VITAMIN A STATUS OF LACTATING WOMEN IN SOUTHERN ETHIOPIA

By

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VITAMIN A STATUS OF LACTATING WOMEN IN

SOUTHERN ETHIOPIA

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CHAPTER I

INTRODUCTION

Vitamin A deficiency (VAD) has long been recognized as a major public health problem. The United Nations Children's Fund (UNICEF, 2006) estimates that between 250,000 to 500,000 children are blinded due to VAD each year; many of these children die each year from vitamin A deficiency related diseases. Participants in conferences such as The World Summit for Children, the Policy Conference on Ending Hidden Hunger, and the International Conference on Nutrition all agreed upon the international goal of eliminating VAD and all its consequences by the year 2000 (WHO, 1996). This goal has yet to be achieved, and so VAD remains an important focus area for research and intervention.

Studies conducted in Ethiopia have shown a high prevalence of VAD (De Sole et al., 1987; Tafesse et al., 1996). Ethiopia is located in the Horn of Africa (Fig. 1), and is the second most populated country in Africa. Nutritional problems in Ethiopia are numerous, and are caused by a wide range of factors, including poverty, civil unrest, and natural disasters ranging from destructive flooding during the rainy season to intermittent droughts throughout the dry season (Opportunities for Micronutrient Interventions).

Figure 1: Location of Ethiopia in Africa

Adapted from: www.rafiki-foundation.org/ethiopia.html

The Ethiopian Health and Nutrition Research Institute has responded to the alarming rates of VAD through their efforts in distributing vitamin A capsules, nutrition education, and food fortification as part of a five year program that began in 1989. Vitamin A capsule distribution has reached over 2.5 million children. In selected high prevalence areas, widespread distribution of vitamin A capsules has reduced the prevalence of xerophthalmia from 11% to 2% (Taffesse et al., 1994).

Vitamin A deficiency primarily affects sight and is manifested in such conditions as xerophthalmia, Bitot's spots, and night blindness. However, VAD also has a negative effect on the immune system and is associated with an increased prevalence of diarrhea, respiratory infections, and even death. In a study conducted by De Sole et al. (1987) in Southern Ethiopia, children with xerophthalmia were twice as likely to have diarrhea ($p < 0.001$) and respiratory diseases ($p < 0.02$) than children without xerophthalmia. In another study, conducted by Tafesse et al. (1996) in central Ethiopia, 147 children were examined for signs of xerophthalmia. Night blindness, Bitot's spots, corneal ulceration, and corneal scars were observed in 17.0%, 26.5%, 2.7% and 0.7% of the children respectively. Serum retinol concentrations categorized 31.9% of the children as being

deficient (< 0.35μmol/l) and 48.9% as having low levels (0.35-0.69μmol/l) (Tafesse et al., 1996).

While VAD is very common in children, other high-risk groups include pregnant and lactating women (WHO, 1996). The nutritional status of the mother may be reflected in her child; thus, pregnant, lactating, and other fertile women are important groups in which research and interventions regarding VAD should be targeted.

PURPOSE

The purpose of this study was to determine the vitamin A status of lactating women in Wondo Genet and Arsi Negele, two villages located in Southern Ethiopia.

OBJECTIVES

1. To assess the vitamin A status of lactating women through serum retinol measurements, breast milk vitamin A concentrations, and dark adaptation threshold.
2. To determine the efficacy of measuring impaired dark adaptation, a functional test of vitamin A status, with the Scotopic Sensitivity Tester-1 (SST-1) instrument as compared with serum retinol concentrations.
3. To determine the efficacy of the CRAFTi portable fluorometer, a new field instrument for measuring serum vitamin A concentrations, as compared with serum vitamin A concentrations measured by high-performance liquid chromatography (HPLC).

NULL HYPOTHESIS

Ho1. There will be no relation between serum retinol, breast milk vitamin A concentrations, and dark adaptation thresholds.

Ho2. There will be no relation between serum retinol and dark adaptation thresholds.

Ho3. There will be no relation between the CRAFTi portable fluorometer and HPLC serum vitamin A concentrations.

ASSUMPTIONS AND LIMITATIONS

It is assumed that the participants answered the questionnaire honestly. It is also assumed that the vitamin A in the blood and milk was stable throughout the collection, transport, and storage of the samples. Finally, it is assumed that the breast milk samples obtained are representative of the mother's typical milk fat secretion. Results from this study are based on these assumptions.

There were several limitations of this study. One limitation is that a convenience sample, rather than a random sample, was utilized. Another limitation was that there was no control group. This was a cross-sectional study with no treatment; therefore, there was no opportunity to measure change.

This paper has been referenced in the style of The American Journal of Clinical Nutrition, except for Chapter IV, which has been formatted in the style accepted by The East African Medical Journal

CHAPTER II

REVIEW OF LITERATURE

OVERVIEW OF VITAMIN A

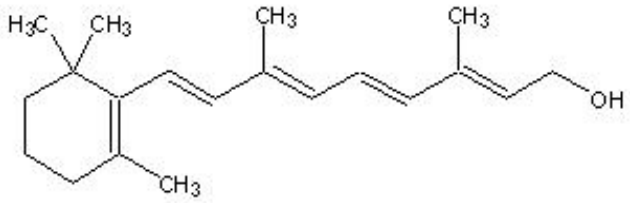
Structure and Functions

Vitamin A is a fat-soluble vitamin that is essential for normal vision, gene expression, reproduction, embryonic development, and growth (Institute of Medicine, 2001). However, in recent years, new physiological functions of vitamin A have been identified. These include its potential in reducing morbidity in those affected by measles, respiratory illnesses, and possibly even HIV infections. Vitamin A has also been shown to play a role in gene regulation and cell differentiation and morphogenesis (Gerster, 1997).

Vitamin A, a 20-carbon structure, refers to a family of molecules with many different forms depending on the group attached at carbon-15. These include a hydroxyl group (retinol) (Fig. 1), an aldehyde group (retinal), a carboxylic acid group (retinoic acid) (Fig. 2), or an ester group (retinyl ester) (Fig. 3). Dietary sources of vitamin A are found in the form of preformed vitamin A or provitamin A carotenoids. Although over 600 forms exist, β-carotene (Fig. 4), α-carotene, and β-cryptoxanthin are the three most

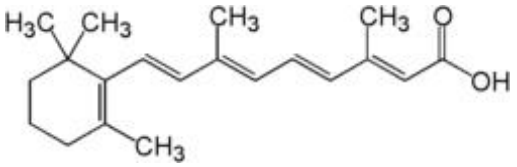
common provitamin A carotenoids (Institute of Medicine, 2001). Provitamin A carotenoids are most abundantly found in darkly colored fruits and vegetables, and oily fruits and red palm oil, while preformed vitamin A is abundant in some animal-derived foods (Institute of Medicine, 2001).

Figure 1: Structure of Retinol



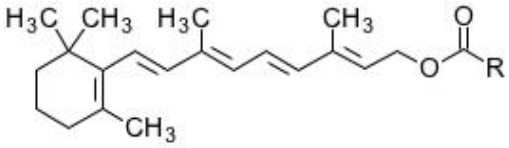
Adapted from: vitamina.quickseek.com/

Figure 2: Structure of Retinoic Acid



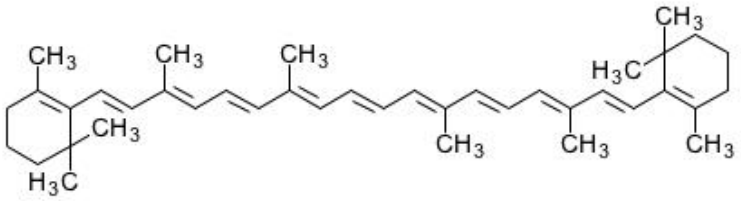
Adapted from: www.answers.com/topic/retinoic-acid

Figure 3: Structure of Retinyl Ester



Adapted from: www.genome.ad.jp/…/www_bget?compound+C02075

Figure 4: Structure of β-carotene



Adapted from: www.genome.ad.jp/…/www_bget?compound+C02094

Absorption and Metabolism

Preformed vitamin A is absorbed primarily through the intestinal lumen, following the processing of retinyl esters. Bile salts help form water-miscible micelles, where solubilized retinyl esters and triglycerides are hydrolyzed to retinol and products of lipolysis (Harrison, 1993). Provitamin A carotenoids are also solubilized into micelles in the intestinal lumen. From here they are absorbed into duodenal mucosal cells by passive diffusion (Institute of Medicine, 2001). The newly synthesized retinyl esters and nonhydrolyzed carotenoids are transported from the intestine to the liver in chylomicrons and chylomicron remnants. Retinoic acid, derived from dietary retinoids, is absorbed via the portal system bound to albumin (Blaner & Olson, 1994; Olson, 1991). Retinol that is needed in the tissues binds to retinol-binding protein (RBP) for release into the circulation. Retinol that does not need to be released into the circulation immediately is reesterified and stored in the liver until it is needed.

Absorption of preformed vitamin A is relatively high, somewhere between 70-90 percent (Sivakumar and Reddy, 1972). In addition, its absorbability remains high as intake increases. Provitamin A carotenoids are not absorbed as well, however, with a

range from 9-22 percent. It should also be noted that absorption efficiency of carotenoids decreases as consumption increases (Brubacher & Weiser, 1985; Tang et al., 2000).

Recommended Intake

Vitamin A requirements differ both by age and between genders, according to the Recommended Daily Allowance (RDA). Men typically have a higher daily requirement than do women (900 µg RAE vs. 700 µg RAE). While adults have a higher daily requirement than do children (700-900 µg RAE vs. 300-600 µg RAE), children's needs are actually higher than those of adult's relative to their body weight. (Institute of Medicine, 2001). Retinol activity equivalents (RAE) are the preferred method for reporting levels of vitamin A in food or dietary analyses. One RAE is equivalent to 12 µg of dietary all-trans-β-carotene, and 24 µg of other dietary provitamin A carotenoids. International Units (IU) are sometimes used to express amounts of vitamin A. The ratio of RAE to IU is 1:3.33 (1 µg retinol = 3.33 IU) (Institute of Medicine, 2001). Table 1 shows the interconversion of vitamin A and carotenoid units, thus emphasizing the importance of reporting food content in amounts of each carotenoid if possible, in order to accurately calculate the amount of dietary and supplemental vitamin A consumed.

Table 1:

2001 Institute of Medicine Interconversion of Vitamin A and Carotenoid Units

1 retinol activity equivalent (RAE)
= 1 µg of all-*trans*-retinol
= 2 µg of supplemental all-*trans*-β-carotene
= 12 µg of dietary all-*trans*-β-carotene
= 24 µg of other dietary provitamin A carotenoids

It should be noted that 1 μg retinol equals 3.33 IU vitamin A activity from retinol (WHO, 1966), and 10 IU β-carotene equals 3.33 IU retinol (WHO, 1966). For β-carotene, 10 IU is based on 3.33 IU vitamin A activity x 3, which is the relative vitamin activity of β-carotene in supplements versus in diets. Consequently, when converting from IU β-carotene from fruits or vegetables to μg RAE, IU is divided by 20 (2 x 10).

Retinol equivalents (RE) have been used previously to report dietary amounts of vitamin A; however, research has shown that RE's overestimated the absorption of dietary all-*trans*-β-carotene as well as other dietary provitamin A carotenoids. (Institute of Medicine, 2001).

Sources

A variety of different foods provide vitamin A. When present in foods, dietary carotenoids have a red, orange, or yellow color; however, this color may be concealed by chlorophyll in leafy green vegetables. Preformed vitamin A is obtained from animal sources. Good food sources of carotenoids include sweet potatoes, carrots, mangoes, spinach, cantaloupe, apricots, kale, and pumpkin; liver is an excellent source of preformed vitamin A (Institute of Medicine, 2001).

VITAMIN A TOXICITY

While consuming adequate amounts of vitamin A is essential, consuming excess amounts can result in vitamin A toxicity. According to the Institute of Medicine, the Tolerable Upper Level (UL) for vitamin A intake is 3,000 μg RAE per day. The UL is the maximum amount of a nutrient that appears safe for most healthy people to consume;

however, consuming these amounts on a regular basis is not recommended due to the relative uncertainty of its effects on an individual basis. Vitamin A toxicity, or hypervitaminosis A, may be characterized as acute or chronic. Acute hypervitaminosis A occurs as a reaction from taking in too much vitamin A over a short period of time; chronic hypervitaminosis A occurs as a result of consuming too much vitamin A over longer periods of time. Symptoms of acute toxicity may include headache, nausea, vomiting, dizziness, and blurred vision (Olson, 1983).

Chronic excessive intake of vitamin A can result in more severe reactions. Teratogenic effects of excessive vitamin A have been recorded (Rothman et al., 1995). Risks of excessive vitamin A intake during pregnancy include birth defects such as craniofacial malformations and abnormalities of the central nervous system, thymus, and heart. In pregnant women, a teratogenic risk exists during the first 60 days following conception (WHO, 1998a). For this reason, vitamin A supplements are not given to pregnant women during the first trimester unless there is evidence of a deficiency. At any time during pregnancy, the maximum daily supplement given should not exceed 10,000 IU (3,000 μg RE) (WHO, 1998a). Hepatic damage may also occur as a result of chronic hypervitaminosis A. Damage may range from elevated liver enzymes to cirrhosis, and even death. Chronic excessive intake of alcohol may also enhance hepatic toxic reactions to vitamin A. In children, vitamin A toxicity may result in intracranial and skeletal abnormalities (Persson et al., 1965).

VITAMIN A DEFICIENCY

Vitamin A has been recognized as a critical factor in reducing the morbidity and mortality of children worldwide (WHO, 1996). A study conducted in India showed that mortality was lowered by 54% among preschool aged children when small, weekly doses of vitamin A were distributed (Humphrey et al., 1992). Another study in Nepal found that periodic distribution of megadoses of vitamin A (200,000 IU) every four months for a year reduced child mortality by almost 30% (West et al., 1991). Studies such as these reflect efforts by researchers to identify appropriate public health strategies in areas where vitamin A deficiency is a problem.

Vitamin A deficiency affects women as well as children. The mother's vitamin A status is of concern, particularly during breast-feeding. During pregnancy, the mother must have adequate intake and/or stores of vitamin A in order to support the growing fetus; during lactation, the vitamin A status of the mother is quantitatively more important (Stoltzfus & Underwood, 1995). Normally at least 60 times more vitamin A is transferred from the mother to the infant during 6 months of lactation compared with the amount accumulated by the fetus in 9 months of gestation. This may be, in part, due to the relatively small amount of vitamin A allowed to pass through the placenta to the fetus as a defense mechanism against toxic amounts. For this reason, infants are usually born with small hepatic reserves of vitamin A (Stoltzfus & Underwood, 1995). They must therefore rely on adequate amounts of vitamin A from breast milk as long as this is their primary food source.

The World Health Organization (WHO, 1996) has identified three main groups in which it is appropriate to measure vitamin A status as an indicator for VAD surveillance.

These groups (children, pregnant, and lactating women) are further broken down into a total of six categories: newborns and infants under 6 months of age; infants > 6 months and children up to 6 years; school-aged children; pregnant women at MCH clinics; lactating women preferably within 4-6 weeks (or at most 8 weeks) from birth; and lactating women from 2 months onward and other fertile women.

ASSESSING VITAMIN A STATUS

Several indicators of VAD may be used when assessing the status of an individual or population. These can be broken down into clinical and subclinical indicators. Clinical indicators include the deficiency syndrome known as xerophthalmia. Subclinical indicators may be further broken down into functional and biochemical indicators. Night blindness is a functional indicator, while biochemical indicators include serum retinol and breast milk vitamin A concentration (WHO, 1996).

Clinical Indicators

Xerophthalmia is the clinical deficiency syndrome that affects the eye. It occurs most frequently in children (Stoltzfus & Underwood, 1995). According to the World Health Organization (WHO, 1996), xerophthalmia can be characterized by night blindness, conjunctival xerosis, Bitot's spots, corneal ulceration, and scarring (WHO, 1996).

Conjunctival xerosis refers to drying of the conjunctiva, the eye's outer membrane. This condition may be followed by Bitot's spots, which are superficial, irregularly shaped foamy gray or white patches that appear on the conjunctiva. These

spots, or patches, are composed primarily of keratinized epithelial debris and secretions that have accumulated. If untreated, ulcers may develop and cause the cornea to soften, leading to irreversible blindness.

A mother's vitamin A status directly affects her child's risk of acquiring xerophthalmia. According to several studies, children are more likely to be affected at a younger age if duration of breast-feeding is short, while children aged 3-4 years are more likely to be affected in cultures where breast-feeding extends to several years (Stoltzfus & Underwood, 1995).

Subclinical Indicators

Night Blindness

Night blindness is the first clinical manifestation of VAD that can potentially be measured (WHO, 1996). Therefore, developing reliable field instruments to measure night blindness accurately has been the focus of much research (Christian et al., 2000; Congdon et al., 2000; Congdon et al., 1995; Haskell et al., 2005; Shrestha et al., 2000; Taren et al., 2004; Wedner et al., 2004; Wondmikun, 2002). There are two different types of cells in the eyes that work under different conditions. Rods are primarily responsible for both peripheral vision and vision at low levels of light, while cones primarily see color and in daylight. Night blindness, or poor ability to adapt to the dark, occurs due to slowed regeneration of rhodopsin molecules in the rods of the eye. Vitamin A in the form of 11-*cis* retinal is an integral structural component of rhodopsin (Institute of Medicine, 2001).

Shrestha et al. (2000) developed a new, simple, and inexpensive field tool called the Night Vision Threshold Test (NVTT) that can be used to determine night blindness in field settings. In a preliminary field-test, 150 middle-school children from Sankhu, Nepal were surveyed for the presence of xerophthalmia. The NVTT was then utilized. When testing the children for night blindness, they were dark adapted in a completely darkened room for at least 10 minutes. The tester then randomly directed the light from the NVTT in one of three different directions: directly in front or to the left or right side of the wall. The child must have correctly identified the direction of light at the same intensity on two different occasions. The lowest intensity of light observed by the child was recorded as their NVTT score (from 1-5). The researchers then measured stored retinol levels by using a modified retinol dose response test (MRDR). The NVTT and MRDR test were both done prior to vitamin A supplementation and were repeated three weeks after supplementation. Anthropometric, demographic, and nutritional data were also collected. The researchers found that only 3 of the 150 children surveyed (2%) reported night blindness. However, when the NVTT was utilized, 19 out of the 150 children (12%) were shown to be night blind. After supplementation, 15 out of the 19 children who failed the NVTT returned for retesting. Fourteen of these children (93%) improved their NVTT scores by at least 1 point. No correlation between NVTT scores and serum retinol was found. Nutritional data showed that cases consumed significantly less vitamin A containing foods than did controls ($p<0.05$). Serum retinol is affected by many factors, such as acute and chronic infections and zinc deficiency, which may be why no correlation was found between NVTT scores and serum retinol levels. Another reason may be because the liver tends to maintain homeostatic control over serum retinol

concentrations until these concentrations drop precipitously due to a deficiency (Underwood 1990). This study did find MRDR test results to be associated with NVTT scores. The MR/R (modified retinol/retinol) ratio decreased from 0.04 ± 0.02 to 0.03 ± 0.01 ($p = 0.0665$), showing an improvement in vitamin A status following supplementation. However, a larger sample size would be necessary to assess significant associations between these two tests. Another limitation to this study was the difficulty of getting young children to cooperate. Dark adapting for 10 minutes can be very inconvenient, as can setting up a dark room in the field. In the future, refinement of the NVTT as well as testing with adequate sample sizes needs to be done.

