evidence of vitamin A deficiency. *Nutrients*, *9*(3), 190. <https://doi.org/10.3390/nu9030190>
- [5] UNICEF. (2016). UNICEF data: monitoring the situation of children and women. *New York: UNICEF*. <https://data.unicef.org/topic/nutrition/vitamin-a-deficiency/>
- [6] Ahmed, F., & Darnton-Hill, I. (2004). Vitamin A Deficiency. In Gibney, M.J., Margetts, B.M., Kearney, J.M and Arab L. (Eds). *Public Health Nutrition* (pp:192-215). Blackwell Science. John Wiley & Sons. ISBN 0-632-05627-4
- [7] Gannon, B. M., Jones, C., & Mehta, S. (2020). Vitamin A requirements in pregnancy and lactation. *Current Developments in Nutrition*, *4*(10), nzaa142. <https://doi.org/10.1093/cdn/nzaa142>
- [8] da Silva Ribeiro, K. D., De Araújo, K. F., De Souza, H. H. B., Soares, F. B., da Costa Pereira, M., & Dimenstein, R. (2010). Nutritional vitamin A status in northeast Brazilian lactating mothers. *Journal of Human Nutrition and Dietetics*, *23*(2), 154-161. <https://doi.org/10.1111/j.1365-277X.2009.01026.x>
- [9] Panpanich, R., Vitsupakorn, K., Harper, G., & Brabin, B. (2002). Serum and breast-milk vitamin A in women during lactation in rural Chiang Mai, Thailand. *Annals of Tropical Paediatrics*, *22*(4), 321-324. <https://doi.org/10.1179/027249302125001976>
- [10] De Pee, S., & Dary, O. (2002). Biochemical indicators of vitamin A deficiency: serum retinol and serum retinol binding protein. *The Journal of Nutrition*, *132*(9), 2895S-2901S. <https://doi.org/10.1093/jn/132.9.2895s>
- [11] Dror, D. K., & Allen, L. H. (2018). Retinol-to-fat ratio and retinol concentration in human milk show similar time trends and associations with maternal factors at the population level: a systematic review and meta-analysis. *Advances in Nutrition*, *9*(suppl\_1), 332S-346S. <https://doi.org/10.1093/advances/nmy021>
- [12] Kim, S. Y., & Yi, D. Y. (2020). Components of human breast milk: From macronutrient to microbiome and microRNA. *Clinical and Experimental Pediatrics*, *63*(8), 301. <https://doi.org/10.3345/cep.2020.00059>
- [13] Ene-Obong, H. N., Alozie, Y., Abubakar, S., Aburime, L., & Leshi, O. O. (2020). Update of the Nutrition Situation in Nigeria. *The North African Journal of Food and Nutrition Research*, *4*(9), S63-S74. <https://doi.org/10.51745/najfnr.4.9.S63-S74>
- [14] Williams, I. O., Essien, E. U., & Eka, O. U. (2011). Socioeconomic factors and vitamin A status of pregnant women in Calabar urban, southeastern Nigeria. *Maternal and Child Health Journal*, *15*, 943-948. <https://doi.org/10.1007/s10995-009-0541-7>
- [15] Ugwa, E. A. (2015). Vitamins A and E deficiencies among pregnant women attending antenatal care at general hospital Dawakin Kudu, North-West Nigeria. *International Journal of Preventive Medicine*, *6* (65). <https://doi.org/10.4103/2008-7802.161078>
- [16] Ajose, O. A., Adelekan, D. A., & Ajewole, E. O. (2004). Vitamin A status of pregnant Nigerian women: relationship to dietary habits and morbidity. *Nutrition and Health*, *17*(4), 325-333. <https://doi.org/10.1177/026010600401700>
- [17] Ndau, E. L., Walters, D., Wu, D., Saleh, N., Mosha, T. C. E., Horton, S., & Laswai, H. S. (2016). Factors influencing vitamin A status of lactating mothers in Manyara and Shinyanga Regions of Tanzania. *Tanzania Journal of Agricultural Sciences*, *15*(1). <https://www.ajol.info/index.php/tjags/article/view/164875>
- [18] Engle-Stone, R., Haskell, M. J., Nankap, M., Ndjebayi, A. O., & Brown, K. H. (2014). Breast milk retinol and plasma retinol-binding protein concentrations provide similar estimates of vitamin A deficiency prevalence and identify similar risk groups among women in Cameroon but breast milk retinol underestimates the prevalence of deficiency among young children. *The Journal of Nutrition*, *144*(2), 209-217. <https://doi.org/10.3945/jn.113.179788>



- [19] Fujita, M., Lo, Y. J., & Brindle, E. (2017). Nutritional, inflammatory, and ecological correlates of maternal retinol allocation to breast milk in agro-pastoral Ariaal communities of northern Kenya. *American Journal of Human Biology*, 29(4), e22961. <https://doi.org/10.1002/ajhb.22961>
