

Anthropometry and Biochemical Assessments of Women of Childbearing Age and Children 6–59 Months of Age from Rural Areas in Akwa Ibom State, Nigeria

B.B. Maziya-Dixon, R.A. Sanusi, and E.B. Oguntona



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N. Sanginga Director General International Institute of Tropical Agriculture December 2012

Preface

This report summarizes the findings of the 2011 Akwa Ibom State Nutrition Survey conducted by IITA Ibadan, in collaboration with the Ministry of Health, Universities of Uyo, Ibadan, and Agriculture-Abeokuta. Financial support and technical assistance were provided by HarvestPlus, Washington DC.

The field data were collected in October 2011. The laboratory analyses for biochemical indices were compiled in 2012; data analysis and report writing were completed in December 2012.

Additional information on the Akwa Ibom survey may be obtained from the State Ministry of Health, Nutrition Division, Idongesit Nkanga Secretariat, Uyo, Akwa Ibom State, and from IITA, PMB 5320, Oyo Road, Ibadan, Oyo State, (Telephone 08034035281; Fax +44 2087113786; email: iita@cgiar. org).

Executive summary

The Nutrition Survey was a project in Akwa Ibom State looking at the nutritional status of children 6-59 months and their mothers (women of childbearing age) through biological sample collection and anthropometric measurements to assess the extent of malnutrition and micronutrient deficiencies, especially in vitamin A and iron.

A listing of all local government areas was obtained, grouped according to the level of urbanization as defined by the Federal Government of Nigeria. The current official designation of rural, medium, and urban is based mainly on population. A community with fewer than 5000 people is regarded as rural, 5000-20000 as medium, and more than 20000 as urban. A total of 10 Local Government Areas (LGAs) were selected using the probability proportionate to size sampling technique so that the likelihood of an LGA being selected is proportional to its population size. A total of 570 households were selected with women of childbearing age and children 6-59 months of age making a total of 1,140 participants.

The main data collection instrument had anthropometry and biochemical measurements. Data collection took place in October 2011. The survey was implemented by IITA and funded by HarvestPlus, with partners from Nigerian national universities and the Ministry of Health.

Anthropometric indices showed a high level of stunting (43.1%), wasting (12.0%), and underweight (17.2%). As malnutrition contributes over 50% to mortality in the under-5s, concerted effort should be made to take a second look at why malnutrition remains intractable despite several programs and projects in the past decades. Furthermore, underweight (9.8%), overweight (21.9%), and obesity (9.3%) among the women of childbearing age demand attention. This scenario where children are predominantly under-nourished and their mothers are overweight demands a shift in the thinking of how to address this double burden of malnutrition.

At the time of the survey, 18.9% of children under-5 had biochemical vitamin A deficiency (VAD). As VAD affects morbidity, the severity of other infections, and mortality these consequences make this level a trigger point with potential for action, particularly since the survey was undertaken exactly three months after a State-wide vitamin A supplementation had been conducted. Paradoxically, only 3.4% of women of childbearing age had VAD.

Iron deficiency as measured by serum ferritin was greater than 10% in both children under-5 and their mothers. For children under-5, 18.6% suffer from iron deficiency (ID) or iron depleted stores and 29.1% for women of childbearing age. Because the percentage is higher than 10, ID is a problem of public health significance in the rural population of this State.

The prevalence of anemia as measured by hemoglobin was 32.8% in children under-5 and 48.6% in women of childbearing age. These are high as one in three children under-5 and approximately one in two ID women of childbearing age are anemic. It is worth noting that usually iron deficiency anemia does not occur until after iron store depletion. The issue of ID hereby needs to be revisited to find out whether it is due to inadequate intake, inadequate absorption, or excessive demand. The effect of ID and anemia on child growth and cognitive responses and productivity as well as the propensity to infection should guide the actions for prevention.

Malaria infection as determined by the malaria parasite load on microscopy and categorized into three groups showed that 65% (n=584) had no malaria parasite on their slides, 30% had a mild/moderate malaria parasite load, and only about 5% had a severe malaria parasite load as shown by their slides. It was surprising that the majority (44.2%) of these children who were negative for malaria and

about 17% of those with mild/moderate malaria parasite load were in the group with low acute phase proteins.

An overwhelming majority (90.2%) had a negative malaria parasite load with only 9.8% in the mild/ moderate category in all the subjects and 8.8% after adjustment for infection/inflammation. It is also sufficient to summarize that malaria was not a serious problem among these women. At the level of these analyses serious relationships have not been found in terms of malaria parasite load and the status of vitamin A or iron.

Results of the 2011 Akwa Ibom State Nutrition survey indicate that VAD and anemia are serious health problems among children and that the situation has not changed and may be getting worse, especially for children under-5. In addition, stunting and wasting are also prevalent. The micronutrient and nutritional status assessments reveals high prevalence of ID in women of reproductive age and practically no VAD, compared to their children. Based on these findings, urgent action is needed both to strengthen current programs and target efforts in the prevention and control of micronutrient deficiencies, especially of vitamin A and iron in children under-5 and overweight/obesity in women of childbearing age.

Introduction

Justification

Data from the 2001-2003 national survey show that 42% of children under-5 were stunted, 9% were wasted, and 25% were underweight, indicating high levels of protein energy malnutrition. The micronutrient deficiencies were also alarming with 29.5% of children under-5 with VAD, 28% with ID, and 29.6% with iodine deficiency. The proportions of children with VAD differed among the agro-ecological zones with 31.3% in the dry savanna, 24.0% in the moist savanna, and 29.9% in the humid forest. More children with severe deficiency (serum retinol < 0.35 umol/L) lived in the humid forest (7.1%) than in the dry savanna (3.1%) and moist savanna (2.4%). The distribution of VAD in children under-5 was 25.6% in the rural sector, 32.6% in the medium, and 25.9% in the urban (Maziya-Dixon et al., 2004). In the same survey, only 1.2% of women of childbearing age were deficient in vitamin A and 29.7% in iron.

In the more recent Nigeria Demographic and Health Survey (NPC and ICF Macro., 2009), anthropometric indicators for young children were collected to provide outcome measures of nutritional status. The percentage of children in Nigeria who were stunted (height-for-age), a condition reflecting the cumulative effect of chronic malnutrition, was 41. A higher proportion of males (43%) than females (38%) were stunted. In the rural areas, 45% of children were stunted, versus 31% of children in the urban areas. About half (53%) of the children residing in the North West were stunted and 49% in the North East. Stunting was most among children in Kebbi State (64%) and least (12%) among children in Anambra State.

The link between food and health is the diet and therefore the nutrition of the people. Diet quality and diversity and indeed the nutrient adequacy have been suggested as determinants of malnutrition in several populations (Arimond and Ruel, 2004). This situation calls for urgent action that is effective, sustainable, and culturally acceptable.

Cassava (*Manihot esculenta Crantz*), variously known as manioc, mandioc, tapioca, or yuca, is grown principally for its swollen roots but its leaves are also consumed in some developing countries. The roots contain 25-35% starch and the leaves contain a significant amount of proteins, vitamins, and minerals. Currently, about half of the world's production of cassava comes from Africa with Nigeria as the largest producer. In Africa, total consumption of cassava has more than doubled in the last 30 years, from 24 million t/yr (1961 - 1965) to 58 million t/yr (1994 - 1998) after accounting for waste (Nweke et al., 2002)

From a nutritional point of view, the chief advantage of cassava is that it is an inexpensive source of carbohydrate. The roots contain a negligible amount of protein although substantial amounts are derived from the cassava leaves (Montagnac et al., 2009). Serious consideration had previously not been given to the enhancement of cassava's nutritive value, either in breeding research or in processing. However, recent research by the Consultative Group for International Agricultural Research (CGIAR) and international partners have focused on increasing the protein, mineral, and vitamin contents of cassava storage roots.

The Bill and Melinda Gates Foundation (BMGF) has provided financial support since 2004 to HarvestPlus (H+), a consortium of organizations working to develop bio-fortified varieties of this important staple in sub-Saharan Africa. The goal of H+ is to develop bio-fortified varieties with substantially increased content of pro-vitamin A carotenoid (β -carotene). The health impact of these varieties depends on a host of factors including the micronutrient content of bio-fortified cassava (after processing), the prevalence and severity of VAD, the adoption rate and quantity produced by farmers,

the quantity and frequency of consumption of bio-fortified cassava by the target groups, the other foods with which it is consumed, and the health status of the persons eating it, among others.

HarvestPlus and its partners are looking towards bio-fortified cassava with increased levels of provitamin A that is estimated to be capable of contributing at least 50% of the mean daily vitamin A requirement if consumed daily.

Study objectives

Limited information is available about the current micronutrient status (vitamin A and iron), nutritional anthropometric status, and rate of infection in children aged 6-59 months and women of childbearing age residing in cassava-based production and consumption areas. Therefore, a survey was conducted to determine the vitamin A, iron, and nutritional anthropometric status of childbearing age 6-59 months and women of childbearing age in households residing in relevant areas of the target State.

Methodology

Survey design and sampling

Criteria for Selection of State

The first consideration in selecting the target State was the area where cassava is most consumed. The Nigeria Food Consumption and Nutrition Survey (2001-2003) was conducted in 12 States, and the *per capita* amount of cassava consumption was greater than 300g/d in four of those surveyed (Kwara, Imo, Akwa Ibom, and Bayelsa). These results showed that the South Region from east to west is a relevant area. The second parameter, once we had selected States that are "possible candidates", was to consider the vitamin A status of the population of interest. The State with a greater prevalence of VAD was a preferred candidate. The vitamin A status from the Nigeria Survey (2001-2003) is shown in **Figure 1** below.



Figure 1: Vitamin A deficiency in children 0-59 months and household cassava consumption in selected States in Nigeria. Key: CU5Y- children under the age of 5 years, WCBA - women of childbearing age.

With the set criteria, Akwa Ibom in the South South geo-political zone and the humid forest agroecological zone was selected as the project State. It has a population of 3,902,051 and 31 LGAs.

Akwa Ibom has the highest cassava consumption among the cassava producing States. According to Phillip et al. (2005), using the frequency of consumption within one week, consumption was estimated

at 409.2 g/person/day. Results of the Nigeria Survey 2001-2003 showed that 33% of the respondents reported consuming cassava products over four times a week (Maziya-Dixon et al., 2004) and that 90% consume it as *gari*, 8% as *fufu*, and 2% as *abacha* (Phillip et al., 2005). In the same survey, using the 24 hr dietary recall method, children aged 6-59 months in the humid forest agro-ecological zone consumed 198.6g of *eba*170.5g of *fufu*, and 98.6g of *gari*. Children in the rural sector consumed 202.5g of *eba* (*gari*, *ckd*), 171.9g of *fufu*, and 113.9g of *gari* (Maziya-Dixon, unpublished). The major cassava products are *gari* and *fufu* in the same agro-ecological zone, where consumption of palm oil was found to be 21.3g with an apparent contribution of 355 RAE micrograms.