Another study, conducted by Taren et al. (2004), made use of the NVTT. The purpose of this study was to validate the NVTT as an indicator of night blindness. The researchers also wanted to determine how the NVTT compares with serum vitamin A concentrations in pregnant women. This study was conducted in Katmandu, Nepal, with a total of 1401 women recruited. Maternity nurses conducted an interview with the prospective subjects. In this interview, the nurse asked the women if they had trouble seeing in the dark now, versus before they became pregnant. The NVTT was conducted following the interview. If the women failed the NVTT, a blood sample was obtained using a standard venipuncture method. Scores for the NVTT ranged from 1-5; a score of 5 meant the subject passed the NVTT. Failure of the NVTT meant the subject could not see the lower intensities of light (0.3 mlux) and had a score of 1-4. Control subjects were also utilized, in which case a blood sample was obtained if the women passed the NVTT. The NVTT currently utilizes seven different pre-set settings of light. Methodology for performing the NVTT has been explained above. A total of 89 women (6.4%) reported

having night blindness. However, the NVTT identified 224 women (16.0%), an additional 135 women who did not report night blindness, as having night blindness. Only 32.6% (29 out of 89) of the women who reported night blindness failed the NVTT. Women who failed the NVTT had a lower ($p < 0.05$) serum vitamin A concentration than did women who passed the NVTT. The serum vitamin A concentrations did not differ between the women who reported night blindness and the women who did not. These results indicate that the NVTT can be used in conjunction with serum vitamin A concentrations to predict vitamin A deficient pregnant women. They also indicate that the NVTT was a better predictor of night blindness than self-reported night blindness in these pregnant Nepalese women. There is a need to test this instrument further under different conditions, such as rural settings. One reason the NVTT found more night-blind women than were self-reported may be because this research was conducted in an urban setting. There are more sources of light in urban settings, which may result in night blindness being more difficult for women to notice, as opposed to more rural areas where fewer sources of light are available. Also, using a single question to obtain a woman's night vision status could under or overestimate the actual prevalence of night blindness, because of the chance of confusion about the question. Additional probing could be used. The NVTT is very efficient and takes about one minute to administer following dark adaptation. This instrument could be very useful when applied in field settings.

A study by Congdon et al. (1995) evaluated pupillary and visual thresholds in 295 Indonesian children aged 1-5 years using a prototype scotopic sensitivity machine. Subjects were drawn from a larger study of children who initially had noncorneal xerophthalmia or were the sibling or near neighbor of a child with xerophthalmia

(Humphrey et al., 1994). In the larger study, all participants received a high dose of vitamin A (105 or 210 μmol) at baseline. They were then examined 3, 6, and 9 months later. Pupillary and visual testing was completed at the 6 and 9-month follow-ups. In this study, two separate hand-held illuminators were used. Machine 1 ("high intensity) was used to measure pupillary threshold. The illumination on this machine ranged from –4.16 to 0.44 log cd/m^2. Machine 2 ("low-intensity) was used to measure the visual threshold. The illumination on this machine ranged from –8.75 (calculated value) to –3.00 log cd/m^2.

Participants were subjected to binocular partial bleaching and then underwent 10 minutes of dark adaptation. The visual threshold was measured first, using the low-intensity machine, which was placed over the subject's left eye. Both a stimulus and nonstimulus were presented for each level tested; the subject was required to choose between the two. Visual threshold was defined as the lowest level at which the subject was able to correctly differentiate between stimulus and nonstimulus on three separate trials.

Next, pupillary threshold was measured using the high-intensity machine. The machine was placed over the participant's left eye, and the right eye was observed using a 2.5 x loupe under illumination from a red light emitting diode (LED). The subject's attention was directed forward while the test took place. The lowest intensity of light was presented first (1-s) and was increased at 10-s intervals until a pupillary response was clearly visible to the observer on two successive trials.

Results from this study show that children with abnormal pupillary scores had significantly lower mean serum retinol concentrations ($p < 0.05$) at 9 months than

children with normal pupillary threshold scores. Overall, both pupillary and visual threshold tests showed a significant improvement in children receiving vitamin A supplements, although there was no significant difference for the pupillary or visual threshold tests between children receiving vitamin A and children receiving a placebo. Results from this study correlate well with other indicators of vitamin A status. Use of these instruments should be considered in future studies assessing the vitamin A status of populations.

In a study conducted by Congdon et al. (2000), acceptability of the dark adaptation protocol to a group of pregnant and lactating women living in rural Nepal was tested. This protocol was initially developed for children, and has been described above. In addition to determining acceptability of the protocol, researchers also compared the responsiveness of dark adaptation thresholds and serum retinol concentrations to vitamin A and β-carotene supplementation in these women. The study was conducted in Sarlahi District in the southern, rural plains of Nepal. Pregnant and breastfeeding women aged 15-45 years who went to the local clinic between July 1995 and July 1997 were invited to return to have their blood drawn; dietary and anthropometric data were also collected. Half of these women (n =345) were randomly selected to participate in dark adaptation testing. A total of 298 of the randomly selected women completed the testing. Scotopic sensitivity was assessed by using a battery operated, handheld instrument with a light-emitting diode (LED) light source with 11 settings of light. Prior to dark adapting, the subjects underwent binocular partial bleaching and were then allowed to dark adapt for 10 minutes in a completely darkened room. The pupillary threshold was measured with the apparatus over the subject's left eye while the right eye was observed. Stimulus

intensity was increased until a contraction of the pupil in the right eye was clearly visible on two consecutive trials of the same intensity.

Supplementation was divided into three different treatment groups: 7000 µg retinol equivalent (RE) preformed vitamin A (retinyl palmitate) (n=98), 7000 µg RE *all-trans*-β-carotene (n=113), or an identical-appearing placebo in peanut oil (n=87). All supplements contained 5 mg α-tocopherol and were given weekly for 5 months. Pregnant women receiving vitamin A (retinyl palmitate) had higher serum vitamin A concentrations (1.36 µmol/L ± 0.36) than did women receiving β-carotene (1.12 µmol/L ± 0.41) or the placebo (1.11 µmol/L ± 0.35). They also had better dark adaptation thresholds (-1.24 log cd/m^2) than did the group receiving β-carotene (-1.13 log cd/m^2; p = 0.05) and the group receiving a placebo (-1.11 log cd/m^2; p = 0.03). Women with higher serum vitamin A concentrations had better dark adaptation thresholds. In the placebo group, dark adaptation thresholds were better in the first trimester and progressively worsened in the second and third trimesters. The authors concluded that the pupillary threshold obtained from the dark adaptation testing is a valid indicator of population vitamin A status. They also concluded that vitamin A supplementation during pregnancy and lactation is associated with an improvement in pupillary thresholds.

Serum Retinol

Serum vitamin A (SVA) concentration may be used as a biochemical indicator of a population's vitamin A status; but there are several disadvantages to its use. Due to hepatic homeostatic control of vitamin A in the blood over a wide range of body stores, variations of SVA are not reflective of body stores unless they are very low or very high

(WHO, 1996). Therefore, SVA concentrations do not determine overall vitamin A status very well, and should not be used to assess an individual's vitamin A status. However, when a large sample is utilized, serum retinol concentrations may give a better indication of overall vitamin A status when looking at frequency distributions (WHO, 1996). Specificity of serum retinol values can be confounded by both acute and chronic infections. Retinol in serum is attached to retinol binding protein (RBP), an acute phase protein. RBP levels can be depressed by such infections, causing serum retinol to appear low, even in individuals with adequate body stores (Reddy et al., 1986; Filteau et al., 1993; Louw et al., 1992; Butler et al., 1993). This can lead to overestimation of vitamin A deficiency. Therefore, when an acute or chronic infection is present, these values should be interpreted with caution (WHO, 1996). Measurement of an acute-phase protein should accompany serum retinol analyses in order to better quantify the role an illness plays in low serum retinol values. Several acute-phase proteins are elevated in the presence of an infection; these proteins differ in their maximum concentration peaks. C-reactive protein increases within the first six hours of an infection and reaches its maximum concentration within 24-48 hours (Thurnham et al., 2003). Concentrations of α-1-acid-glycoprotein (AGP), however, rise more slowly and reach a maximum concentration 2-5 days after infection (Thurnham et al., 2003). Another difference between these two acute-phase proteins is that C-reactive protein has a rapid turn-over time; once the immediate stimulus disappears, concentrations fall. Concentrations of AGP remain elevated for longer than other acute-phase proteins (Thurnham et al., 2003). Therefore, measurements of AGP are more accurate in identifying the presence of an infection over a longer period of time. Various thresholds have been used to define

abnormal values of AGP. However, a value of 1.0 g/L has been used in several large studies as the cutoff between normal and raised AGP concentrations (Filteau et al., 1993; Thurnham et al., 2003).

Serum retinol concentrations of < 0.35 μmol/l and 0.35-0.69 μm/l have been used to indicate deficient and low status, respectively (Arroyave et al., 1989). Serum retinol is commonly measured by high performance liquid chromatography (HPLC), which has both a high specificity and sensitivity. However, this technique is fairly expensive and requires the use of equipment that most laboratories in developing countries are unlikely to have (WHO, 1996).

Serum retinol may also be measured using the fluorometric method described by Futterman et al. (1975). In this method, the fluorescence of retinol (excitation 335 nm; emission 460 nm) is measured in unextracted, diluted serum (Driskell et al., 1986). Retinol bound to retinol-binding protein (RBP) has an enhanced fluorescence 14 times greater than retinol itself (Goodman & Leslie, 1972). This property of retinol bound to RBP allows for fluorescence measurements using relatively small samples of serum (Futterman et al., 1975). Fluorometry is a relatively simple procedure that produces results comparable in precision and accuracy to those produced using HPLC (Driskell et al., 1986). However, a disadvantage of using fluorometry is that serum retinol concentrations may be underestimated in samples that have hemolyzed (Marinovic et al., 1997). Marinovic et al. (1997) found that serum retinol concentrations obtained from HPLC and direct fluorometry were significantly different in samples that were severely hemolyzed.

There are several other factors that may interfere with fluorometric determination of serum vitamin A and should be kept in mind. Retinyl esters, which have the same fluorescence properties as retinol, may be found in the serum within two hours following consumption of a meal high in vitamin A (Bubb & Murphy, 1973). Phytofluene is a colorless carotenoid found in tomatoes and tomato products that also has fluorescence properties similar to retinol (Bubb & Murphy, 1973; Thompson et al., 1971). Therefore, serum should not be obtained from participants who have consumed a meal high in vitamin A or tomato products until at least two hours afterwards (Bubb & Murphy, 1973; Thompson et al., 1971). Another possible confounding factor may be the usage of certain pharmaceutical drugs that have fluorescent properties (Bettaieb & Aaron, 2001; Futterman et al., 1975). Researchers should find out if participants are taking any such drugs before conducting fluorometric analyses. Despite these disadvantages, fluorometry remains an inexpensive and reliable method that can be used in field settings to determine serum vitamin A concentrations (Marinovic et al., 1997).

Breast Milk Vitamin A concentrations

Breast milk vitamin A concentrations may be used as an indicator of maternal vitamin A status (Stoltzfus & Underwood, 1995). A study by Butte et al. (1981) found a correlation between serum and milk vitamin A concentration in lactating women ($r = 0.526$, $p < 0.009$). Most of the vitamin A found in breast milk is in the form of retinyl esters (mainly retinol palmitate) (WHO, 1998a). Infants are able to absorb breast milk vitamin A very well due to the presence of lipoprotein lipase, which aids in the digestibility of retinyl esters by breaking down the fat into smaller globules (Fredrikzon

et al., 1978). Concentrations of vitamin A are related to the fat content of the breast milk, which varies depending on the maturity of the milk as well as the time of day and the fullness of the breast (WHO, 1996; WHO, 1998; Stoltzfus & Underwood, 1995). Colostrum, the milk secreted in the first 4-6 days postpartum, contains the highest concentrations of vitamin A, approximately 7 μmol/l in mothers with adequate vitamin A stores (Stoltzfus & Underwood, 1995). Transitional milk, which is secreted 7-21 days postpartum, also contains high concentrations of vitamin A (WHO, 1996). Vitamin A concentrations in breast milk stabilize after about 21 days in the mature milk, and contain approximately 2.3 μmol/l in mothers with adequate vitamin A stores (WHO, 1996; Stoltzfus & Underwood, 1995). However, mature milk may only contain approximately 1.0 μmol/l (range 0.4-1.8 μmol/l) in mothers who live in parts of the world where VAD is common (WHO, 1985). A concentration of 1.0 μmol/l of milk vitamin A will only meet the infant's immediate needs and doesn't allow for accumulation and storage of the vitamin (Underwood, 1994). Due to the variation of breast milk vitamin A concentrations in the early stages of lactation, only mature milk should be used in research studies evaluating vitamin A status of mothers and infants.

The time of day during which breast milk samples are obtained should also be considered, because the fat content (and therefore the vitamin A content) varies throughout the day (Stoltzfus & Underwood, 1995). In general, the highest milk fat concentrations can be found mid-morning; however, this pattern may not be consistent (Lawrence, 1989). In fact, variations in fat concentrations throughout the day may depend more upon the pattern of breast-feeding and the fullness of the breast, rather than the time of day (Stoltzfus & Underwood, 1995). The milk in a full breast that has not yet

been suckled on contains less fat, and therefore less vitamin A, than the last milk expressed (WHO, 1996).

When assessing breast milk vitamin A in individuals, these variations must be taken into consideration. A sample should be obtained that is representative of the mother's typical milk fat secretion (Stoltzfus & Underwood, 1995). Another option is to control for the milk fat concentration when the sample is analyzed (Stoltzfus & Underwood, 1995). Methodology for analysis of the fat content in breast milk will be discussed below.

When assessing breast milk vitamin A in populations, samples should be collected at random times throughout the day and at different periods following the last feed (WHO, 1996). This variation in the collection of breast milk samples from many mothers should be a fair representation of the population, because the variation will be randomly distributed among all the samples as long as the collection period is randomly distributed as well (Stoltzfus & Underwood, 1995; WHO, 1996).

Acceptability of obtaining breast milk samples is generally high, especially if it is explained to the mothers that expressing their breast milk will stimulate the breast to produce more and will not decrease the amount of milk their baby will receive (WHO, 1996). Also, mothers may better understand vitamin A status in terms of the quality of their breast milk as opposed to serum levels. Because of the interest most mothers have in the quality of their breast milk, use of breast milk to assess vitamin A status may lead to increased cooperation on the part of mothers (WHO, 1996).

Vitamin A in breast milk may be analyzed using high-performance liquid chromatography (HPLC). In this procedure, milk samples must first be saponified by

incubation in an alkaline solution (Stoltzfus & Underwood, 1995). This converts the retinyl esters to retinol. The samples are then washed to remove the alkaline solution, and the retinol is extracted into an organic solution, such as hexane (Stoltzfus & Underwood, 1995). This procedure produces both accurate and precise results; however, it requires expensive equipment and technical expertise (Stoltzfus & Underwood, 1995).

When assessing an individual's breast milk vitamin A concentration, results should be expressed in relation to the volume of fat present in the milk (Stoltzfus & Underwood, 1995; WHO, 1996). Milk fat may be determined using the creamatocrit method (Stoltzfus & Underwood, 1995; WHO, 1996). In this method, milk samples are centrifuged in haematocrit capillary tubes. The cream layer should separate and rise to the top. The length of the cream layer is then measured to the nearest 0.05 mm using vernier calipers. The fat concentration should be expressed as a percentage of the length of the milk column in the tube (Lucas et al., 1978). The vitamin A concentration is then expressed as a ratio to the fat concentration in percent (Stoltzfus & Underwood, 1995). The World Health Organization (WHO, 1996) recommends using a cut-off of ≤ 1.05 µmol/l, or ≤ 8 µg/g milk fat to identify low breast milk vitamin A concentrations.

INDICATORS ASSOCIATED WITH VITAMIN A DEFICIENCY

While the clinical and subclinical indicators described above may be used to assess a population's vitamin A status, there are other, indirect indicators that are also associated with vitamin A deficiency. These may not be used to assess vitamin A status; however, they may be useful when identifying and describing populations where VAD

occurs frequently (WHO, 1996). These indicators include nutritional status and diet-related indicators, illness-related indicators, and socioeconomic indicators.

Nutritional Status and Diet-Related Indicators

Several indicators may be used to assess the nutritional status of a population. Stunting and wasting are associated with VAD and an increased risk for VAD in children, respectively (WHO, 1996). Stunting (< -2 Z-scores for height-for-age) represents a measure of chronic under-nutrition and food deprivation, while wasting (< -2 Z-scores for weight-for-height) represents acute inadequate dietary intake (WHO, 1996). Infants born with a low birth weight (< 2.5 kg) may be reflective of maternal malnutrition (WHO, 1996).

Food availability and dietary and food consumption patterns may also be used as indicators. Information on the availability of vitamin A rich foods may give an indication of the vitamin A status of a population (WHO, 1996). Care should be taken to obtain information on availability of foods during different seasons, because some vitamin A rich foods may not be as accessible for certain populations depending on the time of the year. Dietary patterns may also give insight into the status of a population. If vitamin A rich foods are available, information should be obtained concerning the frequency of consumption, portion sizes, food practices during pregnancy and lactation, and complementary feeding of infants (WHO, 1996).

Illness-Related Indicators

Several illnesses may be associated with an increased risk of VAD in children (WHO, 1996). These include protein-energy malnutrition, measles, diarrhea, and respiratory diseases (WHO, 1996). In a study conducted by Semba et al. (2003), diarrhea within the two weeks prior to the study was associated with self-reported night blindness in women of childbearing age. Another study, conducted by Sommer et al. (1983), found that the mortality rate of children with mild xeropthalmia was on average four times the rate of children without xerophthalmia. Mild xerophthalmia was defined as having night blindness and/or Bitot's spots. This was a prospective longitudinal study of 4,600 preschool-aged children in rural Indonesia. A total of 3,481 children completed the study. Every three months for a total of 18 months, the researchers met with the children and their families to conduct interviews and obtain anthropometric data. In addition, a pediatrician conducted a physical examination, and an ophthalmologist conducted an ocular examination at each interval. These results showed that children with mild xerophthalmia at the start and end of a three month interval were twice ($p < 0.001$) and three times ($p < 0.001$) as likely to develop respiratory infections and diarrhea as children without xerophthalmia during the same interval (Sommer et al., 1984). Results also showed that the risk of developing a respiratory infection and diarrhea was more closely associated with vitamin A status than with overall nutrition status (Sommer et al., 1984).