- [20] Suri, S., Kumar, D., & Das, R. (2017). Dietary deficiency of vitamin A among rural children: community-based survey using food frequency questionnaire. *The National Medical Journal of India*, 30(2), 61-61. <http://www.nmji.in>
- [21] World Health Organization. (1995). *Physical status: The use of and interpretation of anthropometry, Report of a WHO Expert Committee*. World Health Organization. [https://www.who.int/childgrowth/publications/physical\\_status/en/](https://www.who.int/childgrowth/publications/physical_status/en/)
- [22] Alam, D. S., van Raaij, J. M., Hautvast, J. G., Yunus, M., Wahed, M. A., & Fuchs, G. J. (2010). Effect of dietary fat supplementation during late pregnancy and first six months of lactation on maternal and infant vitamin A status in rural Bangladesh. *Journal of Health, Population, and Nutrition*, 28(4), 333. <https://doi.org/10.3329/jhpn.v28i4.6039>
- [23] Kumar, A., Kamboj, M., Virender (2021). A review on photometric methods for the quantitation of vitamin A. *Microchemical Journal*, 171, 106791. <https://doi.org/10.1016/j.microc.2021.106791>
- [24] Deminice, T. M. M., Ferraz, I. S., Monteiro, J. P., Jordao, A. A., Ambrósio, L. M. C. S., & Nogueira-de-Almeida, C. A. (2018). Vitamin A intake of Brazilian mothers and retinol concentrations in maternal blood, human milk, and the umbilical cord. *Journal of International Medical Research*, 46(4), 1555-1569. <https://doi.org/10.1177/0300060518757155>
- [25] Ncube, T. N., Malaba, L., Greiner, T., & Gebre-Medhin, M. (2001). Evidence of grave vitamin A deficiency among lactating women in the semi-arid rural area of Makhaza in Zimbabwe. A population-based study. *European Journal of Clinical Nutrition*, 55(4), 229-234. <https://doi.org/10.1038/sj.ejcn.1601138>
- [26] Ahmed, F., Azim, A., & Akhtaruzzaman, M. (2003). Vitamin A deficiency in poor, urban, lactating women in Bangladesh: factors influencing vitamin A status. *Public Health Nutrition*, 6(5), 447-452. <https://doi.org/10.1079/PHN2002454>
- [27] Nimmannun, K., Davis, C. R., Srisakda, P., Gannon, B. M., Tanumihardjo, S. A., & Udomkesmalee, E. (2022). Breast Milk Retinol Concentrations Reflect Total Liver Vitamin A Reserves and Dietary Exposure in Thai Lactating Women from Urban and Rural Areas. *The Journal of Nutrition*, 152(12), 2689-2698. <https://doi.org/10.1093/jn/nxac223>
- [28] Kaliwile, C., Michelo, C., Sheftel, J., Davis, C. R., Grahn, M., Bwembya, P., Simpungwe, E., Mwanza, S., Chileshe, J., & Tanumihardjo, S. A. (2021). Breast milk-derived retinol is a potential surrogate for serum in the <sup>13</sup>C-retinol isotope dilution test in Zambian lactating women with vitamin A deficient and adequate status. *The Journal of Nutrition*, 151(1), 255-263. <https://doi.org/10.1093/jn/nxaa320>
- [29] Daniels, L., Gibson, R. S., Diana, A., Haszard, J. J., Rahmannia, S., Luftimas, D. E., Hampel, D., Shahab-Ferdows, S., Reid, M., Melo, L., Lamers, Y., Allen, L. H., & Houghton, L. A. (2019). Micronutrient intakes of lactating mothers and their association with breast milk concentrations and micronutrient adequacy of exclusively breastfed Indonesian infants. *The American Journal of Clinical Nutrition*, 110(2), 391-400. <https://doi.org/10.1093/ajcn/nqz047>
- [30] Tokuşoğlu, Ö., Tansuğ, N., Akşit, S., Dinç, G., Kasirga, E., & Özcan, C. (2008). Retinol and  $\alpha$ -tocopherol concentrations in breast milk of Turkish lactating mothers under different socio-economic status. *International Journal of Food Sciences and Nutrition*, 59(2), 166-174. <https://doi.org/10.1080/02699200701539171>
- [31] Mello-Neto, J., Rondó, P. H., Oshiiwa, M., Morgano, M. A., Zacari, C. Z., & Domingues, S. (2009). The influence of maternal factors on the concentration of vitamin A in mature breast milk. *Clinical Nutrition*, 28(2), 178-181. <https://doi.org/10.1016/j.clnu.2009.02.002>
- [32] Klevator, M. K., Haskell, M. J., Lartey, A., Adu-Afarwuah, S., Zeilani, M., & Dewey, K. G. (2016). Lipid-based nutrient supplements providing approximately the recommended daily intake of vitamin A do not increase breast milk retinol concentrations among Ghanaian women. *The*

- Journal of Nutrition*, 146(2), 335-342.  