Selection of Local Government Areas (LGAs)

Within the State, a list of all LGAs was obtained, grouped according to the level of urbanization (urban, medium, and rural) as defined by the FGN. In Nigeria, the current official designation of rural, medium, and urban is based mainly on population. A community with fewer than 5,000 people is regarded as rural, 5,000-20,000 as medium, and more than 20,000 as urban. Akwa Ibom State has 31 LGAs made up of five urban, 16 rural, and 10 medium. The choice of probability proportional to size as a sampling technique was based on the following considerations; first, malnutrition and VAD are public health problems even in urban and medium areas. Secondly cassava products are consumed by all sectors of the population. A total of 10 LGAs was selected using the probability proportionate to size sampling technique so that the likelihood of an LGA being selected is proportional to its population size. Ten LGAs were selected as shown in **Table 1** and their distribution in the State is presented in **Figure 2**.

| LGA code | Name of LGA | Community code | Name of Community |
|----------|-------------------|----------------|-------------------|
| 01 | Eastern Obolo | 01 | Afuku |
| | | 02 | Mbaja |
| | | 03 | Okoro-Mbokho |
| 02 | Essien udim | 01 | Ikot Otu |
| | | 02 | Ukana Ikot Ntuen |
| | | 03 | Ukana Mbak Ukot |
| 03 | lbeno | 01 | Akata |
| | | 02 | lwuo-kpom |
| | | 03 | Ntafre |
| 04 | lbiono ibom | 01 | Ibiaku Ikot Amba |
| | | 02 | Ibiaku Ikot Udo |
| | | 03 | Omu-Ekene |
| 05 | Ikono | 01 | Atai Obio Ediene |
| | | 02 | Ikot Akpa-Ekpuk |
| | | 03 | Ikot Efre-Itak |
| 06 | Mkpat enin | 01 | Esa Ekpo |
| | | 02 | Ibot |
| | | 03 | Ikot Etefia-Minya |
| 07 | Nsit ubium | 01 | Ikot Akpabin |
| | | 02 | Ikot Akpatu |
| | | 03 | Ntit-Oton |
| 08 | Onna | 01 | Awa-Iman |
| | | 02 | Ikwe |
| | | 03 | Ndon Eyo |
| 09 | Oruk anam | 01 | Ikot Etim-Ibesit |
| | | 02 | Ikot Omono |
| | | 03 | Nung Ikot Asanga |
| 10 | Urue offong oruko | 01 | Edok-Oruko |
| | | 02 | Mbukpo Uko-Akai |
| | | 03 | Oyuku-Ebighi |

Table 1: Selected LGAs, communities within an LGA, and their codes.



Figure 2: Akwa Ibom State showing selected (LGAs.

Sample size calculation

The calculation of sample size was based on estimating the vitamin A level in children aged 6-59 months and women of childbearing age to enable inferences at the State level only. Since Akwa Ibom was included in the 2001-2003 Nigeria Food Consumption and Nutrition Survey 2001-2003, sample size calculation was determined using the prevalence data on deficiencies of vitamin A, iron, and zinc based on the State's results in that survey. Levels of wasting, stunting, and underweight were based on the results of NDHS 2008.

The sample size was calculated based on the following criteria and assumption: (a) confidence level of 93.5 (precision of 6.5%), (b) power of 80%, (c) estimated VAD prevalence of 30%, among children, (d) design effect of 2.5, (e) individual response rate at 85%, and seven individuals per household with children 6-59 months of age forming 16% of the population. The sample was selected using a multi-stage selection scheme consisting of three levels (LGA, enumeration area (EA), and household). A total of 570 households were selected with women of child-bearing age and children 6-59 months of age making a total of 1,140 participants.

Enumeration Areas (EAs)

A comprehensive list of all the EAs in a selected LGA was obtained from the National Population Commission, Abuja. The EA is the smallest geographical cluster of households as delineated by the NPC for the purposes of enumeration. Three were randomly selected from the list of EAs within a selected LGA.

Selection of household/Household listing workshop

As the vitamin A status survey was a component of the Socioeconomic and Dietary Intake surveys which were conducted between July and August 2012, household listing was covered during a twoday training workshop on community mobilization and household listing in preparation for the surveys. The workshop was held on 1-2 July 2011 at Sky Cap Hotel, Uyo, Akwa Ibom. The objectives were as follows; train the potential interviewers on identifying the selected locations/localities using the EA maps, construct a sample frame and select respondents using a random number table. A total of 36 potential interviewers were trained. The same households listed and selected for Socio-economic and Dietary Intake surveys were used to assess serum samples for retinol and iron.

Selection of subjects for biochemical measurements

The nutrition focal persons (mobilizers) in each LGA visited the communities to remind them of the pending biochemical survey. Two days before data collection, the mobilizers visited each community to meet the women about the survey. During this meeting, all those who participated in the DI survey were invited and urged to participate in the biochemical survey as these are linked and related. The women were requested to come to the health facility. Because of the time lag (three months) between the two surveys, not all those who participated in the DI survey were available during the biochemical survey. To fulfill the minimum sample size for making inferences at the State level, some eligible mother/child pairs in these communities who did not participate in the DI but were present during the mobilization meeting and came to the health facility were also included in the survey.

Ethical clearance

IITA submitted the application for Ethical Clearance through the Nutrition Division to the National Health Research Ethics Committee. The process required to obtain the ethical clearance approval involved submitting the proposal of the proposed study, research protocols, and a sample of the consent form to the NHREC based in the Federal Ministry of Health, Federal Secretariat, Abuja. The same documents were also submitted to the State Health Research Ethics Committee in the Akwa Ibom State Ministry of Health. An ethical clearance for the conduct of both the pilot and main survey, including collection of biological samples, was obtained after due process (see appendix 1).

Training workshop for Phlebotomists

A separate training workshop for 11 laboratory technologists/scientists was conducted from 26 to 29 September 2011 at La Mem Hotel, Uyo, Akwa Ibom. The objectives of the workshop were to (a) highlight and address problems that might be encountered with respect to biological sample collection during the survey; (b) reach a consensus on procedures for sample collection so as to ensure uniformity in all the LGAs as well as standard sample analysis and validity of results; (c) familiarize the participants with the equipment and materials to be used during the survey; and (d) discuss other relevant logistic and administrative issues. The resource person Mr Eldrich Harmse came from the South African Medical Research Council and was assisted by a consultant, Dr R.A. Sanusi of the University of Ibadan, Nigeria.

Pilot study

A pilot study involving the collection of biological samples was conducted at the Primary Health Care Center, Uyo, Akwa Ibom on 30 September 2011. Blood samples were collected from 20 mother/child pairs selected from a group of mother/child pairs attending the Primary Health Care center for growth monitoring and immunization exercises. The result of the pilot study and the experience of using these tools and procedures enabled the survey team to modify and improve them appropriately for the main study. The pilot study provided an excellent opportunity to further improve the efficiency of survey operations. Issues of inadequate sample collection, processing, labeling, ensuring adequate supplies, and constraints such as sample handling and transportation of biological samples were highlighted. To capture experiences and provide feedback on procedures and materials, a debriefing meeting was conducted after the pilot study. The phlebotomists, Principal Investigator, consultant, resource person, representatives from the State Ministry of Health, and nutrition focal persons participated in the debriefing meeting.

Mobilization and briefing of communities

The selected LGAs and EAs to be surveyed were mobilized and briefed for the survey. The State Nutrition Officer used the inter-governmental communication channels to notify and obtain the consent of the Local Government Chairpersons. The nutrition focal person in each of the selected LGAs assisted by a local guide identified and mobilized the selected households. The households used for this biological sample survey were those selected for Dietary Intake/ Food Consumption/Nutrient Intake interviews conducted three months earlier.

Survey team

The survey team was made up of three teams: mobilization, biological sampling, and monitoring. The responsibilities of the mobilization team were as follows.

- Ensure ability to locate the LGA, community, and participants whose dietary intake had been assessed three months earlier
- Book an appointment with these participants
- Motivate each of them to keep the appointment
- Ensure informed consent to participation in the biological sampling was obtained

The team was made of the nutrition focal person, a local guide, and personnel from each of the LGAs.

The biological sampling team was made of phlebotomists. There were two teams with four phlebotomists per team and a supervisor. Their main responsibility was to collect and process biological samples.

The survey monitoring team composed of two officials from the State Ministry of Health and the Principal Investigator. Their responsibility was to monitor field data collection, storage of collected, processed samples. In addition, the team ensured the safety of participants and compliance with ethical issues.

Biochemical sample collection, handling, storage, and shipping

Blood samples (5 ml) were drawn from each of the children and their mother by venipuncture at a health facility close to the survey site using appropriate techniques and precautions. Where there was no health facility, arrangements were made with the village head for the use of a community hall or the village head's compound. Sample collection commenced on 2 October 2012. A total of 1,270 samples were collected (640 mother/child pairs).

The blood samples were placed in cold boxes for about 30-45 minutes and allowed to clot after which the *vacutainer* containers were centrifuged at 200g for 10 minutes to obtain clear serum. The serum samples were pipetted using a Pasteur pipette into two coded *eppendorf* safe-lock serum vials and wrapped in aluminum foil for protection from light. Sample processing was done in a subdued light environment. The coded and wrapped samples were placed in their respective cold chain boxes which were labeled for the different laboratories. The temperature in the cold box was maintained at -4 °C with a frozen cooler ice pack. At the end of each day, the collected serum samples were transferred to the cold store at the Primary Health Care clinic that served as a temporary place for collection/storage in the State. The process of sample collection was performed following standard procedures of antisepsis and safety. The State Ministry of Health through the State Primary Health Care Development Agency obtained permission from the Primary Health Care centers in the State and LGAs to use their cold store facilities for the temporary storage. The samples were at the designated centers for a

maximum period of 14 days. They were then transported frozen to IITA Ibadan, where they were kept at -20 °C for a period of 30 days. The samples for serum retinol, CRP and 1-AGP were air freighted in dry ice to the Medical Research Council, Cape Town, South Africa for analyses. Samples for ferritin, transferrin receptors, AGP, CRP, and RBP were air freighted in dry ice to DBS-Tech Laboratory, Willstaett, Germany.

Laboratory methods of analysis and cut-off points

Serum retinol: Serum retinol was determined by a reversed-phase high-performance liquid chromatographic (HPLC) method with wavelength-programmed ultraviolet-visible absorbance detection based on the method described by Catignani and Bieri (1983). The variability was 2.5% (inter-batch) and 3% (intra-batch).

Serum C-reactive protein (CRP): An enzyme immuno-assay procedure was used for the quantitative determination of C-reactive protein in human serum. Commercially available external controls were used as quality measures and were obtained from BIO-RAD Clinical Diagnostics Group, BIO-RAD Liquichek Immunology Control Levels 1, 2 and 3, Ref no. 590X. The kit Cat. No. EIA-1952 was obtained from DRG Diagnostics, DRG International, USA. The variability was 7% (inter-batch) and 5% (intra-batch).

Serum α_{γ} -Glycoprotein (AGP):Alpha 1-acid glycoprotein (AGP) was measured using commercially available ELISA kits no. ABIN414455, GmbH, Schloß-Rahe-Str. 15, 52072 Aachen, Germany. Commercially available external controls were used as quality measures. The variability was 7% (inter-batch) and 5% (intra-batch).

*Retinol Binding protein (RBP), Ferritin, sTfR:*_These parameters were measured using a simple and inexpensive sandwich ELISA technique to measure vitamin A and iron status together in a small serum sample (Erhardt et al., 2004). In addition, CRP and AGP as indicators of infection/inflammation were also measured using the same technique.