Information on occurrence and frequency of such illnesses may be obtained through surveys or reports issued by the country's government. However, these surveys may not be completely accurate because many of these illnesses are not reported. A high prevalence of these illnesses may be indicative of populations at an increased risk for

VAD (WHO, 1996). In Ethiopia, a total of 22,101 cases of acute watery diarrhoeal syndrome with 219 related deaths have been reported as of September 28, 2006 (WHO, 2006). The Ethiopian Central Statistical Authority (2001) reported 24.4% of children under five surveyed (n = 2,623) as having symptoms of acute respiratory infections in the two weeks prior to the survey. Of these children, only 15.8% (n = 414) were taken to a health care provider for treatment. Other survey results showed that 23.6% (n = 2,537) of children under five had experienced diarrhea in the two weeks prior to the survey. Many of the children with diarrhea (45%, n = 1,141) were given oral rehydration therapy treatment of some sort. However, 38.5% (n = 976) were not given any treatment (Ethiopian Central Statistical Authority, 2001).

Socioeconomic Indicators

The socioeconomic status of a population may also be helpful when identifying populations vulnerable to VAD (WHO, 1996). Socioeconomic indicators are often used to support evidence found from using clinical and/or subclinical indicators. Maternal education and literacy consistently have shown an inverse relationship with VAD prevalence (WHO, 1996). The United Nations has addressed gender disparity and women's lack of empowerment in their Millennium Development Goals, with the goal being to "eliminate gender disparity in primary and secondary education preferably by 2005, and at all levels by 2015". According to the World Food Program's (WFP) Gender Policy Paper, gender disparities that discriminate against women and restrict their economic contributions have a negative impact on the advancement of individuals, households, and societies (2002). According to Quisumbing et al. (1995), when women

control the income rather than men, both economic and nutritional benefits are higher for the entire household. Women are also more likely to spend income on food and child welfare and are less likely to sell or trade food for cash or other non-food items than men (WFP, 2002).

Poverty is another socioeconomic indicator associated with VAD prevalence (WHO, 1996). Characteristics of impoverished societies may include several factors, such as low income and employment, poor water supply and sanitation, lack of access to health and social services, and lack of access to land and agricultural services (WHO, 1996).

SUPPLEMENTATION IN LACTATING WOMEN

One approach to combating VAD that has been the focus of much research is vitamin A supplementation. The World Health Organization (1996) advises supplementation for the treatment and prevention of VAD; however, due to its lack of both feasibility and sustainability, WHO recommends that this not be used as a long-term solution. Due to the risk of teratogenic effects from high doses of vitamin A during pregnancy, the maximum daily supplement of vitamin A that should be given to fertile women, independent of their vitamin A status, is 10,000 IU (3,000µg RE) (WHO, 1998a). The WHO has also recommended a dosing schedule for mothers, infants, and children for the prevention of VAD (Table 2) (WHO, 1998b).

Table 2:

The World Health Organization's recommended vitamin A dosing schedule (1998)

Infants < 6 months of age	50,000 IU orally
Infants 6-12 months of age	100,000 IU orally every 4-6 months
Children > 1 year	200,000 IU orally every 4-6 months
Lactating mothers	200,000 IU orally within 8 weeks of delivery

In a study conducted by Bahl et al. (2002), vitamin A supplementation improved breast milk vitamin A levels at two months postpartum. In this randomized, double-blind, placebo-controlled multi-center trial, conducted in Ghana, India, and Peru, a total of 9,424 mother-infant pairs were assigned to receive vitamin A or a placebo. Mothers in the vitamin A group received a single dose of 60 mg retinol palmitate at 18-42 days postpartum, while their infants were given 7.5 mg on three separate occasions (Bahl et al., 2002). Results showed that at two months postpartum, supplementation had improved breast milk vitamin A levels in the vitamin A group (49.8 nmol/g fat ± 24.6) as compared with the placebo group (42.7 nmol/g fat ± 22.1) (Bahl et al., 2002). However, at both 6 and 9 months postpartum, mean breast milk retinol did not differ significantly between the two groups (Bahl et al., 2002).

In another study, conducted by Stoltzfus et al. (1993), lactating mothers were given a single dose of 312 μmol of vitamin A as retinyl palmitate at 1-3 weeks postpartum. This randomized, placebo-controlled, double-blind trial was conducted in Central Java, Indonesia. Mothers were divided into a vitamin A group (n = 77) and a placebo group (n = 76). All infants were given a single dose of 104 μmol vitamin A as retinyl palmitate at the conclusion of the study. Serum retinol concentrations were measured in the mothers at baseline, 3 months, and 6 months postpartum (Stoltzfus et al.,

1993). At 3 and 6 months postpartum, serum retinol concentrations were significantly higher in the vitamin A group (1.39 μmol/l ± 0.49 and 1.23 μmol/l ± 0.34) than in the placebo group (1.24 μmol/l ± 0.43 and 1.08 μmol/l ± 0.37) ($p < 0.05$ and $p < 0.01$). However, both the vitamin A and placebo groups showed a nearly parallel decline in serum retinol concentrations from 3 to 6 months postpartum, although the vitamin A group still had slightly higher serum vitamin A concentrations at 6 months postpartum (1.23 μmol/l ± 0.34) than at baseline (1.17 μmol/l ± 0.45) (Stoltzfus et al., 1993).

According to WHO (1998a), low daily doses and periodic massive doses of vitamin A will increase the vitamin A concentration in milk. While massive doses (300,000 IU) of vitamin A given to mothers in the first few weeks postpartum have shown beneficial effects in both the mothers and their infants, the safe upper limit for vitamin A supplementation is not yet known (WHO, 1998a). Although the safety and efficacy of supplementing both mothers and infants independent of each other has been shown, there is insufficient evidence available to evaluate the safety and efficacy of dosing both a mother and her infant concurrently (WHO, 1998a). Until more is known regarding safe doses of supplemental vitamin A, care should be taken when initiating a research study, community outreach program, or supplementation program.

INTERACTIONS WITH OTHER NUTRIENTS

There are several nutrients that are interrelated closely with vitamin A. Iron and zinc are the two most commonly studied in tandem with vitamin A. Deficiencies of either of these two nutrients may affect the vitamin A status of an individual; likewise, VAD may affect both or either of these two nutrients.

Iron

Vitamin A deficiency and iron deficiency anemia are both highly prevalent in many developing countries (Ettyang et al., 2003). Several studies have shown a direct correlation between hemoglobin and serum retinol concentrations (Kafwembe, 2001; Suharno et al., 1993). In a cross-sectional survey conducted by Kafwembe (2001) in Ndola City, Zambia, serum retinol and hemoglobin levels were measured in 242 lactating women. Anemia was defined as Hb < 12 g/dL, and iron deficiency was defined as iron < 7 μg/dL. Anemia was found in 29% of the participants, while iron deficiency was present in 41%. A significant positive correlation was found between serum vitamin A and hemoglobin concentrations (Kafwembe, 2001). Other studies have shown that supplementation of vitamin A may improve hemoglobin concentrations (Bloem, et al., 1989; Mjia, et al., 1988). In another study conducted in Indonesia, VAD in lactating women decreased their body's response to iron supplementation by 30% (WHO, 1996). A recent study conducted by Zimmermann et al. (2006) measured the effect of vitamin A supplementation on hemoglobin, iron status, and circulating erythropoietin (EPO) concentrations in Moroccan school children with both poor iron and vitamin A status. In this double-blind, randomized control trial, 81 children were given either 200,000 IU vitamin A (as retinyl palmitate) or a placebo at baseline and five months. At the 10 month follow-up, a significant increase in serum retinol concentrations was seen in the vitamin A group compared with the control group ($p < 0.02$). A significant increase was also observed in the geometric mean EPO concentrations of the vitamin A group ($p < 0.05$). Mean hemoglobin concentrations were significantly greater in the vitamin A

group compared with the control group ($p < 0.02$), and anemia (defined as hemoglobin < 120 g/L in children ≥ 12 years and hemoglobin < 115 g/L in children 5-11 years) was significantly reduced in the vitamin A group ($p < 0.01$) (Zimmermann et al., 2006). The researchers concluded that repletion of vitamin A may cause a redistribution of iron from stores to the marrow for erythropoiesis (Zimmermann et al., 2006).

However, in a recent study conducted by Miller et al. (2006), no effect on hemoglobin levels or anemia (defined as hemoglobin < 105 g/l) was seen in Zimbabwean infants who were given a single dose of 50,000 IU vitamin A (as retinyl palmitate). Maternal supplementation of a single dose of 400,000 IU (as retinyl palmitate) also had no effect on hemoglobin or anemia in the infants. The researchers hypothesized that the lack of change in hemoglobin levels post-supplementation may be attributable to iron deficiency in the infants (Miller et al., 2006). Although the relationship between iron and vitamin A is not fully understood, studies suggest that VAD impairs iron mobilization from body stores; therefore, supplementation of vitamin A may improve hemoglobin concentrations (Lynch, 1997).

Zinc

The relationship between zinc and vitamin A has been well documented (WHO, 1996). Zinc is required in the hepatic synthesis and secretion of retinol binding protein (RBP), which is the transport protein for vitamin A (Christian et al., 2001; WHO, 1996). Therefore, production of RBP is reduced when zinc deficiency is present, resulting in a secondary vitamin A deficiency (Christian et al., 2001). In secondary vitamin A deficiency, hepatic stores of vitamin A may be adequate with low serum retinol

concentrations (Christian et al., 2001). Although evidence of the relationship between zinc and vitamin A has been documented, cross-sectional studies in humans have failed to consistently establish a relationship between zinc and vitamin A status (WHO, 1996). In a randomized, double-blind, placebo-controlled trial conducted by Christian et al. (2001), pregnant women who reported night blindness were randomly assigned to receive 25 mg of elemental zinc, or a placebo. This was part of a larger study that divided women who self-reported night blindness into three groups: vitamin A supplementation, β-carotene supplementation, or placebo. Supplements were given as capsules containing either 7,000 μg RE retinyl palmitate or 42 mg all-trans β-carotene. Women receiving a zinc supplement were added to each of the three groups for a total of 202 women participating; therefore, some women received both a zinc and vitamin A supplement (Christian et al., 2001). A total of 28 capsules were given to each woman, with instructions to take one tablet each day. Women were visited twice each week to monitor consumption. Pupillary thresholds and serum retinol and zinc were measured both at baseline and at the end of the trial. Pupillary response was measured using the procedure developed by Congdon et al. (1995) and described previously.

Results showed that zinc supplementation, either alone or in combination with vitamin A or β-carotene, failed to improve the mean pupillary threshold scores of the participants (Christian et al., 2001). However, there was an indication of improvement in pupillary thresholds among the women receiving zinc and vitamin A relative to women receiving a placebo only (Christian et al., 2001). Women in the vitamin A and β-carotene groups developed night blindness in spite of receiving these supplements for three consecutive weeks. The mean pupillary scores among the night-blind pregnant women in

this study (-0.83 to –0.86 log candela/m^2) are lower when compared with the mean score of pregnant women in Nepal receiving a placebo who were not night-blind (-1.11 log candela/m^2) (Christian et al., 2001; Congdon et al., 2000).

As the studies above and many others have shown, vitamin A deficiency is a major problem with far-reaching consequences. In many lesser-developed parts of the world where VAD is common, these consequences include alarmingly high rates of respiratory infections, night blindness, corneal ulcerations, blindness, and childhood death. These consequences could be avoided if the international goal of eliminating VAD is reached. Researchers and community health workers need to work together to develop programs that address VAD both in the short and long term.

CHAPTER III

RESEARCH DESIGN AND METHODS

In this section, the subjects and sampling method will be discussed. This will include procedures used to recruit subjects, the number of subjects participating in the study, and the time frame in which the subjects participated. Full details of blood sampling, dark adaptation testing, and use of a questionnaire will also be explained. Methods regarding analysis of blood samples by HPLC and the CRAFTi portable fluorometer and dark adaptation testing will also be addressed in this section.

This study was part of a cross-sectional investigation of the nutritional status of lactating women in two rural villages in Southern Ethiopia conducted in January, 2006. A convenience sample of 108 women in Wondo Genet and Arsi Negelle was utilized. The investigation was conducted as a joint effort between graduate students and professors from Oklahoma State University and Hawassa University, located in Stillwater, Oklahoma and Awassa, Ethiopia, respectively. Dr. Barbara J. Stoecker was the advisor from Oklahoma State University, and Dr. Yewelsew Abebe was the advisor from Hawassa University. Dr. Yewelsew obtained her doctorate in Nutritional Sciences from Oklahoma State University in 2003. Members of the research team from Hawassa University included Alemzewed Chall, Tafere G/Egziabher, Getahun Ersino, Fikadu Reta, and Meron Girma.

Approval from the Institutional Review Board at Oklahoma State University and the Ethics Committee at Hawassa University was obtained prior to beginning the research.

LOCATION AND RECRUITMENT

The research team from Hawassa University conducts community-based research projects in a variety of locations throughout Southern Ethiopia. The two villages for our study, Wondo Genet and Arsi Negele, were chosen prior to collaborative involvement between the two universities on this particular project. The villages were chosen because of their close proximity to Awassa, which acted as the home base for the project and because they represented two different agroeconomic zones with different staple foods. The vitamin A status of lactating women in these two villages had not been studied prior to this investigation. Vitamin A rich foods did not appear to be readily available in either village, which was another reason these two villages were chosen. In addition, there was a health clinic or health post located in both Wondo Genet and Arsi Negele, which was necessary in the recruitment process.

Both of the villages visited for this research project are located in Southern Ethiopia. The research was conducted in January of 2006, which is the dry season. The land itself was very dry and dusty. Roots and tubers were the major crops grown in Wondo Genet, while cereals and grains were staple crops grown in Arsi Negele. Dr. Yewelsew first made contact with the Wondo Genet College of Forestry, to inform them of the upcoming research project and to receive their cooperation. The team was then directed to the health center in rural Wondo Genet, where contact was made with a local

health worker who scheduled a community meeting. Lactating women and their husbands were encouraged to attend. The study procedures were explained, as well as the level of involvement required, possible risks associated with participation, and the incentives to be awarded at the conclusion of the study. The women and their husbands were encouraged to ask any questions and voice any concerns they might have had. Informed verbal consent was obtained and tape-recorded from the women who agreed to participate in the research. A convenience sample consisting of a total of 56 women in Wondo Genet participated in the study.

Arsi Negele was the second village where the research was conducted. Once again, Dr. Yewelsew contacted a local health worker at the health post in this village. Women were recruited using the same methods as described above. A total of 52 women participated from this village.

The participants were actively involved for 2-3 hours at the community health center. This included the interview with the questionnaire, anthropometric measurements, getting blood drawn, expressing breast milk, and performing the test to measure dark adaptation.

Rationale for Sampling

A convenience sample was used, as it was the most appropriate subject selection method for the investigation. These villages had not been utilized for nutrition research prior to the present study. Therefore, it would not be culturally sensitive or realistic to create a random sample and expect cooperation from members of the community.

Inclusion Criteria

Prospective subjects had to meet certain critera in order to participate in the investigation. They had to be lactating women, whose youngest child was no younger than six months old, and no older than three years of age. This specific criterion was necessary for studying the vitamin D status of the mothers and their children, which was a separate research study conducted as part of the overall investigation. Immunization cards were checked to ensure accuracy of reported child age.

PROGRAM DESCRIPTION

Many different indicators were collected in order to determine the vitamin A status of the participating women. A questionnaire was utilized, which collected information regarding dietary habits, as well as information regarding supplementation and knowledge of vitamin A. Anthropometric measurements of height, weight, and mid-upper arm circumference were taken. Blood samples and breast milk were obtained in order to analyze the vitamin A concentration. Dark adaptation was tested as a functional indicator of vitamin A status.

Each of the above activities was divided into a separate station in and outside of the health center. Women were divided into small groups, with anywhere from four to six in a group. Each group started at a different station. After completion at the first station, the groups rotated on to the next station until each group had participated in all the activities. Incentives were given to participants at the conclusion of each activity. Hair oil and a comb were given to women who completed the questionnaire and anthropometric measurements. Pictures of the participants with their babies were taken

on a digital camera and printed on a portable printer. These were given to the women after obtaining blood and breast milk samples. The participants were given a bottle of water during the testing of dark adaptation and were given a headscarf at the end of the study.

Questionnaire

The students and professors from Oklahoma State University and Hawassa University compiled the questionnaire used in this investigation (Appendix A, B). Questions were divided into six major sections: household and background characteristics, health, nutritional status, and other reproductive behaviors, household food production and consumption patterns, vitamin A supplementation, knowledge of vitamin A deficiency, and vitamin D related questions. Afework Bezabih, a doctoral student at Oklahoma State University, initially translated the questionnaire from English into Amharic, Ethiopia's national language. Trained research assistants and department faculty from Hawassa University helped translate the questionnaire from Amharic into Sidaminga and Oromigna, the local languages spoken in Wondo Genet and Arsi Negele, respectively. In Wondo Genet, the research team from Hawassa University discussed the questions with the participants and recorded their answers. In Arsi Negele, a different dialect was spoken, so translators were employed to assist with the process.

Anthropometric Measurements

The team of trained research assistants and department faculty from Hawassa University obtained anthropometric measurements from each participant after completion of the questionnaire. All measurements were rounded to the nearest tenth. Height was measured in centimeters using a portable wooden stadiometer (Shorr Productions, Olney, MD). Weight was measured in kilograms using a calibrated digital solar-powered scale (Uniscale, UNICEF, Copenhagen). Mid upper arm circumference was measured using a flexible non-stretchable measuring tape. All measurements were taken twice. If the difference between the two measurements was > 0.5, at least one more measurement was taken.

Blood Samples

Blood samples were drawn from all but one of the participants, who was ill. A medical technologist obtained the blood samples using standard venipuncture protocols. Blood was drawn from a vein on the inside of the elbow of each participant. The puncture site was cleaned with an alcohol swab, and a tourniquet was tied around the upper portion of the arm to apply pressure and restrict blood flow through the vein. A sterile needle was used to pierce the vein, and 10 milliliters of blood was collected into a syringe. The puncture site was then covered with a cotton ball and bandage to ensure that bleeding had stopped. All appropriate safety measures were taken to ensure the safety of the personnel and participants, and to ensure that the integrity of each sample was not compromised due to exposure to sunlight. Blood samples were taken in an open-air gazebo in Wondo Genet and inside the health post at Arsi Negele. The sample racks

were covered with aluminum foil and placed on ice packs in an insulated cooler. In Wondo Genet, samples were centrifuged on site to obtain serum; samples obtained in Arsi Negele were taken back to the laboratory at Hawassa University where they were centrifuged to obtain serum. After collection of serum, all samples were frozen at -20°C until further use.