<https://doi.org/10.3945/jn.115.217786>
- [33] Góes, É., Cordeiro, A., Bento, C., & Ramalho, A. (2023). Vitamin A Deficiency and Its Association with Visceral Adiposity in Women. *Biomedicines*, 11(3), 991.  
<https://doi.org/10.3390/biomedicines11030991>
- [34] Ribamar, A., Cruz, S., Bento, C., & Ramalho, A. (2022). Visceral and body adiposity are negatively associated with vitamin A nutritional status independently of Body Mass Index and recommended intake of vitamin A in Brazilian Women. *The Journal of Nutritional Biochemistry*, 109, 109120.  
<https://doi.org/10.1016/j.jnutbio.2022.109120>
- [35] Zerfu, T. A., Umeta, M., & Baye, K. (2016). Dietary habits, food taboos, and perceptions towards weight gain during pregnancy in Arsi, rural central Ethiopia: a qualitative cross-sectional study. *Journal of Health, Population and Nutrition*, 35(1), 1-7.  
<https://doi.org/10.1186/s41043-016-0059-8>
- [36] Cabezuelo, M. T., Zaragozá, R., Barber, T., & Viña, J. R. (2019). Role of vitamin A in mammary gland development and lactation. *Nutrients*, 12(1), 80.  
<https://doi.org/10.3390/nu12010080>
- [37] Kaliwile, C., Michelo, C., Titcomb, T., Moursi, M., Donahue Angel, M., Reinberg, C., Bwembya, P., Alders, R., & Tanumihardjo, S. (2019). Dietary intake patterns among lactating and non-lactating women of reproductive age in rural Zambia. *Nutrients*, 11(2), 288.  
<https://doi.org/10.3390/nu11020288>
- [38] McGuire, S. (2012). WHO Guideline: Vitamin A supplementation in pregnant women. Geneva: WHO, 2011; WHO Guideline: Vitamin A supplementation in postpartum women. Geneva: WHO, 2011. *Advances in Nutrition*, 3(2), 215-216.  
<https://doi.org/10.3945/an.111.001701>
- [39] Dror, D.K., & Allen, L.H. (2018). Overview of Nutrients in Human Milk. *Advances in Nutrition*, 9(Suppl. 1), 2785-2945.  
<https://doi.org/10.1093/advances/nmy022>
- [40] Tanumihardjo, S. A., Russell, R. M., Stephensen, C. B., Gannon, B. M., Craft, N. E., Haskell, M. J., ... & Raiten, D. J. (2016). Biomarkers of Nutrition for Development (BOND)—Vitamin A review. *The Journal of Nutrition*, 146(9), 1816S-1848S.  
<https://doi.org/10.3945/jn.115.229708>
- [41] Tanumihardjo S. A. (2021). Biological evidence to define a vitamin A deficiency cutoff using total liver vitamin A reserves. *Experimental biology and medicine (Maywood, N.J.)*, 246(9), 1045–1053.  
<https://doi.org/10.1177/1535370221992731>
- [42] National Research Council. (2001). Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. *Institute of Medicine/Food and Nutrition Board. National Academy Press. Washington DC.*
- [43] Sheftel, J., Valentine, A. R., Hull, A. K., Fadjarwati, T., Gannon, B. M., Davis, C. R., & Tanumihardjo, S. A. (2021). Findings in 3 clinical trials challenge the accuracy of the Institute of Medicine's estimated average requirements for vitamin A in children and women. *The American Journal of Clinical Nutrition*, 113(5), 1322-1331.  
<https://doi.org/10.1093/ajcn/nqaa132>