Hemoglobin: Hemoglobin was measured on 10 µL whole blood using Hemocue-Hb 201+. This was carried out on the field at data collection. The Hb 201+ system uses a modification of Vanzetti's reagents, utilizing an *azide methemoglobin* reaction yielding results within one minute. Commercially available external controls were used as quality measures.

*Malaria:*_Malaria parasites density was determined by counting the number of parasitized erythrocytes per 2000 erythrocytes from a thin smear and expressed as percentage *parasitaemia* (Das et al., 1997). The slides were prepared at the time of data collection,

Total body iron: It has been reported that ferritin correlates very well with iron stores and the soluble transferrin receptor (sTfR) is increased as a result of iron deficiency. Body iron stores were calculated using the ratio of serum ferritin and soluble transferrin receptor values (Erhardt et al., 2004). Cut-off points used for each biomarker to determine adequacy are presented in **Table 2**.

Table 2: Cut-off points used for classification of adequate and inadequacy/deficiency in children 6-59 months.

| Cut-off points for each biochemical marker | Status |
|--|---|
| Vitamin A status(WHO, 2011) | |
| Serum retinol >0.7µmol/L | Adequate vitamin A/normal |
| Serum retinol <0.7 to ≥0.35 µmol/L | Marginal vitamin A deficiency (VAD) |
| Serum retinol <0.35 µmol/L | Severe VAD |
| Serum RBP >0.7µmol/L | Adequate vitamin A/normal |
| Serum RBP <0.7 to ≥0.35 µmol/L | Marginal vitamin A deficiency (VAD) |
| Serum RBP <0.35 µmol/L | Severe VAD |
| Iron status (WHO, 2011) | |
| Serum ferritin >30 μg/L | Adequate iron status/normal |
| Serum ferritin <30 μg/L | Iron deficiency |
| sTFR >8.3 mg/L (<i>Erhardt et al., 2004</i>) | Iron deficiency |
| sTFR <8.3 mg/L | Adequate iron status/normal |
| Hemoglobin >11g/dl (<i>WHO, 2011</i>) | No anemia |
| Hemoglobin <11g/dl | Anemic |
| Acute phase proteins (Erhardt et al., 2004) | |
| C-reactive protein (CRP) >10 mg/L | Presence of sub-clinical infection/inflammation |
| C-reactive protein (CRP) <10 mg/L | Absence of sub-clinical infection/inflammation |
| Alpha-1-acid glycoprotein (α ₁ -AGP) >1.2 g/L | Presence of sub-clinical infection/inflammation |
| Alpha-1-acid glycoprotein (α_1 -AGP) <1.2 g/L | Absence of sub-clinical infection/inflammation |
| Malaria parasite load | |
| 0 | Negative or no malaria parasite seen |
| 1, 2, and 3 | Mild/moderate parasite infestation |
| 4 | Severe parasite infestation |

For malaria parasite load 0= negative/no malaria parasite seen; 1= fewer than 10 malaria parasites in 100 microscope field; 2= fewer than 10 but more than 6 per 100 microscope fields; 3= more than 10 in 10 microscope fields; 4=more than 20 in 100 microscope fields.

Anthropometry:

Anthropometric indicators for young children and women of child-bearing age in Akwa Ibom were collected in the 2011 survey to provide outcome measures of nutritional status. Stunting (Height-for-age), underweight (Weight-for-age), and wasting (Weight-for height) was determined using WHO-Anthro software (WHO, 2007). Evaluation of nutritional anthropometry in this survey was based on the comparison of these three indices for the population of children in the survey with those reported for a reference population of well-nourished children. The indices are expressed as standard deviation units from the median for the reference group as indicated below (WHO, 2007). For women of childbearing age, Body Mass Index (BMI) was calculated as Weight (kg)/Height² (WHO, 2000). The cut-off points for nutritional status are presented in **Table 3**.

| Cut-off | Classification | |
|--|------------------------------------|--|
| Malnutrition (stunting, wasting, and unc | lerweight) in children (WHO, 2007) | |
| >-2SD to +2SD | Normal | |
| -3SD to -2SD | Mild/Moderate | |
| <-3SD | Severe | |
| Nutrition status of women (BMI) WHO, | 2000) | |
| <18.5 | Thin/underweight | |
| 18-24.9 | Normal/ideal weight | |
| 25-29.9 | Overweight | |
| >30 | Obese | |

Data processing and Statistical analysis

Data processing and analysis was done centrally at IITA. Data entry was accomplished using *MS*-*ACCESS* and *MS*-*Excel*. Data verification, screening, and editing were carried out to ensure that entry errors are corrected. The double data entry technique was used for data quality and verification (Elliott et al., 2007). The double-entered data were compared using the *COMPARE* Procedure of the SAS system to identify erroneously entered data which usually cannot be easily verified or corrected. Serum retinol and ferritin concentrations were adjusted to remove the effects of subclinical inflammation in the assessment of vitamin A and iron deficiency using two markers of infections (CRP and AGP) as indicated below (Thurnham et al., 2010; Thurnham and McCabe, 2012). This resulted in four groups:

- 1. Healthy/reference group (CRP <10 mg/L and AGP<1.2 g/L)
- 2. Incubation group (CRP > 10mg/L and AGP< 1.2 g/L)
- 3. Early convalescence group (CRP >10 mg/L and AGP>1.2g/L)
- 4. Late convalescence group (CRP <10 mg/L and AGP>1.2 g/L)

The frequency of each variable was conducted to assure that the values are within an acceptable range. Essential basic descriptive statistics and plots on distribution were conducted using the Statistical Analysis System (SAS) version 9.2 (SAS, 2001), Cary, NC, USA. Results are presented as means and standard deviations. Pearson's correlation was used to examine relationships among variables and concordance analysis to determine degree of agreement between the two laboratories that conducted the CRP and AGP analyses. The significance of differences in proportion was tested by χ 2 test and p<0.05 was taken as significant. Analysis of variance was used to test for significant differences after log transformation of the data and means separation was done using Tukey's test at p<0.05. Data processed for statistical analysis for each of the investigated parameters are presented in **Table 4**.

| Variable/Parameter | Children 6-59 months | Women of childbearing age (mothers) |
|--|----------------------|--|
| Age | 640 | 643 |
| Height | 639 | 641 |
| Weight | 633 | 638 |
| Stunting | 580 | - |
| Underweight | 586 | - |
| Wasting | 544 | - |
| Body Mass Index (BMI) | - | 638 |
| Vitamin A | 581 | 636 |
| Retinol binding protein (RBP) | 542 | 622 |
| Ferritin | 548 | 622 |
| sTRF | 547 | 622 |
| Iron store | 549 | 622 |
| Hemoglobin | 637 | 639 |
| C-Reactive protein (CRP) | 617 | 622 |
| $\boldsymbol{\alpha}_1$ -Acid glycoprotein ($\boldsymbol{\alpha}_1$ -AGP) | 617 | 622 |
| Malaria | 635 | 624 |

Table 4: Data sets processed for analysis.

Results for Children 6-59 months

The Dietary Intake (DI) study (the result of which is not included in this report) was completed three months before the biological sampling survey (BS) was started. Just as the D1 was being undertaken, a State-wide vitamin A supplementation (part of a national program) was conducted in Akwa Ibom. It was then decided with H+ to allow a three-month gap between the vitamin A supplementation and the commencement of data collection for the BS. The same mother/child pairs that were recruited during the DI survey were visited during the BS. It should be noted that all the subjects in the first survey were not available to participate in the second.

Presented in **Tables 5 and 6** are the numbers of samples collected in each LGA per location for mothers and children and the numbers obtained at each location within an LGA for both DI and biochemical surveys.

| | | Number | of Mothers/Cl | nildren Assess | sed | | |
|-----|-------------------|---------|---------------|----------------|----------|---------|----------|
| No. | LGA Name | Loca | tion 1 | Loca | tion 2 | Loca | ation 3 |
| | | Mothers | Children | Mothers | Children | Mothers | Children |
| 1 | Eastern Obolo | 22 | 22 | 22 | 22 | 22 | 22 |
| 2 | Essien Uddim | 23 | 23 | 24 | 24 | 22 | 21 |
| 3 | Ibeno | 17 | 17 | 19 | 19 | 14 | 14 |
| 4 | Ibiono Ibom | 20 | 20 | 20 | 20 | 22 | 22 |
| 5 | Ikono | 22 | 22 | 24 | 24 | 22 | 22 |
| 6 | Mkpat Enin | 23 | 23 | 22 | 22 | 22 | 22 |
| 7 | Nsit Ubium | 22 | 22 | 20 | 20 | 22 | 22 |
| 8 | Onna | 22 | 22 | 20 | 20 | 22 | 22 |
| 9 | Oruk Anan | 22 | 22 | 23 | 23 | 22 | 22 |
| 10 | Urue Offong Oruko | 22 | 22 | 22 | 22 | 22 | 22 |

Table 5: Number of samples collected at the 10 LGAs from mothers and children in each of the three locations.

Table 6: Number of samples obtained at LGAs by the two surveys.

| | | Location ? | 1 | Location 2 | 2 | Location 3 | 3 |
|-----|----------------------|-------------------|----------------------|-------------------|----------------------|-------------------|----------------------|
| No. | LGA | Dietary intake | Biological Survey | Dietary intake | Biological Survey | Dietary intake | Biological Survey |
| 1 | Eastern Obolo | 20 | 22 | 20 | 22 | 17 | 22 |
| 2 | EssienUdim | 19 | 23 | 20 | 24 | 21 | 22 |
| 3 | Ibeno | 21 | 17 | 19 | 19 | 19 | 14 |
| 4 | lbionolbom | 22 | 20 | 17 | 20 | 18 | 22 |
| 5 | Ikono | 19 | 22 | 20 | 24 | 20 | 22 |
| 3 | MkpatEnin | 19 | 23 | 19 | 22 | 20 | 22 |
| 7 | NsitUbium | 18 | 22 | 16 | 20 | 20 | 22 |
| 3 | Onna | 17 | 22 | 18 | 20 | 22 | 22 |
| 9 | Oruk Anan | 19 | 22 | 19 | 23 | 19 | 22 |
| 10 | Urue Offong Oruko | 19 | 22 | 20 | 22 | 24 | 22 |
| | Total | 193 | 215 | 188 | 216 | 200 | 212 |

The total number of subjects sampled for D1 was 581 and for the BS was 643.

Children 6-59 months

Subjects' characteristics

The mean (\pm standard deviation) age of children surveyed was 30.1 months and the mean age was 30.1 \pm 13.2 months (**Table 7**). Although the mean age for boys was slightly higher than that of girls, it was not significant (*p*=0.117). Overall the mean weight of the subjects was 12.5 \pm 3.7 kg and similar for boys and girls. The mean height for all the children was 86.6 \pm 13.2 cm and although the mean height for boys was more than that of girls, it was not significantly different (*p*=0.104).

| | All subjects | Boys | Girls | P value |
|--------------|-----------------|------------------|------------------|---------|
| | | Mean±SD (N)¹ | | |
| Age (months) | 30.1±13.2 (640) | 30.9± 13.5 (316) | 29.3± 12.9 (324) | 0.117 |
| Weight (kg) | 12.5± 3.7 (633) | 12.7± 3.7 (313) | 12.2± 3.7 (320) | 0.072 |
| Height (cm) | 86.6±13.2 (639) | 87.5± 14.2 (316) | 85.8± 12.2 (323) | 0.104 |

Table 7: Mean age, height, and weight of children 6-59 months.