Breast Milk Samples

Breast milk samples were also collected from each participant. The participants were each given a disposable plastic drinking glass, which they used to express ~5 milliliters of breast milk. The women took the containers to a private location to express the milk if they wished. After collection, each breast milk sample was poured into a smaller plastic container and immediately put on ice packs in an insulated cooler. At the end of each day, the samples were transferred to a freezer at Hawassa University where they were frozen at -20°C until further use.

Dark Adaptation

Dark adaptation was tested using a modified technique developed by Congdon et al. (1995). The Scotopic Sensitivity Tester-1 (SST-1, LKC Technologies, Inc.), a portable field instrument, was used to test participants' pupillary response to various intensities of light. The SST-1 uses a slowly oscillating (2 Hz) light. The intensity of light ranges from 0 dB (lowest) to 30 dB (highest). It consists of two units: the hand held stimulator and the control unit. The bulb in the SST-1, used in pupillary response testing, is a tungsten-halogen bulb. Its expected lifetime is approximately 5,000 tests. Because

the bulb retains 90% of its initial brightness throughout its lifetime, replacement of the bulb is not necessary until it fails. The SST-1 was calibrated at LKC Technologies prior to shipment.

Either dark adaptometry or scotopic sensitivity may be tested using this machine. For this investigation, the dark adaptometry test was used in order to measure each participant's final dark-adapted threshold. As stated above, the SST-1 consists of both a handheld stimulator and a control unit. The control unit displays the test type that has been selected, the brightness of the stimulus, and the time (in seconds). It also has a dimmer button to dim the glow of the display, as well as a timer-reset button. The handheld stimulator allows the stimulus to be presented. The "up" button increases the intensity by increments of 5 dB, while the "down" button decreases the light intensity by increments of 1 dB. A button labeled "STIM" presents the stimulus when pushed, and the reset button sets the intensity back at 0 dB.

Dark adaptation testing requires a completely dark room shielded from any outside light. This can pose a problem when conducting field research. Dr. Yewelsew's husband, Ato Tesfaye, built a portable dark room out of thick black canvas and metal poles. This dark room was assembled at each location for use in testing dark adaptation. Thick blankets were pinned along the bottom of the tent to prevent light from entering. Blankets were also pinned along the flap, which opened and allowed access both in and out of the tent. The blankets were layered in such a way so that someone could enter or leave the tent without allowing light inside. Should a participant become ill, they would be able to leave the dark room without sacrificing the integrity of the entire test.

Figure 1: Portable Dark Room

Groups of anywhere from four to six participants were allowed to dark adapt together in order to manage time efficiently. This also allowed the women to socialize and helped to make the experience less threatening overall. Prior to dark adaptation, each participant in the group was subjected to binocular partial bleaching with a digital camera flash (Sony Cyber-shot DSC-W5). This was done as a control to ensure that each participant began on the same baseline. For instance, one participant may have been sitting in the shade and another standing in direct sunlight. Bleaching makes sure that no one participant has begun to dark adapt prior to the actual testing. A cone was used during bleaching to ensure that the entire back of the eye was bleached. The cone was made from a piece (1 m^2) of thick cardboard and was covered with aluminum foil with the shiny side out. The cardboard was then rolled up to form a cone, with the aluminum foil on the inside. The larger of the two ends was just big enough to fit around a participant's head. The smaller of the two ends was just large enough for the flash (4x8 cm). Participants were told that they would see a bright flash of light, but that it would not hurt their eyes. The foil cone was put over the participant's face, the camera flash

unit was placed at the opposite end of the cone, and the flash was then triggered. The camera was recharged each night to ensure consistency in the intensity of the flash.

Following the bleaching procedure, participants were immediately taken inside the dark room where they were given bottles of water and guided to seats. The procedure was explained once again, and the participants were allowed to ask any questions they might have thought of since the initial community meeting. Most of the women seemed apprehensive at first, but soon began visiting with each other. Two fans were placed inside the dark room to provide air circulation and prevent the room from becoming too hot.

Participants were allowed to dark adapt for 20 minutes. During this time, the eyes switch from using the cones, which see higher intensities of light, to using the rods. The rods are the part of the eyes that see the lower intensities of light. This switch from using cones to rods is called the rod-cone break (Dark Adaptation Testing Manual).

A red light-emitting diode (LED) was placed at the opposite end of the tent, facing the participants. The participants were asked to look towards the red LED during testing in order for the researcher to be able to get a clearer view of the pupil. Red lights are acceptable for use, as they do not interfere with dark adaptation (Dark Adaptation Testing Manual). Once the eyes have begun to become more sensitive to lower levels of light (once they have begun to dark adapt), the rods in the eyes are used for vision. Rods distinguish blue and green light better than other colors, such as red, which is why a red LED will not interfere with dark adaptation (Dark Adaptation Testing Manual).

Two researchers from Oklahoma State University and Tafere G/Egziabher from Hawassa University worked as a team in the dark room. Tafere acted as a translator and

helped the participants feel more at ease. One researcher was in charge of presenting the stimulus to each participant. This was done by holding the handheld stimulator up against the participant's left eye. The other researcher was in charge of watching for the pupil in the right eye to contract, which is the pupillary response. The observer looked through a night vision scope (ELF-1, LOMO America Inc.) in order to be able to see the contraction while in the darkroom. Communication between the two researchers was vital and required proper training and practice prior to field-testing. The two researchers began training several months prior to the investigation. Dr. Stoecker had been trained previously on use of the SST-1 and helped the researchers with the initial training. In order to become proficient in using the instrument, practice sessions were held at least twice a week for several hours at a time. The researchers worked together during these practice sessions to improve communication and to finalize the protocol.

The stimulus was first presented at the lowest intensity, 0 decibels (dB). The intensity was increased by increments of 5 until the observer could see a contraction of the pupil. Once this occurred, the intensity was decreased by increments of 1 dB until the observer could no longer see a contraction of the pupil. The resulting value on the display was recorded as the participant's final dark adapted threshold. If a contraction of the pupil was seen at 0 dB, the participant's threshold was recorded as less than 0 dB. If a contraction was not seen by 30 dB (the highest intensity of light available on the instrument) then the participant's threshold was recorded as greater than 30 dB.

To prevent the possibility of disease and/or bacterial infection, the handheld stimulator was cleaned with a Clorox disinfectant wipe between uses. Both researchers also wore disposable gloves as an extra precaution.

DIETARY ASSESSMENT

Vitamin A consumption was assessed based on participants' answers from the Household Food Production and Consumption Patterns section of the questionnaire. The question asked, "How often do you eat the following food items?". The nine vitamin A source foods listed were liver, eggs, butter, mango, papaya, sweet potato, pumpkin, carrot, and kale. There were five possible answers: daily, several times per week, weekly, occasionally, and never. To assess the adequacy of consumption of vitamin A source foods, women were grouped into four separate categories. These categories were: consumption of at least two vitamin A source foods daily, consumption of one vitamin A source food daily, consumption of one vitamin A source food at least weekly, and consumption of one vitamin A source food only occasionally.

ANALYSIS OF SERUM

Two separate methods were used in analyzing the vitamin A concentration of the blood. The Micronutrient Initiative of Canada provided partial funding for the investigation in return for field-testing of a new piece of equipment for measuring vitamin A concentrations in blood plasma. This instrument is called the CRAFTi Portable Fluorometer (Craft Technologies, Inc., Wilson, NC) and uses fluorescence spectroscopy to measure retinol bound to retinol binding protein (RBP). Because of its superior fluorescence properties compared with apo-RBP, accurate measurements of retinol-RBP may be taken using small samples of plasma (Futterman et al., 1975).

Fluorometry

The CRAFTi fluorometer must be calibrated against a standard prior to each use. This instrument uses a plastic block with a fluorescent dye to be inserted as the calibration standard. The CRAFTi consists of an LCD screen on which values are displayed, a numeric key pad, and a cuvette well where samples are placed for testing. Before testing the samples, the instrument must be turned on and allowed to warm up for 15 minutes. During this time, the internal microcomputer performs a diagnostic test. Upon completion, the Main Menu appears on the screen, showing that the instrument is ready to receive input. Function 1 > Read should be selected to calibrate the instrument. The first step in calibrating the CRAFTi fluorometer is to insert the solvent blank (500 µL NaCl) into the sample well. The solvent blank should be pipetted into a cuvette (round Durham tube, 6 X 50 mm). The operator should press <ENTER> followed by <ZERO>. The fluorometer sets the blank as zero. Next, the blank is removed and the plastic calibration block is placed in the sample well. The calibration block should be placed in the same orientation each time the instrument is calibrated. After closing the compartment lid, the operator should press <CALIB> and then enter the standard calibration block value, followed by <ENTER>. These steps should be repeated until stable readings for both the blank and the calibration block are achieved.

After calibrating the instrument, each cuvette was marked with the appropriate subject code and filled with 500 µL of the dilution buffer (1 M NaCl) using a micropipette. Then, 25 µL of serum were transferred into each appropriate cuvette. Samples were then covered with Parafilm and mixed by inversion, using gloved hands for

protection. Samples were inverted three times to ensure adequate mixing. After mixing and before the initial fluorescence readings were taken, samples were allowed to sit for 5-10 minutes in order for the sample to mix thoroughly and stabilize. The cuvettes were wiped clean with a Kimwipe to ensure that no liquid or fingerprints were present, which could affect the reading. Each cuvette was then placed in the sample well with a small cuvette adapter in place to hold the tube steady. After placing the cuvette in the compartment and shutting the cover, a reading appeared on the display. The compartment cover was opened and closed again to re-initiate the test. The second value displayed is often more stable than the first, so that was the value recorded.

Following the first reading, 20 µL of alkali solution (1 M NaOH) was added to the diluted sample. The samples were again mixed by inversion, allowed to stand at room temperature for 15 minutes, and read as described above. Each cuvette was placed in the sample well in the same orientation as for the previous reading. The second (alkali-treated) fluorescence reading was lower than the first. The difference between the two readings was then calculated and recorded. This value was the participant's serum vitamin A concentration in µg/dL.

Prior to beginning the study, researchers from Oklahoma State University obtained serum from their own blood samples in order to practice using the CRAFTi instrument. Samples from the participants were analyzed in the laboratory at Hawassa University on the same day on which they were obtained in the field. Serum from each sample was also analyzed at Oklahoma State University, with 10-20 samples analyzed each day. Tests were run both in the field and at the laboratory at Oklahoma State

University in order to compare field-tested results with results obtained under ideal conditions.

High Performance Liquid Chromatography

Serum vitamin A was also analyzed by use of high performance liquid chromatography (HPLC). Samples were transported to Craft Technologies in Wilson, North Carolina, where they were analyzed for serum vitamin A concentrations, retinol-binding protein, and alpha 1-acid glycoprotein (AGP).

ANALYSIS OF BREAST MILK

Milk vitamin A was also analyzed using high performance liquid chromatography (HPLC). Afework Bezabih, a doctoral student from Oklahoma State University, Department of Nutritional Sciences, performed the extraction and subsequent analysis at the laboratory at Oklahoma State University. The internal standard, 3,4-didehydroretinyl acetate (DRA), was obtained from Dr. Tanumihardjo (Department of Nutritional Sciences, University of Wisconsin-Madison). From the DRA stock solution, 50 µl was mixed with 25 ml isopropanol in a volumetric flask. Ten µl of this solution was injected into the HPLC system (4.6 x 150 mm, 5 um Waters C-18 Reversed Phase Analytical column) and eluted. The eluting solvent was 89:11 methanol and water at a flow rate of 1.0 ml/min. The internal standard was treated independently for each of the milk samples. The extraction efficiency was obtained by dividing the integrator area obtained for the dehydroretinol (DR) by the area obtained with the internal standard (DRA). Final values were corrected by dividing the vitamin A value obtained from the peak areas by

the extraction efficiency. All analyses were conducted in a dark room. To ensure complete mixing of breast milk, samples were completely thawed and mixed thoroughly prior to aliquotting the samples.

Fat content of the breast milk was analyzed using the creamatocrit method developed by Lucas et al. (1978). Approximately 75 µl of breast milk was drawn into glass capillary tubes (75 x 1.5 mm) and centrifuged for 15 minutes. The tubes were immediately removed and placed vertically with the cream layer on top. The cream layer was measured using vernier calipers to the nearest 0.05 mm. Results were expressed as a percentage of the total length of milk in the tube (Lucas et al., 1978).

STATISTICAL ANALYSIS

Data was compiled into a spreadsheet and was analyzed using the PC Statistical Analysis System (SAS) for Windows (Version 9.1 SAS, Inst. Inc., Cary N.C.). Means, standard errors of the mean, ranges and frequency distributions of variables were determined. Pearson correlation coefficients were calculated for all appropriate variables. Significance was set at $p < 0.05$.

Dark adaptation threshold was measured in log candela/m^2 (log cd/m^2), the SI unit of luminance. Participants with better dark adaptation could see lower levels of light, and thus had a smaller luminance value.

CHAPTER IV

VITAMIN A STATUS OF LACTATING WOMEN IN SOUTHERN ETHIOPIA

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ABSTRACT

Scope and Method of Study: The purpose of this study was to assess the vitamin A status of lactating women in Wondo Genet and Arsi Negele, two villages located in southern Ethiopia. A total of 108 women participated in this cross-sectional investigation. Anthropometric measurements were taken, blood samples and breast milk were analyzed for vitamin A content, dietary habits and socioeconomic data were obtained, and dark adaptation testing was performed. Dark adaptation threshold was measured in log candela/m^2 (log cd/m^2), the SI unit of luminance. Pearson correlation coefficients were calculated for all appropriate variables. Significance was set at $p < 0.05$.

Findings and Conclusions: The population studied had a mean age of 25 ± 0.4 years. Mean body mass index (BMI) was 20.8 ± 0.3 kg/m^2. BMI was highly correlated with serum retinol concentrations for both HPLC ($p = 0.0038$) and fluorometry ($p =$

0.0084). Milk fat concentration was highly associated with BMI, although this association was not significant ($p = 0.052$). The mean serum retinol level in participants as determined by HPLC and fluorometry was 1.49 ± 0.04 and 1.68 ± 0.04 μmol/L, respectively. Results from analysis of serum retinol concentrations by HPLC and fluorometry were highly correlated ($r = 0.63$, $p < 0.0001$). Participants were grouped into four categories based on their responses to frequency of consumption of vitamin A source foods (VASF). Based on these groups, 11% ($n = 12$) of the women reported eating any VASF only occasionally. While no significant relation was found between pupillary threshold and serum retinol concentrations, a trend was seen in the expected direction. A trend was also seen between pupillary threshold scores and breast milk vitamin A concentrations. In the future, clear guidelines need to be established for interpretation of such data. The results of this study suggest that the CRAFTi portable fluorometer may be used in the future to analyze serum retinol concentrations in settings where analysis by HPLC is not feasible.

INTRODUCTION

Vitamin A deficiency (VAD) has long been recognized as a major public health problem. The United Nations Children's Fund (UNICEF) estimates that between 250,000 to 500,000 children are blinded from VAD each year (1); many of these children die each year from vitamin A deficiency related diseases. Participants in conferences such as The World Summit for Children, the Policy Conference on Ending Hidden Hunger, and the International Conference on Nutrition all agreed upon the international goal of eliminating VAD and all its consequences by the year 2000 (2). This goal has yet

to be achieved, and so VAD remains an important focus area for research and intervention.

While VAD is very common in children, other high-risk groups include pregnant and lactating women (2). The nutritional status of the mother may be reflected in her child; thus, pregnant, lactating, and other fertile women are important groups in which research and interventions regarding VAD should be targeted.

Night blindness is the first clinical manifestation of VAD that potentially can be measured (2). Therefore, developing reliable field instruments to measure night blindness accurately has been the focus of much research (3-10). Studies conducted in Ethiopia have shown a high prevalence of VAD (11,12). However, to our knowledge, there have been no studies conducted in Ethiopia that test pupillary response in lactating women as a functional indicator of vitamin A status. The purpose of this study was to determine the vitamin A status of lactating women in two villages in southern Ethiopia. Pupillary response was measured using the Scotopic Sensitivity Threshold instrument (SST-1, LKC Technologies, Inc., Gaithersburg, MD). Serum retinol and breast milk vitamin A concentrations were analyzed, and frequency of consumption of vitamin A source foods was collected from questionnaires. This paper reports the methodology and results obtained from this study.

MATERIALS AND METHODS

Study design/sample size: This study was part of a cross-sectional investigation of the nutritional status of lactating women in two rural villages in Southern Ethiopia conducted in January, 2006. A convenience sample of 108 women in Wondo Genet and

Arsi Negele was utilized. A convenience sample was used, as it would not have been culturally sensitive to create a random sample and expect cooperation from members of the community.

Sampling method: Contact was made with local health workers in each of the two villages, who scheduled a community meeting. Lactating women and their husbands were encouraged to attend. Study procedures were explained in the local language, as well as the level of involvement required, possible risks associated with participation, and the incentives to be awarded at the conclusion of the study. The women and their husbands were encouraged to ask any questions and voice any concerns they might have. Informed consent was obtained from the women who agreed to participate in the research. A total of 56 women from Wondo Genet and 52 women from Arsi Negele participated in the study. Approval from the Institutional Review Board at Oklahoma State University and the Ethics Committee at Hawassa University was obtained prior to beginning the research.

Questionnaire: Questions for this study were divided into four major sections: household and background characteristics, health, nutritional status, and other reproductive behaviors, household food production and consumption patterns, and knowledge of vitamin A deficiency. Trained research assistants and department faculty from Hawassa University translated the questionnaire from Amharic into Sidaminga and Orominga, the local languages spoken in Wondo Genet and Arsi Negele, respectively.

Anthropometry: Weight, height, and mid-upper arm circumference measurements of all study participants were obtained. Height was measured to the nearest 0.1 cm using a portable wooden stadiometer (Shorr Productions, Olney, MD) and repeated if the first

two measurements were more than 0.5 cm apart. Weight was measured in kilograms to the nearest 0.1 kg using a calibrated digital solar-powered scale (Uniscale, UNICEF, Copenhagen) and repeated if the values were more than 0.1 kg apart. Mid upper arm circumference was measured to the nearest 0.1 cm using a flexible measuring tape; measurements were repeated if the values were more than 0.1 cm apart.

Sample collection: Blood samples were drawn from all but one of the volunteers who was excluded due to illness. A medical technologist obtained the blood samples using standard venipuncture protocols. Blood samples were drawn in an open-air gazebo in Wondo Genet and inside the health post at Arsi Negele. The sample racks were covered with aluminum foil and placed on ice packs in an insulated cooler. At the end of each day, samples were taken back to the laboratory at Hawassa University. Samples were centrifuged to obtain serum, which was then collected and frozen at -20°C until further use. Breast milk samples were also collected by each participant. The participants were each given a plastic container, which they used to express ~5 milliliters of breast milk. Neither the time of day for sample collection nor the time since last feed were controlled. After collection, the containers of breast milk were put on ice packs in an insulated cooler. At the end of each day, the samples were transferred to a freezer at Hawassa University where they were frozen at -20°C for future analyses.

Pupillary response testing: Dark adaptation was tested using a modified technique developed by Congdon et al. (5). The Scotopic Sensitivity Tester-1 (SST-1), a portable field instrument, was used to test participants' pupillary response to various intensities of light. This test requires a very dark room shielded from any outside light, which can pose a problem for field research. A portable darkroom was built from thick black canvas and

metal poles. This dark room was assembled at each location for use in testing dark adaptation. Thick blankets were pinned along the bottom of the tent to prevent light from entering.

Groups of four to six participants were allowed to dark adapt together in order to manage time and potential apprehension efficiently. Prior to dark adaptation, each participant in the group was subjected to binocular partial bleaching with a digital camera flash (Sony Cyber-shot DSC-W5). An aluminum foil covered-cone was used during bleaching to ensure that the entire back of the eye was bleached.

Participants were allowed to dark adapt for 20 minutes. Three researchers worked as a team in the dark room. One acted as a translator and helped the participants feel at ease. One presented the stimulus to each participant by holding the handheld stimulator very near the participant's left eye. The other researcher watched for the pupil in the right eye to contract, which is the pupillary response. This observer looked through a night vision scope (ELF-1, LOMO America Inc., Northbrook, IL) to better see the contraction while in the darkroom. The stimulus was first presented at the lowest intensity (0 dB). The intensity was increased by increments of 5 until the observer could see a contraction of the pupil. Once this occurred, the intensity was decreased by increments of 1 dB until the observer could no longer see a contraction of the pupil. The resulting value was recorded as the participant's final dark-adapted threshold. If a contraction was not seen by 30 dB (the highest intensity of light available on the instrument) then the participant's threshold was recorded as greater than 30 dB.

Dietary assessment: Vitamin A consumption was assessed based on participants' answers from the Household Food Production and Consumption Patterns section of the

questionnaire. The question asked, “How often do you eat the following food items?”. The nine vitamin A source foods listed were liver, eggs, butter, mango, papaya, sweet potato, pumpkin, carrot, and kale. Participants answered: daily, several times per week, weekly, occasionally, or never. To analyze consumption of vitamin A source foods, responses were grouped into four categories: consumption of at least two vitamin A source foods daily, consumption of one vitamin A source food daily, consumption of one vitamin A source food at least weekly, and consumption of one vitamin A source food only occasionally.

Analysis of serum: Serum vitamin A was analyzed by use of high performance liquid chromatography (HPLC). Samples were transported to Craft Technologies in Wilson, North Carolina, where they were analyzed for serum vitamin A concentrations, retinol-binding protein, and alpha 1-acid glycoprotein (AGP). Serum vitamin A concentrations were also measured in Ethiopia by fluorescence spectroscopy using the CRAFTi Portable Fluorometer (Craft Technologies, Inc., Wilson, NC), which measures retinol bound to retinol binding protein (RBP).

Analysis of breast milk: Breast milk vitamin A concentrations were analyzed by HPLC (4.6 x 150 mm, 5 um Waters C-18 Reversed Phase Analytical column). The eluting solvent was 89:11 methanol and water at a flow rate of 1.0 ml/min. The extraction efficiency was obtained by dividing the integrator area obtained for the dehydroretinol (DR) by the area obtained with the internal standard 3,4-didehydroretinyl acetate (DRA). Final values were corrected by dividing the vitamin A value obtained from the peak areas by the extraction efficiency.

Statistics: Data were compiled into a spreadsheet and analyzed using the PC Statistical Analysis System (SAS) for Windows (Version 9.1 SAS, Inst. Inc., Cary N.C.). Means, standard errors of the mean, ranges and frequency distributions of variables were determined. Pearson correlation coefficients were calculated for all appropriate variables. Significance was set at $p < 0.05$.

Dark adaptation threshold was measured in log candela/m^2 (log cd/m^2), the SI unit of luminance. Participants with better dark adaptation could see lower levels of light, and thus had a smaller luminance value.

RESULTS

A total of 108 women were recruited for this study. One woman was sick and withdrew from the study. Blood samples from three other women were unable to be analyzed. Thus, a total of 104 blood samples were analyzed for vitamin A concentrations. Each of the 108 women participated in the dark adaptation testing. However, due to technical problems in the field concerning the dark room on the first day of testing, scores from three participants have been excluded from analysis. Dark adaptation values from 105 participants were used in the final analysis.

The participants' mean age was 25 ± 0.4 years, and their mean body mass index (BMI) was 20.8 ± 0.3 kg/m^2 (Table 1). The mean age of the last child was 12 months $\pm$ 0.6. A total of 21 women (19.6%) were underweight (BMI < 18.5), while 10 women (9%) were overweight (BMI $\geq$ 25). A majority of the women (68% n = 73) had never been to school or received formal education. Land ownership was quite high, with 70%

(n = 75) of the women reporting that their family owned some amount of land. However, household electricity was available in only 29% (n = 31) of the homes. Knowledge of vitamin A was also quite low. Only 23% (n = 24) of the women answered "yes" to the question, "Have you ever heard about vitamin A?". Forty percent (n = 42) of the women said that they have trouble seeing in the dark.

The mean serum retinol level in participants as determined by HPLC and fluorometry was 1.49 ± 0.04 and 1.68 ± 0.04 μmol/L, respectively (Table 2). There were 16 women (15%) with serum retinol concentrations < 1.05 μmol/L when analyzed by HPLC; however, only 6 women (6%) had serum retinol concentrations < 1.05 μmol/L when analyzed by fluorometry (Figures 1 & 2). Results from analysis of serum retinol concentrations by HPLC and fluorometry were highly correlated ($r = 0.63$, $p < 0.0001$) (Figure 3). Retinol binding protein (RBP) was significantly associated with serum retinol concentrations ($p < 0.0001$ for both HPLC and fluorometry; $r = 0.942$ for HPLC, $r = 0.521$ for fluorometry). Body mass index (BMI) was significantly correlated with serum retinol concentrations for both HPLC ($p = 0.0038$) and fluorometry ($p = 0.0084$).

Alpha 1-acid glycoprotein (AGP), a marker of infection, was measured. A cut-off of ≥ 1.0 g/L was used. The mean (± SEM) level of AGP was 0.84 ± 0.05; twenty six percent of participants had AGP levels of ≥ 1.0 g/L.

The mean (± SEM) concentration of serum retinol in the breast milk was 9.10 μg/g milk fat ± 1.53 (Table 3). Forty one percent of participants had breast milk vitamin A levels < 8 μg/g milk fat. Values for milk retinol concentrations are expressed as a ratio to the fat concentration of the milk. The mean (± SEM) concentration of milk fat was 6.01

g/dL ± 0.46. Milk fat concentration was highly associated with body mass index, although this association was not significant (p = 0.052).

The median pupillary threshold was 18 dB, which corresponds to a value of –3.56 log cd/m^2. No significant associations were observed between pupillary threshold values and serum retinol concentrations. However, a negative association was seen between pupillary threshold values and milk vitamin A concentrations (µmol/l) (p = 0.0724).

Participants were grouped into four categories based on their responses to frequency of consumption of vitamin A source foods. Based on these groups, 11% (n = 12) of the women reported eating any vitamin A source food only occasionally, 15% (n = 16) reported eating any vitamin A source food at least once weekly, 53% (n = 56) reported eating any vitamin A source food once daily, and 21% (n = 22) reported consuming any vitamin A source food at least twice daily.

DISCUSSION

The purpose of this study was to assess the vitamin A status of lactating women in southern Ethiopia. Overall, our serum retinol concentrations did not show evidence of serious vitamin A deficiency. None of the women had serum retinol levels < 0.70 µmol/L, which is the cutoff level used in many studies to define low vitamin A status (5-9). However, 15% (n = 16) of participants had serum retinol concentrations < 1.05 µmol/L, which is a cut-off level used to define mildly deficient vitamin A status (2,9). The World Health Organization has recommended using < 1.05 µmol/L as a cutoff point

to identify the proportion of the population that may be at an increased risk for vitamin A deficiency (2).

It is widely accepted that serum retinol concentrations do not provide an accurate assessment of a population's vitamin A status when measured alone (2,13). Serum retinol levels are tightly regulated by the liver and are not reflective of body stores unless they are very low or very high (2,13). There are many possible confounding variables that may also affect serum retinol concentrations, such as low iron levels, low zinc levels, and the presence of infection. In this study, AGP was used as a marker of infection. While 26% of participants had AGP levels $\geq$ 1.0 g/L, exclusion of these subjects did not significantly change serum retinol, breast milk vitamin A concentrations, or pupillary threshold scores.

Dark adaptation appears to be able to detect functional levels of low vitamin A status (14), and has been used in many studies to identify individuals and populations with vitamin A deficiency (5-9). Studies have shown significant correlations between poor dark adaptation and low serum retinol concentrations (4-5,8-9), although these findings are not entirely consistent (5,7).

In the present study, no significant association was found between serum retinol levels as measured by either HPLC or fluorometry and pupillary threshold level. However, a trend in the expected direction was seen. A negative association was also seen between milk vitamin A and pupillary threshold scores, although this was not significant ($p = 0.0724$).

Because of the relation between fat content vitamin A content in breast milk, breast milk vitamin A results were expressed relative to the fat content present. The

World Health Organization recommends using < 8 μg/g milk fat to identify low levels of vitamin A (2). Forty one percent of participants in this study had low milk vitamin A concentrations of < 8 μg/g milk fat.

Frequency of consumption of vitamin A source foods was used as an indicator for adequacy of vitamin A in the diet. The highest level of consumption by participants was at least two vitamin A source foods each day. The World Health Organization has suggested that consumption of three or more vitamin A rich foods per week among at-risk populations along with sufficient staples, fats, and some animal source foods should be considered adequate (2). A total of 28 (26%) participants had consumption levels of vitamin A source foods lower than this recommended amount, and thus should be considered at a higher risk for inadequate vitamin A status. Women who consumed two vitamin A source foods weekly had lower (and therefore better) dark adaptation scores than did women who consumed vitamin A source foods only occasionally, although this difference was not significant.

ACKNOWLEDGEMENTS

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Table 1
Anthropometric Characteristics of Lactating Women in Southern Ethiopia[1]:

Characteristic	Mean[2]	Range
Age (y)	25 ± 0.4	16-35
Height (cm)	158.1 ± 0.6	143.0-186.7
Weight (kg)	51.9 ± 0.7	40.4-76.3
Body Mass Index (kg/m^2)	20.8 ± 0.3	15.0-30.0
MUAC[3] (cm)	26.0 ± 0.3	20.6-38.9

[1] n = 104, except for MUAC (n = 102)
[2] Mean ± SEM
[3] Mid-upper arm circumference

Table 2
Vitamin A Related Blood Parameters of Lactating Women in Southern Ethiopia[1]:

	Mean[2]	Range
RBP[3] (µmol/L)	1.29 ± 0.04	0.67-3.44
AGP[4] (g/L)	0.84 ± 0.05	0.31-3.40
Serum Vitamin A[5] (µmol/L)	1.68 ± 0.04	1.05-2.83
Serum Vitamin A[6] (µmol/L)	1.49 ± 0.04	0.79-3.32

[1] n = 100-102
[2] Mean ± SEM
[3] Retinol Binding Protein
[4] Alpha 1-acid glycoprotein
[5] Vitamin A measured by fluorometry
[6] Vitamin A measured by HPLC

Table 3
Vitamin A Related Breast Milk Parameters of Lactating Women in Southern Ethiopia[1]:

	Mean[2]	Range
Milk Fat (g/dL)	6.01 ± 0.46	0.26-21.14
Vitamin A (µg retinol/g milk fat)	9.10 ± 1.53	1.01-58.58
Vitamin A (µmol/l)	1.19 ± 0.13	0.18-6.14

[1] n = 58-83
[2] Mean ± SEM

Figure 1
Distribution of Participants' Serum Vitamin A as Determined by HPLC

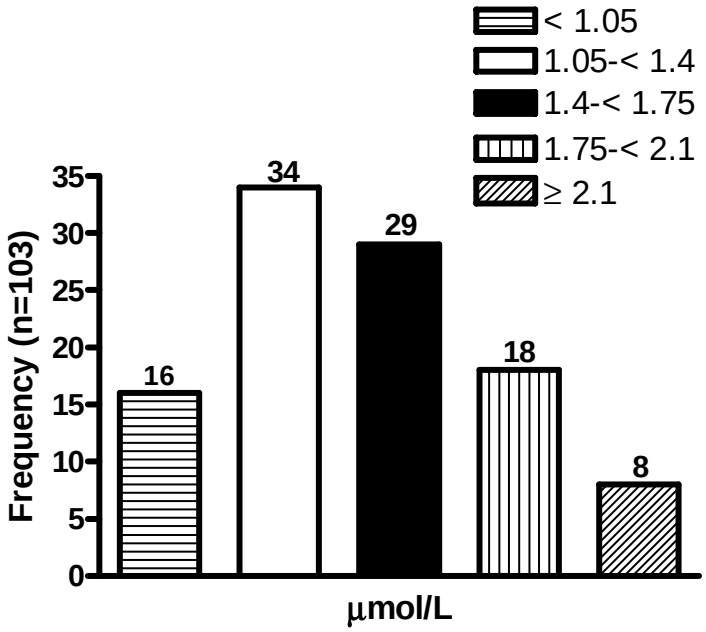


Figure 2
Distribution of Participants' Serum Vitamin A as Determined by Fluorometry

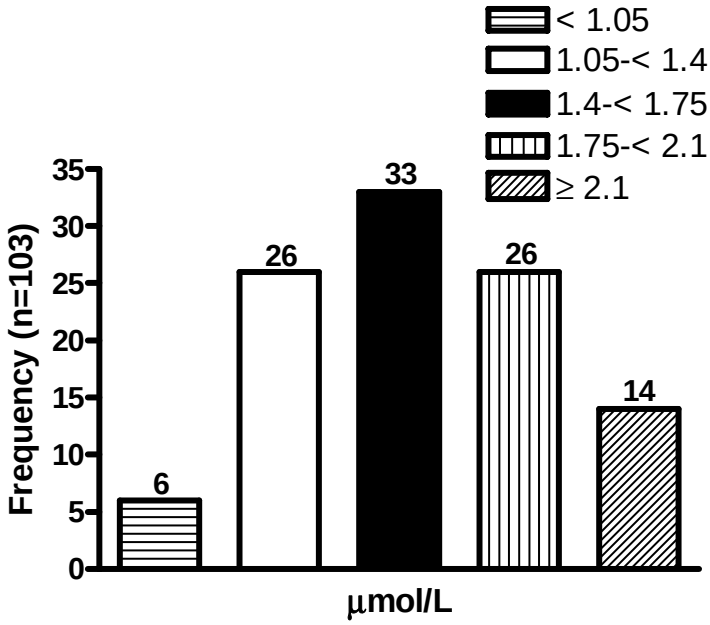


Figure 3
Comparison of Vitamin A Values from Fluorometric and HPLC Analyses

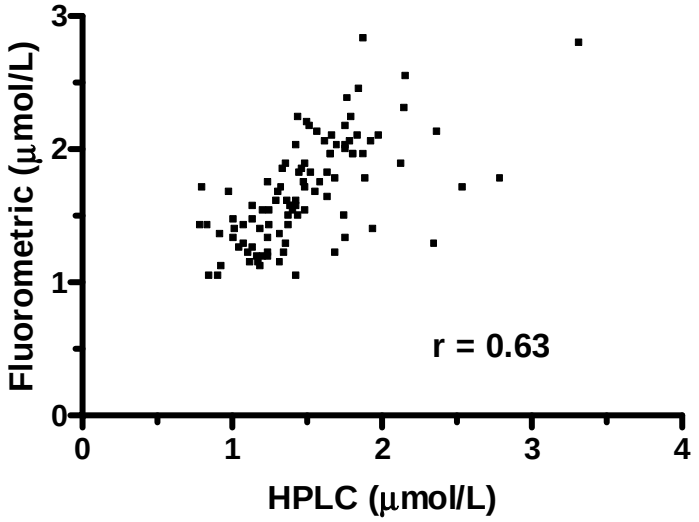


Figure 4
Comparison of Dark Adaptation and Serum HPLC Values

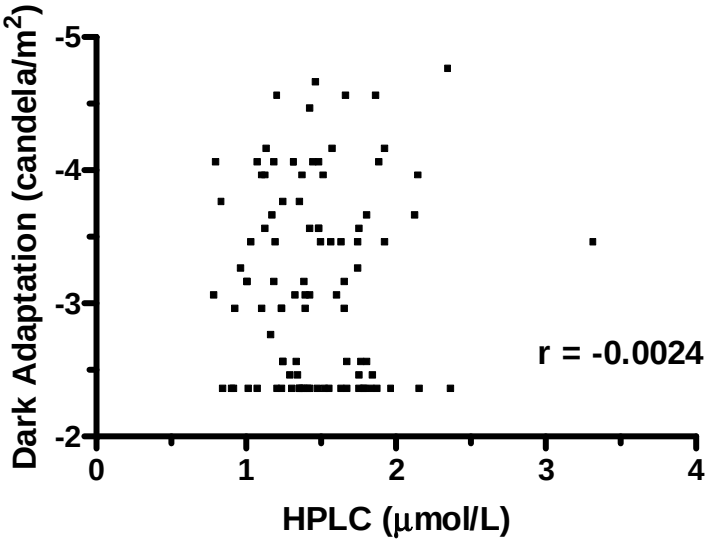
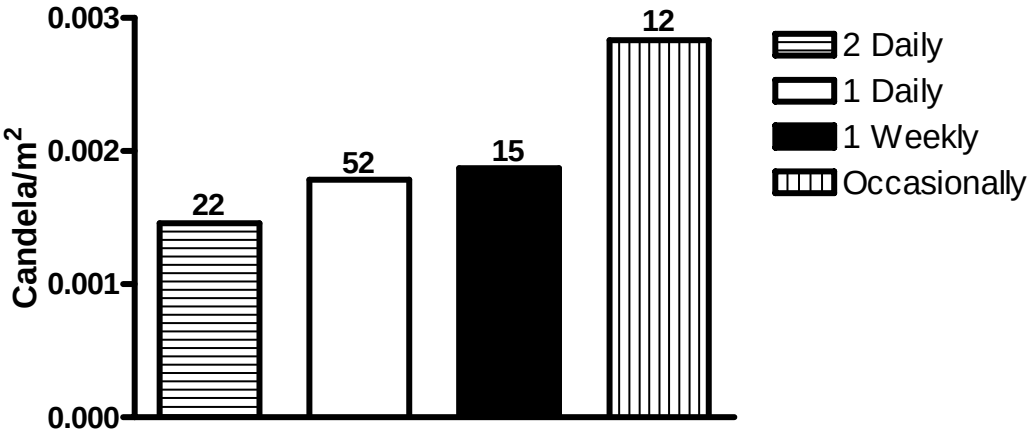


Figure 5
Frequency of Consumption of Vitamin A Containing Foods by Dark Adaptation Score (n = 101)



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CHAPTER V

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

SUMMARY OF FINDINGS

The purpose of this study was to assess the vitamin A status of lactating women in Wondo Genet and Arsi Negele, two villages located in southern Ethiopia. This study evaluated vitamin A status based on ability to dark adapt, serum retinol concentrations, and breast milk vitamin A concentrations.