¹=numbers in brackets are sample sizes

Nutritional status

Stunting is a function of height and age which reflects long-standing deprivation of food and/or chronic debilitating illness. In view of such implications the prevalence of stunting was determined in this study. Overall, 43.1% (n=207) of the children were stunted. Stunting was more prevalent in girls (18.1%, n=88) than in boys (17.6%; n=102). Underweight is a composite index which relates the body weight to the child's age in comparison to reference standards from WHO. Children that are underweight weighs less than they should for the respective age. Prevalence of underweight was estimated at 17.2% (n=101) and was similar for boys and girls. Wasting reflects acute ongoing malnutrition in children. Wasting among all the children under-5 was 12.0% (n=65) and was similar for boys and girls (**Table 8**).

| Category | Stur | nting | Under | weight | Wa | sting |
|---------------------------------|------------|------------|------------|-------------|------------|-------------|
| | | All Su | bjects | | | |
| Normal (>-2SD to +2SD) | 373 (| 56.9) | 485 | (82.8) | 479 (| (88.05) |
| Mild/Moderate (−3SD to −2SD) | 102 (| 21.3) | 60 (| 10.2) | 27 (| (4.96) |
| Severe (<-3SD) | 105 (| 21.8) | 41 (| 7.00) | 38 (| (6.99) |
| All subjects | 580 (| (100) | 586 | (100) | 544 | (100) |
| | | Se | ex | | | |
| | Boys | Girls | Boys | Girls | Boys | Girls |
| Normal (>-2SD) | 183 (32.5) | 190 (32.8) | 241(4.1) | 244 (41.6) | 234 (43.0) | 245 (45.10) |
| Mild/Moderate (−3SD to −2SD) | 51 (8.8) | 51 (8.8) | 30 (5.1) | 30 (5.1) | 15 (2.8) | 12 (2.2) |
| Severe (<-3SD) | 51 (8.8) | 54 (9.3) | 20 (3.4) | 21 (3.6) | 16 (2.9) | 22 (4.0) |
| Total | 285 (49.1) | 295 (50.9) | 291 (49.7) | 295 (50.30) | 265 (48.7) | 279 (51.3) |

Table 8: Nutritional status of children 6-59 months for all subjects and disaggregated by sex.

Biochemical indices

Data on serum concentration of key biochemical indices are presented in **Table 9**. The exclusion of individuals with elevated acute phase proteins has been advocated to improve estimates of vitamin A and iron deficiency prevalence in surveys. In the present study, adjustment of serum retinol and ferritin concentrations to remove the effects of subclinical inflammation was conducted using two acute phase proteins (C-reactive protein [CRP] and α_1 -Acid glycoprotein [AGP]) individually and in combination. This resulted in four groups of subjects (healthy/reference, incubation, early convalescence, and late convalescence).

Serum Retinol: A total of 626 serum samples were collected. Of these, 45 samples were missing due to absence of identification information and inadequate serum. Because of the missing information, the final number of subjects for whom results for serum retinol were available was 581. Mean concentration for all subjects was $0.82\pm0.3 \mu$ mol/L. Overall, mean serum retinol level for boys ($0.83\pm0.3 \mu$ mol/L) was significantly higher (p<0.05) than the mean value for girls ($0.81\pm0.3 \mu$ mol/L).

For the four – group analysis in which the serum retinol concentration in the healthy/reference group was compared with each of the three groups with inflammation, mean serum retinol concentration was significantly (p<0.05) lower in the incubation ($0.75\pm0.3 \mu$ mol/L) and early convalescence ($0.79\pm0.3 \mu$ mol/L) groups than in the healthy/reference group ($0.84\pm0.3 \mu$ mol/L); this trend was also observed when the data were disaggregated by sex. Subjects in the late convalescence stage had serum retinol concentration slightly higher than in the healthy/reference group (**Table 9**).

Retinol Binding Protein (RBP): Retinol binding protein was measured in 549 children. The mean concentration for all subjects was $0.94\pm0.3 \mu$ mol/L. Among the groups, mean RBP concentration was significantly different (p<0.05) among the four groups with the mean concentration of the healthy/ reference group ($1.00\pm0.3 \mu$ mol/L) significantly different than in the incubation ($0.79\pm2.9 \mu$ mol/L) and early convalescence groups ($0.79\pm0.3 \mu$ mol/L). Among the infection/inflammation groups, mean RBP concentration was significantly different in late convalescence ($0.98\pm0.4 \mu$ mol/L) compared with early convalescence ($0.79\pm0.3 \mu$ mol/L) and incubation groups ($0.79\pm2.9 \mu$ mol/L). Mean concentration for girls was $0.95\pm0.3 \mu$ mol/L and significantly (p<0.05) higher than the mean concentration value ($0.93\pm0.4 \mu$ mol/L) for boys. Mean RBP concentration for both boys and girls was significantly lower (p< 0.05) in the incubation and early convalescence groups compared with the healthy/reference and late convalescence groups (**Table 9**).

Serum Ferritin: Blood samples of 549 subjects (276 boys and 273 girls) were used to determine serum ferritin concentrations. The mean concentration for all the subjects was $80.9\pm65.7 \mu g/L$. Mean serum ferritin concentration for girls ($86.3\pm7 \mu g/L$) was significantly (p<0.05) higher than that ($75.7\pm59 \mu g/L$) for boys. However, when the data were corrected for inflammation, 324 subjects (59%) were free of inflammation (i.e., healthy) while 37 subjects (7%) were classified as incubating, 111 were in the early convalescent stage (20%), and 77 were in late convalescence (14%).

In the group analysis, children in early convalescence had significantly higher (p<0.05) mean ferritin concentration (151.8 \pm 8.7 µg/L) compared to those in incubation (102.9 \pm 56.8 µg/L) and healthy (53.7 \pm 38.2 mg/L) groups. Overall, compared to those in the three infection/inflammation groups children in the healthy/reference group had significantly lower ferritin concentration (**Table 9**).

Serum TfR: Mean *sT*fR concentration for all the 549 subjects was 10.2±4.8 mg/L. There was no significant difference between the mean values for boys and girls. Approximately six out of every ten subjects were considered healthy, free of infection/inflammation. The mean sTfR for this group was 8.9±3.9 mg/L and significantly different (p<0.05) than the mean concentrations of the late convalescence (12.8±6.3 mg/L) and early convalescence groups (12.2±4.9 mg/L). When the data

were disaggregated by sex across the four groups, a pattern seems to emerge with the mean sTfR values for the convalescing subjects (12.6 ± 5.8 mg/L) being significantly higher than for those at incubation level (9.9 ± 3.7 mg/L) which, in turn, is significantly higher than that of boys with no inflammation (9.2 ± 4.2 mg/L) f (**Table 9**). This trend was also observed for girls.

Hemoglobin: Hb was determined in 637 subjects. The mean Hb level for all subjects was 9.9 ± 1.6 g/dL, and there was no significant difference (p>0.05) between the mean value for boys and that of girls. There was however, a tendency for the mean Hb value to decrease with the level of inflammation (Table 9). Mean Hb level for the healthy/reference group (10.3±1.4 g/dL) was significantly higher (p<0.05) than the mean Hb concentration for late convalescence (9.4±1.6 g/dL) and early convalescence (9.3±1.8 g/dL). Indeed, the mean Hb level for the healthy/reference group was significantly higher than those of children experiencing early convalescence. Adjusting for inflammation resulted in a slightly higher mean (10.3±1.4 g/dL) compared to the mean of all subjects (9.8±1.6 g/dL).

Iron Store: The sTfR and ferritin ratio can be used to provide an estimate of body iron. The same number of subjects (549) was used to determine serum ferritin and sTfR and for determining iron store. The mean value for all subjects was 5.1 ± 3.4 mg/kg. The mean value for girls (5.4 ± 3.2 mg/kg) was significantly higher (p<0.05) than that (4.8 ± 3.7 mg/kg) of boys. Almost 60% (324) of the children were classified as healthy with mean iron store level of 4.3 ± 3.3 mg/kg. Mean iron store level (6.5 ± 2.9 mg/kg) of the incubation group was significantly higher (p<0.05) than those of the healthy (4.3 ± 3.3 mg/kg) and late convalescence groups (4.6 ± 3.6 mg/kg). In the early convalescence group, the mean iron store in girls (7.8 ± 2.3 mg/kg) was significantly higher than that in the boys (6.5 ± 3.2 mg/kg).

Acute phase proteins

Current investigations of the prevalence of iron deficiency (ID) and VAD often incorporate some measures of infection/inflammation to aid in interpreting some markers of iron and vitamin A status which are altered during acute phase reactions (Beard et al., 2006). The acute phase response is the body's immediate reaction to infection and inflammation. C-reactive protein (CRP) is probably the most used sensitive acute phase protein for monitoring infection and inflammatory activity.

However, CRP concentration may not remain high enough to detect subjects in early convalescence (Young at al., 1991) and thus may not be useful in nutritional studies of apparently healthy individuals. Other acute phase proteins remain elevated for longer than CRP and may therefore be more useful for detecting subjects in whom infection has subsided but whose retinol concentrations may still be depressed. Alpha₁-glycoprotein (α_1 .AGP) is one of such proteins. Because poor vitamin A and iron status and infections co-exist in populations of less developed countries it is difficult to establish whether low plasma retinol and high serum ferritin concentration are due to nutritional or to pathologic causes. In this report CRP and AGP measurements were used to assess sub-clinical infection and the association between this and serum retinol and ferritin levels. Presented in **Table 10** is the concentration of the two acute phase proteins CRP and AGP. Results are shown first for all subjects and then for the healthy/reference and inflammation groups.

C- reactive protein (CRP): CRP serum concentrations were analyzed in two separate laboratories, 549 subjects in Germany and 616 in South Africa. Mean CRP concentration of all the subjects ranged from 9.7±14.9 (n=549) and 12.6±22.4 mg/L (n=616). In the four-group analysis, mean CRP concentrations were significantly higher in all the inflammation groups with mean concentration of 20.0±11.0 mg/L for incubation, 32.2±17.4 mg/L for early convalescence, 4.5±2.5 mg/L for late convalescence, compared with 1.9±2.2 mg/L for the healthy/reference group (**Table 10**). In the groups

| Variable | All subjects | | | Healthy (CKP < 10 mg g/L) | < 10 mg/L ant | 1465 × 1.2 | Incubation (t | /L and AGP < 1.2 Incubation (CRP > 10 mg/L | | Early convale AGP > 1.2 g/l | במוץ convalescence (כגד > זט mg/L and Late convalescence (כגד < זט AGP > 1.2 g/L) mg/L and AGP > 1.2 g/L) | 10 mg/L and | Late convai mg/L and A(| escence (ur 3P > 1.2 g/L | (P < 10 |
|---------------------------------|--------------|------------|----------------------------------|------------------------------|---------------|---------------|--------------------|--|-----------|--------------------------------|---|---------------------------------|----------------------------|-----------------------------|-----------|
| | | | | | | Me | Mean ± std dev (N) | (Z | | | | | | | |
| | All | Boys | Girls | AII | Boys | Girls | All | Boys | Girls | All | Boys | Girls | AII | Boys | Girls |
| Serum retinol (µmol/L) 0.82±0.3 | 0.82±0.3 | 0.83±0.3 | 0.81± 0.3 | 0.84± 0.3 | 0.84±0.3 | 0.83± 0.3 | 0.75± 0.3 | 0.72±0.3 | 0.78± 0.3 | 0.79± 0.3 | | 0.75± 0.2 | 0.86± 0.3 | 0.84± 0.3 | 0.88± 0.3 |
| | (581) | (287) | (293) | | (155) | (145) | (36) | (21) | (15) | (173) | | (66) | (71) | (37) | (34) |
| Retinol Binding Protein | 0.94 ± 0.3 | 0.93±0.4 | 0.95±0.3 | 1.00±0.3 | 0.98±0.3 | 1.01±0.3 | 0.79±2.9 | 0.79±0.4 | 0.78±0.3 | 0.79±0.3 | | 0.81±0.3 | 0.98±0.4 | 0.99±0.5 | 0.97±0.3 |
| (RBP; µmol/L) | (549) | (276) | (273) | (324) | (168) | (156) | (37) | (21) | (16) | (111) | (49) | (62) | (77) | (38) | (39) |
| | | | | | | | | | | | | | | | |
| | 80.98±65.7 | 75.70±59.4 | 80.98±65.7 75.70±59.4 86.32±71.3 | 53.67±38.2 53.70± | 53.70±38.5 | 53.64±38.1 | 102.92±56.8 | 111.73±50.5 | 91.35±6 | 151.79±78.7 | 134.15±77.7 | 165.73±77.4 | 83.29±4.6 | 77.68±7.7 | 88.75±4.6 |
| Serum ferritin (µg/L) | (549) | (276) | | (324) | (168) | (156) | (37) | (21) | (16) | (111) | (49) | (62) | (77) | (38) | |
| sTfR (mg/L) | 10.22±4.8 | 10.40±4.9 | 10.04±4.6 | | 9.24±4.2 | 8.66±3.5 | 9.95±3.7 | 9.90±3.6 | 10.01±3 | 12.21±4.9 | 12.90±5.6 | 11.66±4.3 | 12.77±6.3 | 12.56±5.8 | |
| - - | (549) | (276) | (273) | | (168) | | (37) | (21) | (16) | (111) | (49) | (62) | (22) | (38) | |
| Hemoglobin (g/dl) | 9.84±1.6 | 9.75± 1.6 | 9.94± 1.7 | | 10.12± 1.4 | | 9.53± 1.5 | 9.74± 1.4 | 9.23± 1. | 9.34± 1.8 | 9.19± 1.8 | 9.45± 1.8 | 9.37± 1.6 | 9.42± 1.5 | |
| • | (637) | (314) | (322) | | (164) | (154) | (36) | (21) | (15) | (201) | (89) | (112) | (81) | (40) | |
| Iron store (mg/kg) | 5.10±3.5 | 4.82±3.7 | 5.39±3.2 | 4.32±3.3 | 4.13±3.6 | 4.52 ± 2.9 | 6.51±2.9 | 7.01±2.9 | 5.86±2.9 | 7.26±2.8 | 6.55±3.2 | 7.81±2.3 | 4.59±3.6 | 4.40±3.6 | |
| | (549) | (276) | (273) | (324) | (168) | (156) | (37) | (21) | (16) | (111) | (49) | (62) | (22) | (38) | (39) |

with inflammation, most children were in early convalescence and the fewest were in the incubation group.

AGP serum concentrations of 549 and 617 subjects were analyzed in the same two laboratories that analyzed CRP. The mean AGP values for all subjects was $1.1\pm0.3g/L$ (n=549) and $0.9\pm0.5g/L$ (n=617). Mean AGP was $0.9\pm0.2g/L$ for the healthy/reference group, $1.1\pm0.1g/L$ for incubation, 1.5 ± 0.2 for early convalescence, and 1.4 ± 0.2 for late convalescence. No significant differences were observed for mean AGP among the different groups or between boys and girls (**Table 10**).

The acute phase proteins (CRP and AGP) were analyzed in two separate laboratories. The correlation between laboratories was significantly stronger for CRP (r = 0.93) compared to that of AGP (r = 0.69). We also conducted concordance analysis to determine the degree of agreement between the two laboratories that analyzed the CRP and AGP samples. There was moderate agreement for AGP (r = 0.62) and substantial agreement for CRP (r = 0.80).

| Table 10: N | Table 10: Mean acute phase proteins concentration in children 6 | hase prote | eins conce | ntration in | children 6 | 6-59 months | ŝ | | | | | | | | |
|-------------------------|--|-----------------|-----------------|-------------------|--|-----------------|------------------------|-------------------------------|---------------------|------------------------|---|-----------------|---------------------|--|------------------------|
| Variable | All subjects | ots | | Healthy and AG | Healthy (CRP < 10 and AGP < 1.2 g/L | 0 mg/L _) | Incubatior 10 mg/L) | Incubation (CRP > 10 mg/L) | Early co AGP > 1 | nvalescencı .2 g/L) | Early convalescence (CRP > 10 mg/L and AGP > 1.2 g/L) |) mg/L and | Late con 10 mg/L | Late convalescence (CRP < 10 mg/L and AGP > 1.2 g/L) | e (CRP < • 1.2 g/L) |
| | | | | | | | Mean ± std dev (N) | d dev (N) | | | | | | | |
| | All | Boys | Girls | AII | Boys | Girls | AII | Boys | Girls | AII | Boys | Girls | AII | Boys | Girls |
| CRP | 9.7 | 13.4 | 11.8 | 0.1 0.0 | 3.1 | 2.943 | 20.0 | 25.4 | 22.6 | 32.2 | 35.6 | 25.2 | 4.5 . 0 r | 5.5 | 5.5 |
| (mg/r) | ± 14.9 (549) | ± 23.9 (306) | ± 20.7 (310) | ± 2.3 (320) | ± 4.9 (160) | ± 4.19 (150) | ± 11.0 (36) | ± 14.4 (21) | ± 10.3 (14) | ± 17.4 (112) | ± 30.1 (85) | ± ∠9.5 (106) | ± 2.5 (81) | ± 3.3 (40) | ± 3.1 (40) |
| CRP | 12.6 | 9.7 | 9.7 | 2.9 | 1.8 | 2.076 | 24.3 | 20.8 | 19.0 | 28.9 | 35.5 | 29.55 | 5.5 | 4.4 | 4.6 |
| $(mg/L)^2$ | ± 22.4 | ± 15.5 | ± 14.4 | ±4.6 | ± 2.1 | ± 2.41 | ± 14.9 | ± 12.5 | ± 8.9 | ±32.8 | ± 17.5 | ± 17.0 | ± 3.2 | ± 2.5 | ± 2.5 |
| | (616) | (275) | (273) | (311) | (165) | (164) | (35) | (21) | (15) | (191) | (49) | (63) | (80) | (40) | (41) |
| α-1-AGP | 1.1 | 0.89 | 0.90 | 0.88 | 0.67 | 0.647 | 1.0 | 0.92 | 0.88 | 1.5 | 1.2 | 1.2 | 1.4 | 1.1 | 1.2 |
| (g/L) ¹ | ± 0.3 | ± 0.5 | ± 0.5 | ±0.2 | ±0.3 | ± 0.343 | ± 0.1 | ± 0.5 | ± 0.3 | ± 0.2 | ± 0.5 | ± 0.6 | ± 0.2 | ± 0.3 | ± 0.5 |
| | (549) | (306) | (310) | (320) | (160) | (150) | (36) | (21) | (14) | (112) | (85) | (106) | (81) | (40) | (40) |
| α-1-AGP | 0.89 | 1.1 | 1.1 | 0.66 | 0.89 ± | 0.86 | 06.0 | 1.1 | 1.0 | 1.2 | 1.5 | 1.5 | 1.2 | 1.4 | 1.4 |
| (g/L) ² | ± 0.5 | ± 0.3 | ± 0.4 | ±0.3 | 0.2 | ± 0.2 | ± 0.4 | ± 0.1 | ± 0.1 | ± 0.6 | ± 0.2 | ± 0.3 | ± 0.4 | ± 0.1 | ± 0.2 |
| | (617) | (275) | (273) | (311) | (165) | (154) | (35) | (21) | (15) | (191) | (49) | (63) | (80) | (40) | (41) |
| ¹ Samples an | Samples analyzed at the DBS-Tech Laboratory. Willstaett. Germany | DBS-Tech Le | sboratory. Wi | llstaett. Gerr | nanv | | | | | | | | | | |

¹Samples analyzed at the DBS-Tech Laboratory, Willstaett, Germany ²Samples analyzed at the South Africa Medical Research Council, Tygerberg, Cape Town, South Africa

Vitamin A status

Vitamin A status as measured by serum retinol:

In the present study, data were obtained from persons who were apparently healthy but in whom subsequent analysis using two acute phase proteins (CRP and AGP) showed some presence of inflammation. Most subjects (301) fell in the healthy/reference group category (no elevated acute phase proteins) followed by those in early convalescence (173), late convalescence (91), and incubation (36).

Estimates of vitamin A status in all subjects (581) before adjusting for inflammation indicate that 352 subjects (60.6%) had adequate vitamin A status (>0.7 μ mol/L), 213 subjects (36.7%) had marginal VAD (<0.7 to ≥0.35 μ mol/L), and 16 subjects (2.8%) had severe VAD. When we combined marginal and severe VAD, 229 subjects (39.5%) are vitamin A deficient in all the subjects. In the four-group analysis, marginal VAD in the healthy/reference group was 17.4% (n=101) and severe VAD was 1.5% (n=9). When we combined marginal and severe VAD in this group, 18.9% (n=110) of children had biochemical VAD. Adjustment for inflammation resulted in a reduction of 47.8% in the prevalence of VAD from 39.5% to 18.9% (**Table 11**). Among the subjects of the other three inflammation groups VAD was highest in the early convalescence group 13.1% (n=173) followed by late convalescence at 4.1% (n=71) and the incubation group at 3.3% (n=36). These prevalent values were significant at p<0.05.