The objectives of this study included:

1. To assess the vitamin A status of lactating women through serum retinol measurements, breast milk vitamin A concentrations, and dark adaptation threshold.
2. To determine the efficacy of measuring impaired dark adaptation, a functional test of vitamin A status, with the Scotopic Sensitivity Tester-1 (SST-1) instrument as compared with serum retinol concentrations.
3. To determine the efficacy of the CRAFTi portable fluorometer, a new field instrument for measuring serum vitamin A concentrations, as compared with serum vitamin A concentrations measured by high-performance liquid chromatography (HPLC).

NULL HYPOTHESIS

Null Hypothesis One Stated: There will be no relation between serum retinol, breast milk vitamin A concentrations, and dark adaptation thresholds. There was a relation seen between serum retinol concentrations and dark adaptation scores, although this relation was not significant. No other relations were seen between these variables. Therefore, the researcher fails to reject Null Hypothesis One.

Null Hypothesis Two Stated: There will be no relation between serum retinol and dark adaptation thresholds. After analyzing the data, a significant difference was not seen between serum retinol concentrations and dark adaptation thresholds. Therefore, the researcher fails to reject Null Hypothesis Two.

Null Hypothesis Three Stated: There will be no relation between the CRAFTi portable fluorometer and HPLC serum vitamin A concentrations. A significant relation was seen between serum retinol concentrations as measured by the CRAFTi portable fluorometer and HPLC analyses. Therefore, the researcher rejects Null Hypothesis Three.

The results of this study suggest that the CRAFTi portable fluorometer may provide useful measurements of serum retinol concentrations as compared with traditional analysis by HPLC.

CONCLUSIONS

Vitamin A deficiency remains a global health problem that affects millions of vulnerable people worldwide and is the leading cause of preventable childhood death (WHO, 1996). The international goal of eliminating vitamin A deficiency by the year 2000 has not yet been reached. It is therefore important to target nutrition education and supplementation programs towards children as well as women of childbearing age. When assessing a population's vitamin A status, many different indicators need to be used, including biochemical, functional, and dietary. These indicators by themselves are not truly representative of an individual's or population's vitamin A status. However, the reliability of results from a study is enhanced when several indicators are used in tandem.

While the present study did not find significant correlations between pupillary thresholds and serum retinol concentrations, it should be noted that a trend in the expected direction was seen. Further refinement of the methodology used to assess dark adaptation may lead to a better correlation between the two. It is also worth noting that there are no established guidelines for interpretation of dark adaptation values. Many of the published studies have used children or pregnant women as subjects. Reference values for different age categories and high risk population groups need to be established before results can be interpreted with confidence.

A significant relation was seen between serum retinol concentrations as measured by HPLC and the CRAFTi fluorometer. These results are important findings, in that the CRAFTi fluorometer is a relatively inexpensive and portable instrument that could be used in field settings. Further testing of this instrument needs to be done before

widespread use can be recommended; however, in the future, use of this instrument may assist in the analysis of serum retinol concentrations in places that would otherwise lack the necessary resources to do so.

RECOMMENDATIONS

It should be noted that training and practicing to perform the dark adaptation testing is very time-consuming. Adequate time must be allocated for this before executing the research. Creating a portable tent that can be used for dark adaptation can also be difficult. Logistics, including both the creation and transportation of the tent, need to be carefully thought out prior to the research. It should also be noted that it becomes very hot inside the tent. Use of battery operated fans can help make the dark adaptation testing more comfortable for both the researchers and the participants.

Pretesting of a questionnaire that is used would be helpful in order to ensure that all questions have been worded appropriately and are culturally sensitive. Including more specific questions related to difficulty seeing in the dark would also be helpful, especially in villages with no local term for night blindness.

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APPENDICES

APPENDIX A

QUESTIONNAIRE (ENGLISH)

Hawassa University
Awassa College of Agriculture
Department of Rural Development and Family Sciences

Questionnaire

Name of farmers association_____
Zone _____ Region _____ District_____ Subjects code_____
Date of interview_____. Interviewers name _____
Time the interview-started _____. Time the interview ended_____

I. Household and background characteristics.

	Questions	***Coding***	**Skip.**
01.	Interviewer: fill out the list of all persons who usually live in this household	Male children - - - - ☐☐ Female children- - - ☐☐ Guests ☐☐ Relatives ☐☐ Parents ☐☐ Others ☐☐ Total ☐☐☐	
02.	What is the major source of drinking water for members of your household?	Piped into residence---------- 1 Public tap ----------.- 2 Table well ----------.-- 3 Unprotected well ----------4 River canal ----------5 Spring water ----------. 6 Rain water ----------7 Public tap & spring----------8 Spring & Table well---------- 9	
03.	Do you usually do something to purify the water?	Yes ----------1 No ----------2	

04.	Does the household have any of the following? Electricity Radio Bicycle Sawing machine Cart (Bullock) Kerosene lamp-with glass cover Kerosene lamp-with no glass cover	**Yes** **NO** 1 2 1 2 1 2 1 2 1 2 1 2 1 2	
05	Does the household own agricultural land?	Yes ----------1 No ----------2	
06	How much land does the household owns? In square meter.	<1250m^2 ----------1 1250m^2 ----------2 2500m^2 ----------3 5000m^2 ----------4 >5000m^2 ----------5 Don't know----------6	
07	Does this household own animals? Chicken Oxen Cow Goat Donkey Sheep Horse/mule	Yes No Num 1 2 1 2 1 2 1 2 1 2 1 2 1 2	
08	How old are you?	_____Years old	
09	Have you ever been to school? * If the answer for the question is yes, then ask No. 10	Yes No 1 2	

10	What is the highest grade or level of school that you have completed?	Did not go to school----------0 Elementary school, grade ---------1 Junior school, grade----------2 High school, grade ----------3 Other ----------4	
11	In what language can you read?	Can't read----------0 Amaharic----------1 Sidaminya----------2 Oromic----------3	
12	Can you read this sentence? (What is the name of your child?	Yes No 1 2	
13	How often do you listen to radio?	Almost everyday ----------1 At least once a fortnight----------2 Less than once a week ---------- 3 Not at all ----------4	
14	What is your religion?	Orthodox Christian----------1 Catholic ----------2 Protestant ----------3 Islam ----------4 Others ----------5	
15	What is your current marital status?	Divorced ----------1 Separated----------2 Widowed----------3 Currently married----------4	
16	Does your husband or partner have any other wives beside yourself?	Yes ---------- 1 No ----------2 Divorced/ widowed /separated----3	

17	How many other wives does he have?	☐ None---------- 0 Divorced/ widowed /separated----1	
18	Did your husband (last) ever attend formal school?	Yes ----------1 No ----------2 Divorced/ widowed /separated----3	
19	If the answer for No. 18 is yes, What is (was) the highest grade he completed?	Did not go to school----------0 Elementary (1-6) ----------1 Junior Secondary (7-8)----------2 Secondary (9-12)----------3 College diploma ----------4 College degree ----------5 Others (specify) ----------6 Divorced/ widowed /separated----7	
20	What is (was) his occupation, that is, what kind of work does he usually do?	Does not do any kind of work----0 Self employed ----------1 Civil servant ----------2 Farmer ----------3 Petty trader ----------4 Other (specify) ----------5 Divorced/ widowed /separated----6	
21	Aside from housework, have you done any income-earning jobs in the last 12 months?	Yes ----------1 No ----------2	
22	If the answer for no. 21 is yes, what?	Do not do any kind of work-------0 Petty trading----------1 Employed work----------2 Laborer----------3	

		Selling fire wood----------4 Other----------5	
23	Are you usually involved in decision-making concerning the following issues? Food Expenditure Number of children Social	Yes No 1 2 1 2 1 2 1 2	

II. Health, nutritional status and other reproductive behaviors

	Questions	***Coding***	***Skip***
01	Have you ever had a death of a child? Pregnancy death or other?	Yes No 1 2	
02	Are you pregnant now?	Yes ---------- 1 No ----------2	
03	How many months pregnant are you now?	☐ None----------0 Don't know----------1	
04	During your last pregnancy, did you stop eating specific types of food that you normally eat, for cultural reasons?	Yes ----------1 No ----------2	
05	What did you stop eating? Milk & milk product Butter Any kind of meat	Yes No Not Allowed 1 2 3 1 2 3 1 2 3	

	Any kind of vegetable Any kind of fruit Porridge Sweet potato	1 2 3 1 2 3 1 2 3 1 2 3	
06	Has any one told you what is best to feed your baby?	Yes No 1 2	
07	Did you receive antenatal care?	Yes ---------- 1 No ----------2	
08	What type of antenatal care did you receive?	None----------0 Tablets----------1 Vaccination----------2 Both----------3 Vaccination & examination-----4 Don't know----------5	
09	Who helped you with delivery?	Health professional ----------1 Trained Traditional birth attendant ----------2 Untrained Traditional birth attendant ----------3 No one----------4 Others----------5	
10	During your last pregnancy, were you given any iron, folic acid, tablets, or syrup?	Yes----------1 No----------2	
11	Have you ever received a Vitamin A supplement from health professionals? Or a health facility?	Yes ---------- 1 No ----------2	

	(Show capsule)		
12	Has your last baby received any immunization? (Ask for the card)	Yes ----------1 No----------2	
13	What are neonates/ new born babies in the household given immediately after birth to eat?	Breast milk----------1 Water----------2 Boiled herb----------3 Butter----------4 Water with sugar----------5 Other----------6 Don't know----------7	
14	How many months have you breast-fed your last child?	Months	
15	When did you start to breast feed your last baby?	1. Immediately after birth 2. After one hour of birth 3. After three hours of birth 4. with in one day 5.more than one day _____	
16	How many times per day do you breast feed your child?	No more breast feeding ---------0 Three times----------1 Four times----------2 Five times----------3 Six times----------4 Not fixed----------5	

17	Do you breast feed your child even when you are ill?	Yes ---------- 1 No ----------2	
18	Do you give Colostrum to your newborn baby?	Yes ----------1 No ----------2	
19	Do you bottle feed your child?	Yes ---------- 1 No ----------2	
20	During the **last three months**, have you and your family ever had any of the following health problems? ➢ Malaria ➢ Cough ➢ Diarrhea ➢ Cold	**Yes** **No** 1 2 1 2 1 2 1 2	
21	Which (if any) disease has frequently affected your child during the last three years? Diarrhea Malaria Fever Measles Cold Others	**Yes** **No** 1 2 1 2 1 2 1 2 1 2 1 2	
22	Anthropometrical measurement of the mother ❖ Height in centimeter ❖ Weight in KILO GRAM Mid Upper Arm circumference	**Measurment** **measurement** _____ _____ _____ _____ _____ _____	

23	Anthropometrical measurement of the child ❖ Age in Months ❖ Height in centimeter ❖ Weight in KILO GRAM ❖ Mid Upper Arm circumference ❖ Head circumference in cm	**Measurement measurement** _____ _____ _____ _____ _____ _____ _____ _____	

III. Household food production and consumption patterns

	Questions	***Coding***	***Skip***
01	Do you produce the following food items? 01.Cereals 02.Vegetables 03.Fruits 04.Tubers 05.Legumes 06.Cash crops such as coffee, sugar cane e.t.c	**Yes** **No** 1 2 1 2 1 2 1 2 1 2 1 2	
02	Do you usually purchase the following food items? 01.Cereals 02.Vegetables 03.Fruits 04.Tubers 05.Legumes 06.Cash crops 07.Others	**Yes** **No** 1 2 1 2 1 2 1 2 1 2 1 2	
03	For what purpose do you use your produces? 01.Cereals 02.Vegetables 03.Fruits 04.Tubers 05.Legumes 06.Cash crops 07.Chicken	Consume Income Both Do not produce 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4	

	08. Egg 09. Milk and milk products 10. Sheep and goat 11.Others Dot-do not produce any thing	1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4	
04	From where do you get milk?	Buy----------1 Gift----------2 Owen animals----------3 Other----------4 Don't have milk----------5	
05	Do you give special food to pregnant woman other than the usual food?	Yes No 1 2	
06	Do you give special food to lactating woman other than the usual food?	Yes No 1 2	
07	Do you separately prepare food to children under three?	Yes No 1 2 The child has not started complementary feeding----------3	
08	How often do you give the listed food items to your child? Daily (1) Several times per-week (2) Weekly (3) Occasionally (4) Never (5)	01 .Breast milk 1 2 3 4 5 02 .Liver 1 2 3 4 5 03 .Eggs 1 2 3 4 5 04 .butter 1 2 3 4 5 05. mango 1 2 3 4 5 06 . papaya 1 2 3 4 5 07. sweet potato 1 2 3 4 5 08 .Pumpkin 1 2 3 4 5 09 Carrot 1 2 3 4 5 10 Spinach/kale 1 2 3 4 5	

		11.Others 1 2 3 4 5	
09	How often do you eat the following food items? Daily (1) Several times per-week (2) Weekly (3) Occasionally (4) Never (5)	01.Liver 1 2 3 4 5 02.Eggs 1 2 3 4 5 03.butter 1 2 3 4 5 04. mango 1 2 3 4 5 05 papaya 1 2 3 4 5 06. sweet potato 1 2 3 4 5 07.Pumpkin 1 2 3 4 5 08.Carrot 1 2 3 4 5 09.Spinach/kale 1 2 3 4 5 10 Others 1 2 3 4 5	
10	Do you use oil or butter when preparing food for your child?	None----------0 Butter----------1 Oil----------2 Both----------3 The child has not started eating Complementary food----------4	
11	Do you have trouble seeing in the dark?	Yes ---------- 1 No ----------2	

IV. Vitamin A Supplementation

01	Did your last child ever receive vitamin A supplementation?	Yes ---------- 1 No ----------2	
01	How many times did the child receive vitamin A?	Not received any supplementation----------0 Once----------1 2-3 times----------2 4-6 times----------3 More than 6 times----------4 Do not know----------5	
03	If this child did not receive vitamin A, what are the reasons? Please describe	The child didn't need vitamin A----------1 I did not know that it was being given------2 The clinic didn't have enough vitamin A---3	

		The child was some place else during supplementation ----------4 The child was sick----------5 The clinic was far----------6 I didn't think the child would face any problems if he didn't take vitamin A----------7 Health workers did not give---------- 8 The child received VA----------9	
04	How many months ago did the child take the last dose of vitamin A?	Did not receive VA----------0 < One month----------1 1 month ago ----------2 2 month ago----------3 3 month ago ----------4 > 3 months ago ----------5 Don't remember ----------6	
05	Have you observed any benefit from the vitamin A supplementation?	Did not receive VA----------0 Yes ---------- 1 No ----------2 I do not know----------3	
06	Do you intend to take this child again to get a vitamin A supplementation?	Have not taken any VA----------0 Yes ---------- 1 No ----------2	
Knowledge of Vitamin A deficiency			
07	Have you heard about vitamin A before?	Yes ---------- 1 No ----------2	

Vitamin D related questions

01	Do you work outside in the farm field during the day?	Yes ---------- 1 No ----------2	
02	Do you usually cover your child when you go outside?	Never----------0 Yes ---------- 1 No ----------2	
03	What time of the year do you work outside in the field or	Planting season----------1 Harvesting season----------2	

	garden?	At any time ----------3 Other----------4	
04	Do you try to protect yourself from the sun when you are outside during the day?	Most of the time----------1 Some time ----------2 Rarely ----------3 Never ----------4	
05	How do you protect yourself from the sun?	Umbrella----------1 Clothes covering----------2 Waking in shade----------3 Avoiding mid day----------4 More than one----------5 Don't protect----------6	
06	What type of shoe do you use to cover your foot? (to be observed)?	Bare foot----------1 Thongs ----------2 Cloth shoe----------3 Plastic shoe ----------4 Leather shoe ----------5	
07	Do you have an umbrella?	Yes No 1 2	

Instruction Two

The table below represents the diet diversity of the mother in the past three days. Please ask the mother how many times she has eaten of each food items in the past three days. If she has not eaten the food item in the past three days then put a check on the zero box. If she has only eaten the food item once in the past three days then put a check on the once box. If she has eaten the food item two or more times in the past three days then put a check on the greater than once box. The small portion box is available for detailed descriptions of the portion size of the food item.

Small portions Yes-1

No-2

Nothing – 0

Food Item	Zero=0	Once=1	>Once=2	Small Portion
Grains (Barley, corn, sorghum, wheat)				
Roots/Tubers (Plants with edible roots and stems) Ex. Carrot, potato, kocho.				
Dairy				
Meat				
Egg				
Vitamin A Rich Fruit/Vegetable (Kale, sweet potatoes, carrot)				
Other Fruit/Vegetable				
Legumes/Nuts (Chickpeas, lentil, beans, peas, peanuts)				
Fats & Oils (Butter, nut oil, vegetable oil)				

APPENDIX B

QUESTIONNAIRE (AMHARIC)

ደቡብ ዩኒቨርሲቲ
አዋሳ ግብርና ኮሌጅ
የገጠር ልማትና የቤተሰብ ሳይንስ ትምህርት ክፍል
መጠይቅ

የተጠያቂው ስም_____
የመለያ ቁጥር_____
የቀበሌው ስም_____
ዞን_____ **ክልል**_____ **ወረዳ**_____ **መለያ ቁጥር**_____
ቃለመጠይቁ የተካሄደበት ቀን_____ **የቃለመጠይቅ አድራጊው ስም**_____
መጠይቁ የተጀመረበት ሰዓት_____ **መጠይቁ የተጠናቀቀበት ሰዓት**_____

I. አጠቃላይ የቤተሰቡ ሁኔታ

ተ.ቁ	ጥያቄዎች	መለያ	ይዝለሉ
01	ጠያቂው፡ እባክዎ እዚህ ቤት ውስጥ የሚኖሩትን ሰዎች ዝርዝር ይመዝግቡ	ወንድ ልጆች ሴት ልጆች እንግዶች ዘመዶች ወላጆች ሌሎች ድምር	
02	ቤተሰቡ የሚጠቀመው የመጠጥ ውሃ የሚያገኘው ከየት ነው?	እቤት ውስጥ ካለ ቧንቧ ----------1 የሕዝብ መገልገያ ቧንቧ----------2 ከጉድጓድ/ከተከለለ/----------3 ካልተከለለ ጉድጓድ----------4 ከወንዝ----------5 ከምንጭ----------6 ከዝናብ----------7 ሌላ ካለ ይግለፁ----------8	
03	ውሃውን ለማጣራት የሚጠቀሙት ዘዴ አለ?	አዎ----------1 የለም----------2	
04	ከሚከተሉት ውስጥ በተሰብዎ ያለውን ያመልክቱ	**አለን** **የለንም** መብራት /ኤሌክትሪክ/ ኮረንቲ 1 2 ሬድዮ 1 2 ብስክሌት 1 2 የልብስ ስፌት መኪና 1 2 የፈረስ /የአህያ/ ጋሪ 1 2 ፋኖስ 1 2 ኩራዝ 1 2	
05	የእርሻ መሬት ይዞታ አለዎት?	ኢዎን----------1 የለኝም----------2	
06	ምን ያህል የእርሻ መሬት አለዎት? በጥማድ		

07	እንስሳት አለዎት?	አዎን የለንም ቁጥራቸው ዶሮ 1 2 _____ በሬ 1 2 _____ ላም 1 2 _____ ፍየል 1 2 _____ አህያ 1 2 _____ በግ 1 2 _____ ፈረስ/በቅሎ 1 2 _____	
08	እድሜዎ ስንት ነው?	☐	
09	መደበኛ ትምህርት ቤት /የመንግስት/ ርምህርት ቤት ተምረዋል?	አዎን----------1 አልተማርኩም----------2	
10	ስንተኛ ክፍል ድረስ ተምረዋል?	የመጀመሪያ ደረጃ ----------1 መለስተኛ ሁለተኛ ደረጃ----------2 ሁለተኛ ደረጃ----------3 ኮሌጅ በዲኘሎማ----------4 ኮሌጅ በድግሪ----------5 ሌላ ካለ ይግለፁ----------6	
11	በምን ቋንቋ ማንበብ ይችላሉ?	_____	
12	ይኼንን አረፍተ ነገር ሊያነቡልኝ ይችላሉ ወይ? የልጅሽ ስም ማን ነው?	ትክክል ----------1 ስህተት ----------2	
13	ሬድዮ መቼ መቼ ያደምጣሉ	በየቀኑ----------1 በሁለት ሳምንት አንዴ----------2 ከሳምንት ባነሰ ጊዜ----------3 አዳምጬ አላውቅም----------4	
14	ሀይማኖትዎ ምንድነው?	ኦርቶዶክስ ክርስቲያን----------1 ካቶሊክ----------2 ኘሮቴስታንት----------3 መስሊም----------4 የባህል----------5 ሌላ ካለ ይግለፁ----------6	
15	የጋብቻ ሁኔታ	የተፋቱ----------1 የተለያዩ----------2 ሚስት/ባል የሞተባቸው----------3 ያገቡ----------4	
16	ባለቤትዎ ሌላ ሚስት አላቸው?	አዎን----------1 የለውም----------2	
17	የጥያቄ 21 መልስ አዎን ከሆነ ስንት ሚስት አላቸው	☐	
18	ባለቤትዎ መደበኛ የመንግስት ትምህርት ቤት	አዎን----------1	

	ተምረዋል?	አልተማሩም----------2	
19	ባለቤትዎ ስንተኛ ክፍል ድረስ ተምረዋል?	አንደኛ ደረጃ----------1 መለስተኛ ሁለተኛ ደረጃ----------2 ሁለተኛ ደጃ----------3 ዲኘሎማ----------4 ዲግሪ----------5 ሌላ ካለ ይግለፁ----------6	
20	የባለቤትዎ ስራ ምንድነው?	የግል ተዳዳሪ----------1 የመንግስት ሰራተኛ----------2 ግብርና----------3 የችርቻሮ ንግድ----------4 ሌላ ካለ ይግለፁ----------5	
21	ከቤት ውስጥ ስራ ውጭ ካለፈው አመት ጀምሮ የሰሩት ሌላ ስራ አለ?	አዎን----------1 የለም----------2	
22	ለጥያቄ 28 መልስዎ አዎን ከሆነ የስራው አይነት ምን ነበር?	አነስተኛ ንግድ----------1 የቅጥር ስራ----------2 የጉልበት ስራ----------3 እንጨት መሸጥ----------4 ሌላ ካለ ይግለፁ----------5	
23	በሚከተሉት ጉዳዮች በውሳኔ ሰጪነት በቤትዎ ይሳተፋሉ? ♦ የቤት ወጪን በተመለከተ ♦ ለቤት ቁሳቁስ መግዣ፣ ♦ የቁጠባ ገንዘብና የሚሸጡ ነገሮች ♦ የልጆችዎን ቁጥር መወሰንን በተመለከተ ♦ በእድሮች፣በእቁቦችና በመሳሰሉት መሳተፍን በተመለከተ ♦ ቤተሰብዎንና ጓደኞችዎን መጠየቅን በተመለከተ	እሳተፋለሁ አልሳተፍም 1 2 1 2 1 2 1 2 1 2 1 2	

II. የጤና አመጋገብ ሁኔታና ሌሎች ወሊድን በተመለከተ

ተ.ቁ	ጥያቄዎች	መለያ	
01	በወሊድ ጊዜ ልጅ ሞቶብዎት ያውቃል ወይ?	አዎ----------1 አይደለም----------2	
02	ልጅ ሞቶብዎት ያውቃል?	ያውቃል----------1 አያውቅም----------2	
03	አሁን ነፍሰጡር ነዎት?	ነኝ----------1 አይደለሁም----------2	
04	ነፍሰጡር ከሆኑ አሁን ስንት ወርዎ ነው?		
05	ነፍሰጡር በሆኑ ጊዜ በባህል ምክንያት የማይበሉት ከዚህ ቀደም የሚመገቡት ምግብ	አለ----------1 የለም----------2	

	አለ?		
06	መብላት ያቆሙት ምግብ ምንድነው?	ወተት----------1 ቅቤ----------2 ስጋ /የምን/----------3 አትክልት /ምን ምን/----------4 ፍራፍሬ /ምን ምን/----------5 ሌላ ካለ ይግለፁ----------6	
07	ልጅዎን ምን ምን መመገብ እንዳለብዎት ምክር የሰጠዎት ሰው አለ ወይ? መልስዎ አለ ከሆነ ማን ነው?	አለ----------1 የለም----------2 _____	
08	በአካባቢዎ ምን አይነት የጤና ተቋማት አሉ?	ክሊኒክ----------1 ጤና ኬላ----------2 ጤና ጣቢያ----------3 ሆስፒታል----------4 የሉም----------5	
09	የቅድመ ወሊድ የጤና አገልግሎት ተጠቅመዋል ወይ?	አለ----------1 አይደለም----------2	
10	ምን አይነት ግልጋሎቶችን አግኝተዋል?	_____ _____	
11	የወሊድ ችግር ቢያጋጥምሽ አቅራቢያሽ ወደሚገኝ የጤና ማእከል ለመድረስ ስንት ደቂቃ ይፈጅብሻል?	_____	
12	የመጨረሻ ልጅሽን ያዋለደሽ ማን ነው?	የጤና ባለሙያ----------1 የሰለጠኑ የልምድ አዋላጆች----------2 ያልሰለጠኑ የልምድ አዋላጆች----------3 ማንም አላገዘኝም----------4 ሌላ ካለ ይግለፁ----------5	
13	ባለፈው እርግዝናዎ ወቅት የደም ማነስን የሚከላከል ክኒን ወይም ሽሮኘ ወስደዋል ወይ?	አዎን ----------1 አይደለም----------2	
14	ልጅን ከወለዱ ከሁለት ወር በኋላ እንዲህ ያለ/ያሳዩዋቸው/ የቫይታሚን ኤ ክኒን ወስደው ያውቃሉ?	ወስጃለሁ----------1 አልወሰድኩም----------2	
15	የመጨረሻ ልጅዎ ክብደት ምን ያህል ነበረ?	ክብደት በኪ.ግ----------1 አልተመዘነም----------2 ተመዚንዋል ግን አላውቀውም----------3	
16	ልጅዎ ተከትቧል?	ተከትቧል----------1 አልተከተበም----------2	
17	ልጅዎ የተከተባቻን ክትባቶች ያውቃሉ?	አዎን ----------1 አይደለም----------2	
18	የምን የምን ክትባት ተሰጥቶታል? /እባክዎ ካርዱን ይመልከቱ/	Yes No BCG 1 2 Polio-0 1 2	

		DPT-1 1 2 DPT-2 1 2 DPT-3 1 2 Polio-1 1 2 Polio-2 1 2 Polio-3 1 2 Measles 1 2	
19	ህፃን ልጅዎን እንደተወለደ የሰጡት ምንድነው?	የጡት ወተት----------1 ውሃ----------2 የተፈላ እርጥብ ቅመም----------3 ቅቤ----------4 ውሃ በስኳር----------5 ሌላ----------6	
20	የመጨረሻ ልጅዎን ጡት አጥብተዋል?	አጥብቻለሁ----------1 አላጠባሁም----------2	
21	የመጨረሻ ልጅዎን ምን ያህል ጊዜ አጥብተዋል?	በወራት ☐	
22	ልጅዎን ከወለዱ ከስንት ሰዓት በኋላ ጡት ሰጡ?	ወዲያውኑ----------1 ከአንድ ሰዓት በኋላ----------2 ከሶስት ሰዓት በኋላ----------3 ከሶስት ሰዓት በላይ----------4 ሌላ ይግለፁ----------5	
23	ልጅዎን በቀን ምን ያህል ጊዜ ያጠባሉ?	ሶስት ጊዜ----------1 አራት ጊዜ----------2 አምስት ጊዜ----------3 ስድስት ጊዜ----------4 ቁጥር የለውም----------5	
24	ሕመም በሚሰማዎት ወቅት ልጅዎን ጡት ያጠባሉ ወይ?	አጥብቻለሁ----------1 አላጠባሁም----------2	
25	መጀመሪያ የሚወጣውን ቢጫ ወተት ወይም እንገር ይሰጣሉ?	አሰጣለሁ----------1 አልሰጥም----------2 አልሰጥም ካሉ ለምን _____	
26	ልጅዎን ጡጦ ያጠባሉ?	አጠባለሁ----------1 አላጠባም----------2 የሚያጠቡ ከሆነ ምንድነው የሚሰጡት?_____ _____ _____ _____ ካላጠቡ ለምን? ይግለፁ_____ _____ _____	
27	ባለፉት 3 ወራት ውስጥ እርስዎ ወይም የቤተሰብዎ አባል የሆነ ሰው ከሚከተሉት አንዱ የጤና እክል ገጥሞዎታል?	አዎን አላመመውም	

	♦ ወባ ♦ የሳንባ ነቀርሳ ♦ የልብ ሕመም ♦ የአባላዘር በሽታ ♦ የትርፍ አንጀት በሽታ ♦ ጉንዳን/ሳል ♦ የደም ግፊት ♦ የኩላሊት ሕመም ♦ ሌላ ካለ ይግለፁ	1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2	
28	ከሚከተሉት ውስጥ የትኛው ሕመም እርስዎን ወይም ቤተሰብዎን ባለፉት ሶስት አመታት አጋጥሟችኋል የቤተሰባችሁስ ዋነኛ የጤና ችግር ምንድነው?	ወባ----------1 የሳንባ ነቀርሳ----------2 ሌላ ካለ ይግለፁ----------10	
29	የትኛው በሽታ ልጅዎን ባለፉት ሶስት አመታት በተደጋጋሚ ይዞታል/ታል?	ተቅማጥ----------1 ወባ----------2 ትኩሳት----------3 ኩፋኝ----------4 ሌላ ካለ ግለጸ----------5	
30	የእናት ልኬት ♦ ቁመት በሳሜ ♦ ክብደት በኪሎ ♦ የላይኛው ክንድ ዙሪያ በሳሜ	ልኬት 1 ልኬት 2 ልኬት 3 _____ _____ _____ _____ _____ _____ _____ _____ _____	
31	የሕፃኑ ልኬት ♦ የሕፃኑ እድሜ ♦ ቁመት በሳሜ ♦ ክብደት በኪሎ ♦ የላይኛው ክንዱ ልኬት ♦ የጭንቅላት መጠን ዙሪያ ልኬት	ልኬት 1 ልኬት 2 ልኬት 3 _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____	

III. የቤተሰቡ የምግብ አመራረትና አመጋገብ

ተ.ቁ	ጥያቄዎች	መለያ			
01	የሚከተሉትን ያመርታሉ?	አዎን	አላመርትም	አዎን ካሉ ምን	
	1. የብርዕ እህሎች	1	2	_____	
	2. አትክልት	1	2	_____	
	3. ፍራፍሬ	1	2	_____	
	4. ስራስር ምግቦች	1	2	_____	
	5. ጥራጥሬ	1	2	_____	
	6. የምንዛሪ ተክሎች ለምሳሌ፡ ቡና፣ሽንኩራ አገዳ ወዘተ	1	2	_____	
02	የሚከተሉትን በአብዛኛው ይሸምታሉ?	አዎን	አልሸምትም	አዎን ካሉ ምን	

	1. የብርዕ እህሎች 2. አትክልት 3. ፍራፍሬ 4. ስራስር ምግቦች 5. ጥራጥሬ 6. የምንዛሪ ተክሎች 7. ሌሎች	1 2 _____ 1 2 _____ 1 2 _____ 1 2 _____ 1 2 _____ 1 2 _____	
03	ያመረቱትን ለምን ይጠቀማሉ?	ለቤት ውስጥ ፍጆታ ለገንዘብ ማግኛ	
	1. የብርዕ እህሎች 2. አትክልቶች 3. ፍራፍሬ 4. ስራስሮች 5. ጥራጥሬ 6. የምንዛሪ ተክሎች 7. ዶሮ 8. እንቁላል 9. ወተትና የወተት ውጤቶች 10. በግና ፍየል 11. ሌላ	1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2 1 2	
04	የወተት ከብቶች አለዎት?	አዎን----------1 የለኝም----------2	
05	የለኝም ካሉ ወተት ከየት ያገኛሉ?	በግዢ----------1 በስጦታ----------2 ሌላ----------3	
06	በቤትዎ በአንደኛ ደረጃ በዛ ያለምግብ የሚመገበው ማነው?	ባል----------1 ሚስት----------2 ወንድ ልጅ----------3 ሴት ልጅ----------4 ለምን ይግለፁ----------5	
07	ለነፍሰጡር ሴት ከተለመደው ውጪ ለየት ያለ ምግብ ይሰጣታል?	አዎን----------1 አልሰጥም----------2 አዎን ከሆነ ምንና ለምን? _____ አልሰጥም ከሆነ ለምን? _____	
08	ለምታጠባ እናት ከተለመደው ውጪ ለየት ያለ ምግብ ይሰጣታል?	አዎን----------1 አልሰጥም----------2 አዎን ከሆነ ምንና ለምን? _____ አልሰጥም ከሆነ ለምን? _____	
09	ከአምስት አመት በታች ላሉ ሕፃናት ምግብ ለብቻ ያዘጋጃሉ?	አዎን----------1 አላዘጋጅም----------2 አዎን ከሆነ ምን? _____ _____	

		አላዘጋጅም ከሆነ ለምን? _____ _____	
10	ከተዘረዘሩ ምግቦች ውስጥ ለልጅች በምን ያህል ጊዜ ይመግባሉ? 1. በየቀኑ 2. በሳምንት ከአንድ ጊዜ በላይ 3. በሳምንት 4. አንዳንዴ 5. በፍፁም	1. የጡት ወተት 1 2 3 4 5 2. ጉበት 1 2 3 4 5 3. እንቁላል 1 2 3 4 5 4. አሳ 1 2 3 4 5 5. ቅቤ 1 2 3 4 5 6. ጊህ 1 2 3 4 5 7. ማንጐ 1 2 3 4 5 8. ፓፓያ 1 2 3 4 5 9. ስኳር ድንች 1 2 3 4 5 10. ዱባ 1 2 3 4 5 11. ካሮት 1 2 3 4 5 12. ቆስጣ 1 2 3 4 5 13. ፋርመላ ወተት 1 2 3 4 5 14. የተመጠነ ወተት 1 2 3 4 5 15. የቫይታሚን ኤ ክኒን 1 2 3 4 5 16. ሌላ 1 2 3 4 5	
11	የተዘረዘሩትን ምግቦች በምን ያህል ጊዜ ይመግባሉ? 1. በየቀኑ 2. በሳምንት ከአንድ ጊዜ በላይ 3. በሳምንት 3. አንዳንዴ 4. በፍፁም	1. ጉበት 1 2 3 4 5 2. የእንቁላል አስኳል 1 2 3 4 5 3. አሳ 1 2 3 4 5 4. የአሳ ዘይት 1 2 3 4 5 5. ቅቤ 1 2 3 4 5 6. ጊህ 1 2 3 4 5 7. የበሰለ ማንጐ 1 2 3 4 5 8. የበሰለ ፓፓያ 1 2 3 4 5 9. ስኳር ድንች 1 2 3 4 5 10. ዱባ 1 2 3 4 5 11. ካሮት 1 2 3 4 5 12. ቆስጣ 1 2 3 4 5 13. ፋርመላ ወተት 1 2 3 4 5 14. የተመጠነ ወተት 1 2 3 4 5 15. የቫይታሚን ኤ ክኒን 1 2 3 4 5 16. ሌላ 1 2 3 4 5	
13	በህፃን ልጅዎ ምግብ ዘይት ወይም ቅቤ ይጨምራሉ? 1. ቅቤ 2. ዘይት	አዎን----------1 አልጨምርም----------2	
14	ጨለምለም ሲል ማየት ይቸግርዎታል ወይ?	ይቸግረኛል----------1 አይቸግረኝም----------2 አዎን ከሆነ ለምን ይመስልዎታል? _____	
15	ይህ በሽታ በአካባቢዎ ምን ተብሎ	_____	

	ይጠራል?		