Vitamin A status as measured by retinol binding protein (RBP). Because retinol is carried in the blood in approximately 1:1 molar ratio by RBP, measurement of RBP is used as a surrogate for serum retinol (Beetham et al., 1985). Presented in **Table 11** is the *vitamin* A status of the 542 subjects as measured by RBP; 408 (75.3%) had adequate (70.7 μ mol/L) vitamin A status, 127 (23.4%) had marginal VAD, and 7 (1.3%) had severe VAD. Combining marginal and severe VAD resulted in a prevalence rate of 24.7% (Table 11). Adjusting for inflammation using the acute phase proteins (CRP and AGP) showed a VAD prevalence rate of 12%. Subjects in early convalescence had significantly (p<0.001) higher VAD (7.8%) than those to late convalescence (2.4%) and incubation (2.6%). Among the 542 investigated subjects, 290 (59%) were in the healthy/reference group, 111 (20.5%) were in early convalescence, 74 (13.7%) were in late convalescence, and only 37 (6.3%) were in the incubation group.

| Table 11: Micronutrient status | of children 6-59 months. |
|--------------------------------|--------------------------|
|--------------------------------|--------------------------|

| Variable | All subjects | Healthy (CRP < 10 and AGP < 1.2) | Incubation (CRP > 10) | Early Convalesce (CRP > 10 and AGP > 1.2) | Late Convalesce (CRP < 10 and AGP > 1.2) |
|---|-----------------|---|--------------------------|---|--|
| | | | N (%) | | |
| Subjects with serum retinol > 0.7 mmol/L | 352 (60.6) | 191 (32.9) | 17 (2.9) | 97 (16.7) | 47 (8.1) |
| Subjects with serum retinol <0.7 to ≥0.35 µmol/L | 213 (36.7) | 101 (17.4) | 18 (3.1) | 72 (12.4) | 22 (3.8) |
| Subjects with serum retinol <0.35 µmol/L | 16 (2.8) | 09 (1.5) | 01 (0.2) | 04 (0.7) | 02 (0.3) |
| Total | 581 (100) | 301 (51.8) | 36 (6.2) | 173 (29.8) | 71 (12.2) |
| P value = 0.032 | | | | | |
| Subjects with RBP > 0.7 mmol/L | 408 (75.3) | 225 (47.0) | 23 (4.2) | 69 (12.7) | 61 (11.3) |
| Subjects with RBP <0.7 to ≥0.35 µmol/L | 127 (23.4) | 62 (11.4) | 12 (2.2) | 40 (7.4) | 13 (2.4) |
| Subjects with RBP <0.35 µmol/L | 07 (1.3) | 03 (0.6) | 02 (0.4) | 02 (0.4) | 0.0 (0.0) |
| Total | 542 (100) | 290 (59.0) | 37 (6.3) | 111 (20.5) | 74 (13.7) |
| P value =0.001 | | | | | |
| Subject with serum ferritin> 30 | 427 (77.9) | 221 (40.3) | 34 (6.2) | 107 (19.5) | 65 (11.9) |
| Subject with serum ferritin <30 µg/L | 121 (22.1) | 102 (18.6) | 03 (0.5) | 04 (0.7) | 12 (2.2) |
| Total | 548 (100) | 323 (58.9) | 37 (6.8) | 111 (20.3) | 77 (14.1) |
| <i>P</i> value = 0.000 | | | | | |
| Subjects with STfR < 8.3 mg/L (adequate) | 221 (40.4) | 148 (27.1) | 12 (2.2) | 37 (6.8) | 24 (4.4) |
| Subjects with STfR> 8.3mg/L | 326 (59.6) | 175 (32.0) | 25 (4.5) | 73 (13.3) | 53 (9.7) |
| Total | 547 (100) | 323 (59.0) | 37 (6.8) | 110 (20.1) | 77 (14.1) |
| P value =0.021 | | | | | |
| Subjects with hemoglobin >11 g/dl | 160 (25.1) | 110 (17.3) | 07 (1.1) | 34 (5.3) | 09 (1.4) |
| Subjects with hemoglobin < 11 g/dl | 477 (74.9) | 209 (32.8) | 29 (4.6) | 167 (26.2) | 72 (11.3) |
| Total | 637 (100) | 319 (50.1) | 36 (5.7) | 201 (31.6) | 81 (12.7) |
| P value=0.000 | | | | | |

To classify the population sample according to the severity of the VAD problem in public health terms it is necessary to consider other parameters recommended by WHO (2011). More specifically, the consultation designated VAD as a public health problem requiring intervention when at least one of two specifications is met: (1) The prevalence of low serum retinol is within the range specified and another biological indicator of vitamin A status (including night blindness, breast milk retinol, relative dose response, modified dose response, or conjunctival impression cytology) also indicates widespread deficiency; and/or (2) The prevalence of low serum retinol indicates widespread deficiency and at least four of the demographic and ecologic risk factors are met: (a) infant mortality rate higher than 75/1000 live births and mortality rate for children under-5 is higher than 100/1000 live births; (b) full immunization coverage in less than 50% of children at 12-23 months of age; (c) less than 50% prevalence of breastfeeding in 6-month-old infants; (d) median dietary intake lower than 50% of recommended safe level of intake among 75% of children 1-6 years of age; (e) two-week period prevalence of diarrhea is 20% or higher; (f) measles case fatality rate is 1% or higher; (g) no formal schooling for 50% or more of women 15-44 years of age; (h) less than 50% of households have a safe water source.

Although the survey was not designed and the ethical clearance did not include collection of data to provide for any of the above demographic and ecologic risk factors to determine if VAD is of public health importance, the Nigeria Demographic and Health Survey 2008 reported an infant mortality rate of 84 per1000 live births; an under-5 mortality of 138 per 1000 live births, full immunization coverage of 23% nationally and 32.4% in Akwa Ibom State among children 12-23 months old, and an exclusive

breast feeding rate of 13% (NPopC, ICF Macro, 2009). Based on the level of biochemical VAD and the ecological risk factors, VAD is of public health significance in Akwa Ibom.

Iron status

Iron stores in the body exist primarily in the form of ferritin and small amounts are secreted into plasma or serum. The concentration of this ferritin is positively correlated with the size of the total body iron stores in the absence of inflammation. A low serum ferritin value reflects depleted iron stores, and may not necessarily reflect the severity of the depletion as it progresses (WHO, 2011). However, ferritin is a positive acute phase protein whereby concentrations increase during inflammation. Consequently, in such situations it may no longer reflect the size of iron stores (Gibson, 2005). This makes the interpretation of normal or high serum ferritin values difficult in areas of widespread infection or inflammation (Beard et al., 2006).

In the absence of inflammation or liver disease, high serum ferritin concentrations (>300 µg/L) may indicate iron overload. Erhardt and colleagues (2004) reported that the two best parameters for assessing iron status are ferritin and soluble transferrin receptors (sTfR). As a result of iron deficiency (ID), sTfRs are increased and not significantly affected by inflammation. In the current study, both serum ferritin and sTfR were measured to determine ID.

Iron status as measured by serum ferritin

The measurement of serum ferritin concentrations to assess iron status in populations is the principal recommendation of the World Health Organization (WHO/CDC, 2007). However, it is also recognized that ferritin is a positive acute phase protein and thus it is recommended that ferritin measurements should be accompanied by one or more from the data on acute phase proteins to detect the presence of infection or inflammation.

The prevalence of ID as measured by serum ferritin was 22.1% (n=121) among all subjects. When the data were adjusted for possible inflammation using CRP and AGP concentrations, 18.6% (n=102) of subjects in the healthy/reference group suffered from ID (<30 mg/L) and was significantly different (p<0.000) compared with those in the incubation, early, and late convalescence groups (**Table 11**). In these groups, ID ranged from 0.5% for incubation to 2.2% in late convalescence.

Iron status as measured by serum Transferrin receptor (sTfR)

Values of sTfR concentration > 8.3 are considered consistent with ID. Of the 547 subjects; 326 (59.6%) were considered iron deficient and 221 (40.4%) as having adequate iron status (**Table 11**). Among the investigated subjects, 323 (59%) were in the healthy category, 37 (6.8%) in the incubation group, 110 (20.1%) in early convalescence, and 77 (14.1%) in late convalescence. After adjustment for inflammation, 175 subjects were iron deficient in the healthy/reference group, 73 (13.3%) in early convalescence, 53 (9.7%) in late convalescence, and 25 (4.5%) in the incubation group (**Table 11**). There was a reduction in the prevalence of ID from 326 to 175 subjects (53.7%). The difference among the groups was significant at p<0.05.

Anemia as measured by hemoglobin level

Anemia is amongst the most important contributing factors to the global burden of disease. Although anemia has a variety of causes, it is generally assumed that 50% of cases are caused by iron deficiency (Black et al., 2003). In the present study, a total of 637 children from 6-59 months were investigated for the presence of anemia using hemoglobin with a concentration of <11 g/dl indicative of anemia. Using the concentration of acute phase proteins (CRP and AGP), 319 (50.1%) of the subjects were in the healthy/reference group, 36 (5.7%) in incubation; 201 (31.6%) in convalescence, and 81 (12.7%) in late convalescence. Prevalence of anemia was highly significant at p=0.000 and was estimated at 74.9% (n=477) in all subjects and at 32.8% (n=209) after adjustment for inflammation (**Table 11**). In the remaining three inflammation groups, anemia prevalence ranged from 4.6% in the incubation group to 26.2% in early convalescence.

Micronutrient status of children by sex

The micronutrient status of boys and girls with and without inflammation are presented in **Table 12**. Of the 580 children, there were 287 (49.5%) boys and 293 (50.5%) girls. Significant differences between boys and girls (p<0.05) were observed for VAD, RBP, ID, STfR, and hemoglobin. VAD was more prevalent in girls 20.8% (n=121) and 18.4% (n=107) in all the subjects compared to the healthy/reference group that had VAD of 17.3% (52) for girls and 19.0% (n=57) for boys. VAD was significantly high (p<0.05) between boys and girls in each of the inflammation groups (**Table 12**). There were girls (n=64) in early convalescence followed by those in late convalescence.

Iron deficiency was measured by serum ferritin, and ID was significantly higher (p<0.05) in boys (12.4%) than girls (9.6%) in all subjects. When the data were corrected for inflammation using CRP and AGP, ID was significantly (p<0.05) higher in boys (21%) than in girls (16.2%) and ranged from 0.4 to 2.5% for boys and from 0.7 to 1.8% for girls in the inflammation groups (**Table 12**).

Soluble Transferrin Receptor (sTfR) is increasingly being used to determine iron deficiency in situations where inflammation is common (Biesalski and Erhardt, 2007) as it is less influenced by infection. It is not as sensitive as ferritin but more sensitive than hemoglobin. In the present study, ID (sTfR>8.3mg/L) was similar to boys and girls in all the subjects (**Table 12**). When we adjusted for inflammation, prevalence of ID was higher in boys (31.2%; n=84) compared to girls (26.5%; n=72) in the healthy/reference group. In the inflammation groups, there were more children of both sexes with ID in the early convalescence group.

Anemia

Anemia (hemoglobin <11 g/dl) was significantly different and higher (46.5%) in girls compared to boys (36.5%) in early convalescence (**Table 12**) followed by those in late convalescence 43.2% for boys and 45.7% for girls. Adjustments for inflammation resulted in a slight decrease in anemia and a non-significant difference for boys (from 38.1 to 36.9%) but a significant difference for girls (from 36.9 to 29.2%).

| | All subjects | | Healthy < 10 an < 1.2) | | Incubation > 10) | on (CRP | Early Conv (CRP > 10 > 1.2) | | Late Conv (CRP < 10 > 1.2) | |
|--|----------------|---------------|------------------------------|---------------|---------------------|--------------|-----------------------------------|--------------|----------------------------------|-----------|
| | | | 1.2) | N | (%) | | - 1.2) | | - 1.2) | |
| Parameter | Boys | Girls | Boys | Girls | Boys | Girls | Boys | Girls | Boys | Girls |
| Subjects with serum retinol >0.7µmol/L ^{1*} | 180 (31.03) | 172 (29.6) | 98 (32.7) | 93 (31.0) | 10 (27.8) | 07 (19.4) | 45 (26.0) | 52 (30.1) | 27 (38.0) | 20 (28.2) |
| Subjects with serum retinol <0.7 to >0.35µmol/L ² | 97 (16.7) | 115 (19.8) | 51 (17.0) | 49 (16.3) | 10 (37.8) | 08 (22.2) | 27 (15.6) | 45 (26.0) | 09 (12.7) | 13 (18.3) |
| Subjects with serum retinol <0.35µmol/L ³ | 10 (1.7) | 06 (1.0) | 06 (2.0) | 03 (1.0) | 01 (2.8) | 00 (0.67) | 02 (1.2) | 02 (1.2) | 01 (1.4) | 01 (1.4) |
| Total | 287 | 293 | 155 | 145 | 21 | 15 | 74 | 99 | 37 | 34 |
| Subjects with RBP >0.7µmol/L ^{1*} | 203 (37.0) | 205 (37.4) | 126 (45.5) | 124 (45.8) | 14 (5.1) | 8 (3.0) | 33 (11.9) | 40 (14.8) | 30 (10.8) | 33 (12.2) |
| Subjects with RBP <0.7 to >0.35µmol/ L ² | 69 (12.6) | 64 (11.7) | 38 (13.7) | 26 (9.6) | 05 (1.8) | 07 (2.6) | 16 (5.8) | 24 (8.9) | 10 (3.6) | 07 (2.6) |
| Subjects with RBP | 05 (9) | 02 (3.6) | 01 | 02 | 02 | 00 (0) | 02 (0.7) | 00 (0) | 00 (0) | 00 (0) |
| <0.35µmol/L ³ | | | (0.4) | (0.7) | (0.7) | | | | | |
| Total | 277 | 271 | 165 | 152 | 21 | 15 | 51 | 64 | 40 | 40 |
| Serum ferritin > | 208 (37.9) | 219 | 107 | 108 | 20 | 13 | 48 (17.4) | 62 | 33 (12.0) | 36 (13.2) |
| 30µg/L¹* | | (39.9) | (38.8) | (39.7) | (7.2) | (4.8) | | (22.8) | | |
| Subjects with | 68 (12.4) | 53 (9.6) | 58 | 44 | 1 (0.4) | 2 (0.7) | 02 (0.7) | 02 (0.7) | 07 (2.5) | 05 (1.8) |
| Serum ferritin< 30 | | | (21.0) | (16.2) | | | | | | |
| µg/L⁴ | | | | | | | | | | |
| Total | 276 | 272 | 165 | 152 | 21 | 15 | 50 | 64 | 40 | 41 |
| Subjects with | 106 (19.4) | 112 | 79 | 80 | 9 (3.3) | 06 | 11(4.0) | 14 (5.1) | 7 (2.5) | 12 (4.4) |
| sTfR<8.3mg/L1* | | (20.5) | (28.6) | (29.4) | | (2.2) | | | | |
| Subjects with | 168 (30.8) | 160 | 84 | 72 | 12 | 09 | 39 (14.1) | 50 | 33 (12.0) | 29 (10.7) |
| sTfR>8.3mg/L⁴ | | (29.3) | (31.2) | (26.5) | (4.3) | (3.3) | | (18.4) | | |
| Total | 274 | 272 | 165 | 152 | 21 | 15 | 50 | 64 | 40 | 41 |
| Subjects with | 71 (11.2) | 88 | 48 | 61 | 05 | 02 | 15 (7.5) | 19 (9.5) | 03 (3.7) | 06 (7.4) |
| hemoglobin >11g/ dL¹* | | (13.8) | (15.1) | (19.2) | (13.9) | (5.6) | | | | |
| Subjects with | 242 (38.1) | 234 | 116 | 93 | 16 | 13 | 73 (36.5) | 93 | 37 (45.7) | 35 (43.2) |
| hemoglobin <11g/ dL ⁴ | | (36.9) | (36.5) | (29.2) | (44.4) | (36.1) | | (46.5) | | |

¹=Adequate/normal, ² =marginal, ³ = severe inadequacy, ⁴= inadequate, RBP= retinol binding protein, * p<0.05

Malaria

Malaria commonly afflicts populations that are impoverished and malnourished, and a large proportion of the burden of malaria falls upon children (SanJoaquin and Molyneux, 2009).

A total of 625 children were tested for malaria; however, only 584 are reported here as some of the acute phase protein concentrations were not available to classify the subjects into groups. Out of these 584, about 65% were found to be negative for malaria parasite although 29.9% had mild to moderate parasitaemia and about 5% had severe malaria parasite load. Using acute phase proteins to classify them into healthy, incubation, early and late convalescence groups, 371 (63.5%) were in the healthy category, 90 (15.4%) were in the incubation category, 86 (14.7%) were in early and 37 (6.3) in late convalescence categories (**Table 13**). Of the 371 children in the healthy/reference group, 113 (19.3%) had mild/moderate malaria parasitaemia.

| Malaria Load | Healthy Group | Incubation Group | Early Convalescence | Late Convalescence | All Subjects |
|---------------|------------------|---------------------|------------------------|-----------------------|--------------|
| | | | N (%) | | |
| Negative | 258 (44.2) | 56 (9.6) | 43(7.4) | 22 (3.8) | 379 (64.9) |
| Mild/moderate | 101 (17.2) | 28 (4.8) | 32(5.4) | 14 (2.3) | 175(29.9) |
| Severe | 12 (2.1) | 6 (1.0) | 11 (1.9) | 1 (0.2) | 30 (5.2) |
| Total | 371 (63.5) | 90(15.4) | 86 (14.7) | 37 (6.3) | 584 (100) |

| Table 13: Malaria parasit | e load in | children | 6-59 months. |
|---------------------------|-----------|----------|--------------|
|---------------------------|-----------|----------|--------------|

P< 0.01

The prevalence of malaria between boys and girls as examined in this study is provided in **Table 14.** Although there is a slightly higher prevalence in girls with mild to moderate malaria parasite load, the difference between the sexes is not significant. The difference between the girls in the various groups is also not significant (p=0.158) although significant among the boys (p<0.05).

| Malaria Load | Healthy Group | Incubation Group | Early Convalescence | Late Convalescence | All subjects |
|---------------|---------------|---------------------|------------------------|-----------------------|--------------|
| | | Ν | (%) | | |
| | | В | oys* | | |
| Negative | 128 (44.8) | 28 (9.8) | 19 (6.6) | 13 (4.5) | 188 (65.7) |
| Mild/moderate | 51 (17.8) | 13 (4.5) | 14 (4.9) | 3 (1.1) | 81 (28.4) |
| Severe | 5 (1.7) | 4 (1.4) | 8 (2.8) | 0 | 17 (5.9) |
| Total | 184 (64.3) | 45 (15.7) | 41 (14.3) | 16 (5.6) | 286 (100) |
| p<0.05 | | | | | |
| | | (| Girls | | |
| Negative | 130 (43.8) | 28 (9.4) | 24 (8.1) | 9 (3.0) | 191 (64.3) |
| Mild/moderate | 49 (16.5) | 15 (5.0) | 18 (6.0) | 11 (3.7) | 93 (31.3) |
| Severe | 7 (2.4) | 2 (0.7) | 3 (1.0) | 1 (0.3) | 13 (4.4) |
| Total | 186 (62.6) | 45 (15.2) | 45 (15.2) | 21 (7.1) | 297 (100) |
| p>0.05 | | | | | |

| Table 14: Malaria | parasite load in | n children 6-5 | 59 months | disaggregated b | v sex. |
|-------------------|------------------|----------------|-----------|-----------------|---------------|
| | puraonto roua n | | | alouggiogutou b | J 00A. |

When we examined malaria parasite load and acute phase proteins (**Table 15**) among boys and girls, the data indicate that malaria parasite load was present in 23.4% of boys and 23% of girls in those children having CRP<10mg/L and was lower in children having CRP >10mg/L (10.6% in boys and 12.3% in girls). This trend was also observed for AGP.

| Malaria Load | C-reactive protein < 10 mg/L | C-reactive protein > 10 mg/L | Total | α-1-Acid glycoprotein < 1.2 g/L | α-1-Acid glycoprotein > 1.2 g/L | All subjects |
|---------------|------------------------------------|------------------------------------|------------|---------------------------------------|---------------------------------------|--------------|
| | | | N (%) | | | |
| | | | Boys* | | | |
| Negative | 154 (53.1) | 37(12.8) | 191 (65.9) | 121 (47.5) | 43 (16.9) | 164 (64.3) |
| Mild/moderate | 61 (21.0) | 21(7.2) | 82 (28.3) | 44 (17.2) | 31 (12.0) | 75 (29.4) |
| Severe | 7 (2.4) | 10 (3.4) | 17 (5.8) | 8 (3.1) | 8 (3.1) | 16 (6.3) |
| Total | 222 (76.6) | 68 (23.4) | 290 (100) | 173 (67.8) | 82 (32.2) | 255 (100) |
| | | | Girls | | | |
| Negative | 152 (50.7) | 42 (14.0) | 194 (64.7) | 110 (43.5) | 50 (19.8) | 160 (63.2) |
| Mild/moderate | 60 (20.0) | 33 (11.0) | 93 (31.0) | 42 (16.6) | 38 (15.0) | 80 (31.6) |
| Severe | 9 (3.0) | 4 (1.3) | 13 (4.3) | 6 (2.4) | 7 (2.8) | 13 (5.1) |
| Total | 221 (73.7) | 79 (26.3) | 300 (100) | 158 (62.5) | 95 (37.5) | 253 (100) |

Table 15: Malaria parasite load and acute phase proteins.

Five hundred and eight ferritin data and malaria data were available for the analysis; above 64% of these children had negative malaria parasitaemia. Among them, the level of ferritin was adequate in 240 (47.2%) and inadequate or low in 87 (17.1%) suggesting ID (Table 16). The malaria parasite load between the two categories of ferritin is significant (p=0.010) in this survey.

Table 16: Malaria parasite load and ferritin in children.

| | Adequate ferritin | Inadequate ferritin | All subjects |
|---------------|-------------------|---------------------|--------------|
| Malaria load | >30 µg/L | <30 µg/L | |
| Negative | 240 (47.2) | 87 (17.1) | 327 (64.4) |
| Mild/Moderate | 129 (25.4) | 24 (4.7) | 153 (30.1) |
| Severe | 27 (5.3) | 1 (0.2) | 28 (5.5) |
| Total | 396 (78.0) | 112 (22.0) | 508 (100) |

When the effect of acute phase proteins was completely controlled, the healthy/reference group showed no significant difference (p=0.683) between those who had an adequate ferritin level and those who had inadequate ferritin and a malaria parasite load (**Table 17**).

| Malaria | Adequate ferritin (serum ferritin >30µg/L) | Iron deficient (serum ferritin <30µg/L) |
|---------------|--|---|
| N (%) | | |
| Negative | 147 (50.0) | 74 (25.8) |
| Mild/Moderate | 47 (15.98) | 19 (6.5) |
| Severe | 6 (2.0) | 1 (0.3) |
| Total | 200 (68.0) | 94 (32.0) |

Table 17: Malaria parasite load among the healthy/reference group.