IV. የቫይታሚን ኤ አሰጣጥን በተመለከተ

ተ.ቁ	ጥያቄዎች	መለያ	
01	ሕፃን ልጅዎን በአብዛኛው ሐኪም ቤት የሚወስደው ማን ነው?	እናት----------1 አባት ----------2 ወንድምና እህት ----------3 አያት----------4 ሌላ ዘመድ----------5 ጐረቤት----------6	
02	ልጅዎን የቫይታሚን ኤ እንክብል ወስዶ ያውቃል?	አዎን----------1 አያውቅም----------2	
03	ልጅዎ ምን ያህል ቫይታሚን ኤ ወስዷል?	መጠኑንና ጊዜውን ይመዝግቡ _____	
04	ልጅዎ የቫይታሚን ኤ እንክብል ካልወሰደ ለምን?	ልጁ ስላልፈለገው ----------1 እንደሚታደል አላወኩም----------2 ልወስድ ሄጄ ነበር ቫይታሚን ኤ በማለቁ አልወደስኩም----------3 ሕፃኑ በወቅቱ በመንደር አልነበረም----------4 ሕፃኑ ታምሞ ነበር ----------5 የሚታደልበት ቦታ ሩቅ ነበር----------6 ልጄ ቫይታሚን ኤ ቢወስድ ችግር ኖረዋል ብዬ ስላሰብኩ ነው----------7 ሌላ ምክንያት ----------8	
05	ክስንት ወር በፊት ነው ልጅዎ የመጨረሻውን የቫይታሚን ኤ እንክብል የወሰደው?	1. ከአንድ ወር ያነሰ ----------1 2. ከአንድ ወር በፊት ----------2 3. ከሁለት ወር በፊት----------3 4. ከሶስት ወር በፊት----------4 5. ከሶስት ወር የበለጠ ጊዜ ----------5	
06	የመጨረሻውን የቫይታሚን ኤ እንክብል የወሰደው መቼ ነው?	1. ሕፃኑ ታምሞ ሊታከም በሄደ ጊዜ -------1 2. ክጤና ጣቢያ----------2 3. ሌላ ካለ ይግለፁ----------3	
07	ለልጅዎ የቫይታሚን ኤ እንክብል ከተሰጠው በኋላ የታየ መሻሻል አለ?	1. አዎ ----------1 2. የለም----------2 ሌላ ካለ ይግለፁ_____	
08	ልጅዎን በድጋሚ ቫይታሚን ኤ እንዲወስድ ሊወስዱት ያስባሉ?	አዎ----------1 አላስብም----------2 አዎን ከሆነ መቼ_____	

የቫይታሚን ኤ እጥረት በተመለከተ ያሉ እውቀት

09	ስለ ቫይታሚን ኤ ሰምተው ያውቃሉ?	አዎን ----------1 አላውቅም ----------2	
10	ልጅዎ በቂ ቫይታሚን ኤ ሊያገኝ የሚችለው እንዴት ነው?	1. ክቫይታሚን ኤ እንክብል----------1 2. ክምግብ ----------2 3. ሌላ ካለ ይግለፁ ----------3	

11	ልጅዎ የመጀመሪያውን የቫይታማን ኤ እንክብል መቼ መጀመር አለበት?	1. ከአንድ ወር ያነሰ----------1 2. ከአንድ ወር ጊዜ----------2 3. ከሁለት ወር ጊዜ----------3 4. ከሶስት ወር ጊዜ ----------4 5. ከሶስት ወር በፊት ----------5	
12	ለ9 ጥያቄ መልስዎ አዎን ከሆነ ምን ሰምተዋል? /ተጠያቂዎ በትክክል መጥቀሳቸውን ይመልከቱ/	1. ሕይወት ያድናል [ ] 2. ለልጂ ጤና ጥሩ ነው [ ] 3. ለልጂ አይን ጥሩ ነው [ ] 4. ዳፍንት ይከላከላል [ ] 5. ልጆች እንዳይታወሩ ያደርጋል [ ] 6. የታመሙ ልጆች ቶሎ ይድናሉ [ ] 7. ሌላ_____ 8. አላውቅም [ ] 9. የተለየ ተቃራኒ ሐሳብ ካለ ይግለፁ	
13	ስለ ቫይታሚን ኤ ከየትና እንዴት ነው የሰሙት? የተጠቀሱትን ምልክት ያድርጉና ያልተጠቀሱትን ይጠይቁ	1. ከሬድዮ [ ] 2. ከቲቪ [ ] 3. ከፖስተር [ ] ከበራሪ ወረቀት 4. ከጤና ጣቢያ [ ] 5. ከጐረቤት /ከሌላ ዘመድ/ ጐረቤት [ ] 6. ከሃገሬው [ ] 7. ሌላ_____ 8. አላውቅም [ ]	

ከቪታሚን ዲ ጋር የተያያዙ ጥያቄዎች

01	ከቤት ውጪ መስክ ላይ ስራ ትሰሪያለሽ ወይ?	አዎን----------1 አይደለም----------2	
02	ወደ ውጪ ስትወጪ ልጅሽን ትሸፍኛለሽ ወይ?	አዎን----------1 አይደለም----------2 አዎን ከሆነ ለምን_____	
03	በየትኛው ወራት ውስጥ ብዙ ጊዜ መስክ ላይ ትሰሪያለሽ?	በዘር ወቅት----------1 በአረምና በኩትካቶ ----------2 ምርት ሲሰበሰብ----------3 በማንኛውንም ጊዜ----------4 ሌላ ካለ ይጥቀሱ----------5	
04	ወደ ውጪ በምትወጪበት ወቅት ራስሽን ከፀሐይ ለመከላከል ትሞክሪያለሽ ወይ?	አዎ ሁልጊዜ----------1 አዎን አብዛኛውን ጊዜ----------2 አልፎ አልፎ----------3 መቼም አልሸፍንም----------4	
05	ራስሽን ከፀሐይ እንዴት ትከላከያለሽ?	_____ _____	
06	ተጠያቂው ምን አይነት ጫማ ነው ያደረጉት?	ባዶ እግር----------1 ነጠላ ጫማ----------2 ሽራ ጫማ----------3 ላስቲክ ጫማ----------4	

		የቆዳ ጫማ----------5	
07	ተጠያቂው ጃንጥላ ይዘዋል ወይ?	አዎን----------1 አይደለም----------2	

መመሪያ ሁለት

የሚቀጥለውን ሳጥን ለመመላት እናትየው ባለፉት ሶስት ቀናት የበሉትን ምግብ አይነት ይጠይቃሉ። የምግቡን አይነት ባልፉት ሶስት ቀናት ምንም ጊዜ ካልተመገበች ዜሮ ላይ ምልክት ያድርጉ አንድ ጊዜ ከተመገበች አንድ ላይ ምልክት ያድርጉ። ከሁለት ጊዜ በላይ ከተመገበች ሁለት ላይ ምልክት ያድርጉ። በጣም በአነስተኛ ከተመገቡ አነስተኛ የሚለው ላይ ምልክት ያድርጉ።

የምግብ አይነት	ዜሮ 0	አንድ 1	ከሁለት የበለጠ>2	አነስተኛ
ጥራጥሬ /በቆሎ፣ገብስ፣ስንዴ፣ዘንጋዳ፣ጤፍ/				
ስራስሮች /ካሮት፣ድንች፣እንሰት/				
የወተት ውጤት				
ስጋ				
እንቁላል				
ስኳር ድንች፣ካሮት፣የአበሻ ጉመን				
አትክልትና ፍራፍሬ				
የአበባ እህሎች ምስር፣ሽምብራ፣ቦሎቄ አተር				
የቅባት ውጤቶች ቅቤ፣የኦቾሎኒ ዘይት፣የአትክልት ዘይት				

APPENDIX C

OKLAHOMA STATE UNIVERSITY'S

INSTITUTIONAL REVIEW BOARD APPROVAL

FORM FOR HUMAN SUBJECTS

Oklahoma State University Institutional Review Board

Date:	Thursday, December 22, 2005
IRB Application No	HE0624
Proposal Title:	Vitamin A and D Status of Breastfeeding Ethiopian Women
Reviewed and Processed as:	Expedited (Spec Pop)

Status Recommended by Reviewer(s): Approved Protocol Expires: 12/21/2006

Principal Investigator(s)

Meredith Reilly
814 W. Moore
Stillwater, OK 74075

Amy Pruitt
86 S. Univ. Place #9
Stillwater, OK 74075

Barbara J Stoecker
421 HES
Stillwater, OK 74078

The IRB application referenced above has been approved. It is the judgment of the reviewers that the rights and welfare of individuals who may be asked to participate in this study will be respected, and that the research will be conducted in a manner consistent with the IRB requirements as outlined in section 45 CFR 46.

☑ The final versions of any printed recruitment, consent and assent documents bearing the IRB approval stamp are attached to this letter. These are the versions that must be used during the study.

As Principal Investigator, it is your responsibility to do the following:

1. Conduct this study exactly as it has been approved. Any modifications to the research protocol must be submitted with the appropriate signatures for IRB approval.
2. Submit a request for continuation if the study extends beyond the approval period of one calendar year. This continuation must receive IRB review and approval before the research can continue.
3. Report any adverse events to the IRB Chair promptly. Adverse events are those which are unanticipated and impact the subjects during the course of this research; and
4. Notify the IRB office in writing when your research project is complete.

Please note that approved protocols are subject to monitoring by the IRB and that the IRB office has the authority to inspect research records associated with this protocol at any time. If you have questions about the IRB procedures or need any assistance from the Board, please contact Beth McTernan in 415 Whitehurst (phone: 405-744-5700, beth.mcternan@okstate.edu).

Sincerely,

Sue C Jacobs

Sue C. Jacobs, Chair
Institutional Review Board

APPENDIX D

CONSENT SCRIPT (ENGLISH)

Title: Vitamin A and D status of breastfeeding Ethiopian women

Consent Script for Persons Who Do Not Read English

You have been asked to take part in a research study. The translator has told you the following things about the study:

- why the study is being done
- what will happen to you if you are in the study (tests, etc.)
- how long you will be in the study
- what parts, if any, are experimental
- the possible risks, discomforts, and benefits of the study (there is always a chance that you might have a side effect of a test that we didn't know about before)
- alternatives to being in the study
- how your study records will be kept private
- how you can receive medical care if you are hurt in the study and whether you will have to pay for it
- whether the study will cost you anything
- the situations in which the study doctor could take you out of the study
- what happens if you decide to stop being in the study
- how many people will be in the study.

Invitation for Questions:

You may ask any questions you have now. If you have questions later, you may ask Dr. Fekadu Beyene or Dr. Yewelsew Abebe at Debub University, P.O. Box 5, Awassa or at 462-200-470. They can also contact other project staff as requested. If you have questions regarding your participation in this research, please contact Dr. Adugna Tolera, Chair of the Debub University Ethics Committee and Associate Vice President for Research and Extension at P.O. Box 5, Awassa, Phone #462-200-221. You will be given a copy of this form to keep.

Agreeing to participate in this study means that the research study has been described to you orally, in language you understand. You will have a chance to ask questions about the study. These questions should be answered to your satisfaction before you agree to be in the study. You may choose not to be in the study or you may quit being in the study at any time without loss of any privileges to which you are entitled. If you agree to be in the study, you will say your name and that you want to be in the study, and your words will be taped.

You know what will be done as part of this study. You also know the possible good and bad (benefits and risks) that could happen if you are in this study. You choose to be in this study. You know you can stop being in the study at any time, and you will still get the usual medical support in the community.

Name of Participant (spoken to tape): _____

Name of Witness (spoken to tape): _____

Name of Translator (spoken to tape): _____

Date (spoken to tape): _____

APPENDIX E

CONSENT SCRIPT (AMHARIC)

ዝንክ እና የእናት--ልጅ የአእምሮ ተግባር በድቡብ ኢትዮጲያ

የስምምነት ቅጽ

እንግሊዝኛ ለማያነቡ የተዘጋጀ

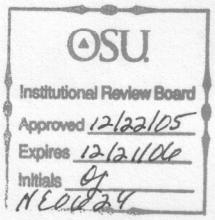
በምርምር ጥናት ላይ እንድሳተፍ ተጠይቂአለሁ ። አስተርጓሚዉ ስለጥናቱ የሚከተሉትን ነገሮች ነግሮኛል ።

- ጥናቱ ለምን እንደሚካሄድ
- በጥናቱ ተሳታፊ ብሆን ምን እንደሚደርስብኝ (ፈተናዎች፤ መከራዎች ..... ወዘተ)
- በጥናቱ ዉስጥ ምን ያህል ጊዜ እንደምቆይ
- ዬትኞች የአካል ክፍሎች ለመከራ እንደሚዉሉ
- በጥናቱ ወቅት ሊያጋጥሙ የሚችሉ አደጋዎች፤ አለመመቸት፤ ጥቅም (መከራ ከመካሄዱ በፊት ያልታወቁ በመከራ ጊዜ ሊከሰቱ የሚችሉ ጎጂ ሁኔታዎች ሊጋጥሙ ይችላሉ ።)
- በጥናቱ ተሳታፊ ከመሆን ሌላ አማራጮች
- የጥናቱ የግል ማህደሬ ሚሰጢራዊነቱ እንዴት እንደሚጠበቅ
- በጥናቱ ጊዜ ጉዳት ቢደረስብኝ እንዴት የህክምና እርዳታ ማግኘት እንደሚችል፤ የህክምና ወጪዉ በማን እንደሚሸፈን
- ጥናቱ በእኔ ላይ ወጪ ያስከትል እንደሆነ
- ጥናቱን የሚያካሂደዉ ሃኪም እኔን ከጥናቱ ዉጭ ሊያደርገኝ የሚችልባቸዉ ሁኔታዎች
- በጥናቱ ላለመቀጠል ብወስን ምን ሊኖር እንደሚችል
- ከጥናቱ የሚፈልቁ አዲስ መረጃዎችን እንዴት እንዳዉቅ እንደሚደረግ፤ በተሰይ መረጃዎቹ በጥናቱ መቀጠል ላይ ተፅዕኖ የሚራቸዉ ከሆነ
- ስንት ሰዎች በጥናቱ እንደሚሳተፉ

አሁን ምንም ዐይነት ጥያቄ ካለዎት መጠየቅ ይችላሉ ። በሌላ ጊዜ ጥናቱን በተመለከተ ጥያቄ ካለዎት ከዚህ በታች የተጠቀሱትን ሰዎች መጠየቅ ይችላሉ ።

ዶ/ር ፈቃዱ በየነ ወይም ዶ/ር የዉልሰዉ አበበ
ደቡብ ዩኒቨርስቲ፤ ፖ. ሣ. ቁ 5
የስልክ ቁጥር 62 00 470
አዋሳ

አስፈላጊ ከሆነ ከሌሎች የፕሮጀክት ሠራተኞች ጋር በእነሱ በኩል መገናኘት ይችላሉ። በጥናቱ ላይ ተሳታፊ መሆንን በተመለከተ ጥያቄ ካለዎት፤



ዶ/ር ግርማ አበበ
የአይ አር ቢ ምርምርና ስርፀት ቢሮ
ደቡብ ዩኒቨርስቲ፤ ፖ. ሣ. ቁ. 5
የስልክ ቁጥር 62 00 221
አዋሳ

ይህንን ቅፅ መፈረም ማለት ስለጥናቱ ምርምር በሚገባኝ ቋንቋ በቃል ተገልፆልኛል ማለት ነዉ ። በጥናቱ ተሳታፊ ለመሆን ከተስማማሁ ፊርማ የሰፈረበት የዚህ ቅፅ ግልባጭና የጥናቱ አጠር ያለ መግለጫ በፅሁፍ ይሰጠኛል ። ስለጥናቱም ጥያቄ የመጠየቅ እድል ይኖረኛል ። ይንን ቅፅ ከመፈረሜ በፊት ለጥያቄዎቼ አጥጋቢ መልስ መሰጠት ይኖርበታል ። በጥናቱ ላለመሳተፈል ወይም በማንኛዉም ጊዜ ለመተዉ ብወስን የሚገባኝን ጥቅማጥቅም አላጣም ።

በጥናቱ ምን እንደሚካሄድ አዉቃለሁ ። እንዲሁም የጥናቱ አካል በመሆኔ ሊያስከትል የሚችለዉን ጥሩና መጥፎ ገፅታዎችን (ጥቅምና ጉዳት) አዉቃለሁኝ ። የዚህ ጥናት አካል ለመሆን መርጨአለሁኝ ። በምንም ጊዜ ከጥናቱ መዉጣት እንደሚችል አዉቃለሁ ። ብወጣም የተለመደዉን የህክምና አገልግሎት አገኛለሁ ።

የተካፋይ ፊርማ፡ ----------

ቀን ---------- / --------- / ----------

የዓይን ምስክር ፊርማ ----------

ቀን --------- / ---------- / ----------

የአስተርጓሚ ፊርማ ----------

ቀን --------- / ---------- / ----------

VITA

Meredith Leah Reilly

Candidate for the Degree of

Master of Science

Thesis: VITAMIN A STATUS OF LACTATING WOMEN IN SOUTHERN ETHIOPIA

Major Field: Nutritional Sciences

Biographical:

Personal Data: Born in Oklahoma City, Oklahoma on December 31, 1981, the daughter of Phillip and Judy Reilly.

Education: Graduated from Mount Saint Mary High School, Oklahoma City, Oklahoma in May, 2000; received a Bachelor's of Science degree in Nutritional Sciences from Oklahoma State University, Stillwater, Oklahoma in December 2004; completed the Dietetic Internship at Oklahoma State University in June 2006; completed requirements for Master of Science in Nutritional Sciences from Oklahoma State University in December, 2006.

Experience: Completed research in Ethiopia; presented research findings at Experimental Biology conference, San Francisco, California, April 2006.

Professional Memberships: American Dietetic Association; Oklahoma Dietetic Association.

Name: Meredith Reilly Date of Degree: December, 2006

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: VITAMIN A STATUS OF LACTATING WOMEN IN SOUTHERN ETHIOPIA

Pages in Study: 120 Candidate for the Degree of Master of Science

Major Field: Nutritional Sciences

Scope and Method of Study: The purpose of this study was to assess the vitamin A status of lactating women in southern Ethiopia. A total of 108 women participated in this cross-sectional investigation. Anthropometric measurements, blood samples, breast milk, dietary habits, socioeconomic data, and dark adaptation were analyzed. Pearson correlation coefficients were calculated for all appropriate variables. Significance was set at $p < 0.05$.

Findings and Conclusions: The mean serum retinol level in participants as determined by HPLC and fluorometry was 1.49 ± 0.04 and 1.68 ± 0.04 μmol/L, respectively. Results from analysis of serum retinol concentrations by HPLC and fluorometry were highly correlated ($r = 0.63$, $p < 0.0001$). Forty one percent of the participants had low levels of breast milk vitamin A. Pupillary threshold scores and serum retinol concentrations tended to be related, as did pupillary threshold scores and breast milk vitamin A.

ADVISER'S APPROVAL: Dr. Barbara Stoecker