Malaria and adjusted vitamin A

Overall, 535 children were considered for this analysis; 347 of these (64.9%) were malaria negative, 158 (29.5%) had mild/moderate malaria load and 30 (5.6%) had severe malaria parasite load (**Table 18**). There is no evidence that there is a difference between malaria parasite load and vitamin A category (p=0.281).

| Malaria | Adequate (serum retinol >0.7 μmol/L) | Marginal (serum retinol <0.7 to ≥0.35 µmol/L | Severe (serum retinol <0.35 µmol/L) | All subjects |
|---------------|---|--|---|--------------|
| Negative | 213 (39.8) | 124 (23.2) | 10 (1.9) | 347 (64.9) |
| Mild/Moderate | 91 (17.0) | 63 (11.8) | 4 (0.75) | 158 (29.5) |
| Severe | 15 (2.8) | 15 (2.8) | 0 | 30 (5.6) |
| Total | 319 (59.6) | 202 (37.8) | 14 (2.6) | 535 (100) |

When this was further examined among the children in the healthy/reference group there was still no statistical evidence (p=0.944) that the levels of vitamin A and malaria parasite load were important (Table 19). This is surprising as febrile infections or inflammations depress vitamin A level. Children recruited to this survey were however afebrile.

| Table 19: Malaria parasite load and adjusted vitamin A in the healthy/reference group. | | | | | |
|--|---|--|--|--------------|--|
| Malaria | Adequate (serum retinol >0.7 µmol/L) | Marginal (serum retinol <0.7 to ≥0.35 µmol/L | Severe (serum retinol <0.35 µmol/L) | All subjects | |
| Negative | 132 (47.5) | 70 (25.2) | 6 (2.2) | 208 (74.8) | |
| Mild/Moderate | 39 (14.0) | 23 (8.3) | 1 (0.36) | 63 (22.7) | |
| Severe | 4 (1.4) | 3 (1.1) | 0 | 7 (2.5) | |
| Total | 175 (62.9) | 96 (34.5) | 7 (2.5) | 278 (100) | |

Correlations (Relationship among biochemical characteristics) for children 6-59 months Although correlation examines the relationship between variables, it does not infer causation. The stronger a correlation (i.e., the higher the r) the more perfect the relationship either in a positive direction or otherwise. Presented in Table 20 are the observed relationships among the different biochemical measurements of children under-5. The results obtained show very strong and negative correlations (P<0.001). Also weak but significant correlations were observed as shown below. Those with a strong positive relationship were iron store and ferritin (r=0.735); CRP and AGP (r=0.616); ferritin and CRP (r=0.586); and ferritin and AGP (r=0.549); and those with strong but negative correlation were hemoglobin and sTfR (r=-0.541); Iron and sTfR (r=-0.421); hemoglobin and AGP (r=-0.420); and hemoglobin and ferritin (r=-0.338). Other positive correlations that are significant but weak include retinol and retinol binding protein (r=0.211); ferritin and sTfR (r=0.153); hemoglobin and retinol binding protein (r=0.242).

| | Retinol | RBP | Ferritin | sTfR | Iron store | Hemoglobin | CRP | AGP |
|------------|-------------|--------------|-------------|--------|------------|----------------|----------------------|-----|
| Retinol | - | | | | | | | |
| RBP | 0.211 (511) | - | | | | | | |
| Ferritin | - | -0.154 (549) | - | | | | | |
| STfR | - | - | 0.153 (549) | - | | | | |
| Iron store | | | | | | | | |
| | | - | | | | | | |
| | | -0.121 (549) | | | - | | | |
| | | 0.735 (549) | | | | | | |
| | | -0.421 (549) | | | | | | |
| Hemoglobin | | 0.242 (548) | -0.338 | -0.541 | 0.086 | - | | |
| петноуюын | - | 0.242 (340) | (548) | (548) | (549) | | | |
| CRP | | 0.285(549) | 0.586 (549) | 0.178 | 0.368 | -0.294 (548) - | | |
| UKF | - | -0.265(549) | 0.560 (549) | (549) | (549) | | - | |
| AGP | | -0.156 (549) | 0.549(549) | 0.390 | 0.281 | -0.420 (548) | 20 (549) 0.616 (540) | |
| AGF | - | -0.150 (549) | 0.549(549) | (549) | (549) | | 0.616 (549) | - |

Table 20: Correlation coefficients (r) serum retinol, ferritin, and acute phase proteins in children 6-59 months.

All correlations were significant at p<0.001.

Results for women of childbearing age (mothers)

Presented in **Table 21** are the cut-off points that were used to determine micronutrient status and malaria parasite load in mothers. All procedures used for the children were also used for their mothers.

| Biochemical marker | Cut-off point | | | |
|---|---|--|--|--|
| Vitamin A status(WHO, 2011) | | | | |
| Serum retinol >0.7µmol/L | Adequate vitamin A/normal range | | | |
| Serum retinol <0.7 to ≥0.35 µmol/L | Marginal vitamin A deficiency (VAD) | | | |
| Serum retinol <0.35 µmol/L | Severe VAD | | | |
| Serum RBP >0.7µmol/L | Adequate vitamin A/normal range | | | |
| Serum RBP <0.7 to ≥0.35 µmol/L | Marginal vitamin A deficiency (VAD) | | | |
| Serum RBP <0.35 μmol/L | Severe VAD | | | |
| Iron status (WHO, 2011) | | | | |
| Serum ferritin >30 μg/L | Adequate iron status/normal range | | | |
| Serum ferritin <30 μg/L | Iron deficiency | | | |
| sTfR >8.3 mg/L (<i>Erhardt et al., 2004</i>) | Iron deficiency | | | |
| sTfR <8.3 mg/L | Adequate iron status/normal range | | | |
| Hemoglobin >12g/dl (<i>WHO, 2011</i>) | No anemia | | | |
| Hemoglobin <12g/dl | Anemic | | | |
| Acute phase proteins (Erhardt et al., 2004) | | | | |
| C-reactive protein (CRP) >10 mg/L | Presence of sub-clinical infection/inflammation | | | |
| C-reactive protein (CRP) <10 mg/L | Absence of sub-clinical infection/inflammation | | | |
| Alpha-1-acid glycoprotein (α_1 -AGP) >1.2 g/L | Presence of sub-clinical infection/inflammation | | | |
| Alpha-1-acid glycoprotein (α_1 -AGP) <1.2 g/L | Absence of sub-clinical infection/inflammation | | | |
| Malaria parasitaemia | | | | |
| 0 | Negative or no malaria parasite seen | | | |
| 1, 2, and 3 | Mild/moderate parasite infestation | | | |
| 4 | Severe parasite infestation | | | |

Table 21: Cut-off points used for classification of adequate and inadequacy/deficiency in mothers.

Subject characteristics

Data on subject characteristics are presented in **Table 22**. Mean age of the subject was 27.3 ± 6.9 yrs (n=643). Mean weight was 58.5 ± 11.8 kg (n=638) and mean height was 157.5 ± 6.6 cm (n= 641). Anthropometric data from 638 women were used to calculate the mean body mass index (BMI) as 23.6 ± 4.8 .

| • | | . , |
|---|--------------------|---------------|
| Variables | Number of subjects | Mean± Std Dev |
| Age (yrs) | 643 | 27.3±6.9 |
| Weight (kg) | 638 | 58.5±11.8 |
| Height (cm) | 641 | 157.5±6.6 |
| Body Mass Index/ BMI (kg/m ²) | 638 | 23.5±4.8 |

Biochemical indices

Mean serum retinol concentration for all the women was $1.5\pm0.5 \mu$ mol/L (n=640), mean RBP was $1.7\pm0.6 \mu$ mol/L (n=622); mean serum ferritin was $59.8\pm49.3 \mu$ g/L (n=622) and mean sTfR was $7.9\pm3.9 \text{ mg/L}$ (n=622). Mean hemoglobin concentration was 11.6 ± 1.7 g/dl (n=643) and mean (derived) iron stores of the respondents was $5.1\pm3.5 \text{ mg/kg}$ (n= 622) (**Table 23**) in all the subjects. In the four-group analysis, significant differences (p<0.05) were observed among the groups. Mothers in the healthy group had significantly higher mean serum retinol concentration ($1.5\pm0.5 \mu$ mol/L) than mothers in incubation ($1.3\pm0.4 \mu$ mol/L) and early convalescence ($1.2\pm0.4 \mu$ mol/L). Mean serum ferritin concentration in the healthy/reference group was significantly lower ($54.9\pm45.5 \mu$ g/L) compared to all in the infection/inflammation groups which was $86.9\pm54.1 \mu$ g/L for incubation, $114.2\pm74.9 \mu$ g/L for early convalescence, and $91.3\pm59.9 \mu$ g/L for late convalescence. No significantly different (p<0.05) between the healthy/reference group and incubation; healthy/reference and early convalescence; incubation and late convalescence; and early convalescence and late convalescence (**Table 23**). No significant differences were observed for mean hemoglobin concentration and iron stores among the different groups.

Acute phase proteins

Presented in **Table 23** are the mean concentrations of acute phase proteins in mothers. For all subjects, mean CRP concentration was 4.5 ± 9.4 mg/L and mean AGP concentration was 0.78 ± 0.3 g/L. Mean CRP concentration for the healthy/reference group after correction for inflammation was 2.0 ± 2.0 mg/L and mean AGP concentration was 0.72 ± 0.2 g/L. There was a significant (p<0.05) difference in the mean CRP concentration of incubation and early convalescence groups; (mean CRP concentration was 20.1 ± 12.4 mg/L for incubation and 41.4 ± 19.0 mg/L for early convalescence). This trend was also observed for AGP. We observed a difference in CRP concentration between the laboratories.
| Acute phase protein | All subjects | Healthy (CRP < 10 mg/L and AGP < 1.2 g/L) | Incubation (CRP > 10 mg/L) | Early Convalescence (CRP > 10 mg/L and AGP > 1.2 g/L) | Late Convalescence (CRP < 10 mg/L and AGP > 1.2 g/L) |
|---|----------------------------------|---|--------------------------------|---|--|
| | | Mear | ns± SD (N) | | |
| Serum retinol (µmol/L) | 1.5±0.5 (636) | 1.5±0.5 (541) | 1.3±0.4 (58) | 1.2±0.4 (22) | 1.4±0.3 (15) |
| Serum RBP (µmol/L) | 1.7±0.6 (622) | 1.7±0.6 (546) | 1.4± 0.4 (40) | 1.3±0.6 (19) | 1.9±0.9 (17) |
| Serum Ferittin (µg/L) | 59.8±49.3 (622) | 54.9±45.5 (546) | 86.9±54.1 (40) | 114.2±74.9 (19) | 91.3±59.9 (17) |
| Serum sTfR (mg/L) | . , | . , | 8.6±3.7 (40) | 9.5±3.2 (19) | 8.5±2.5 (17) |
| 7.9±3.9 (622) 7.8±3.9 (546) | | | | | |
| Iron store (mg/kg) | 5.1±3.5 (622) | 4.9±3.5 (546) | 6.4±3.4 (40) | 6.9±3.3 (19) | 6.6±2.9 (17) |
| Haemoglobin (g/dl) | 11.6±1.7 (639) | 11.8±1.6 (544) | 10.5±2.0 (58) | 10.5±1.8 (22) | 11.6±1.2 (15) |
| CRP (mg/L) ² | 4.5 ±9.4 (622) | 2.0±2.0 (546) | 20.1±12.4 (40) | 41.4±19.0 (19) | 5.2±2.8 (17) |
| AGP (g/L) ² | 0.78±0.3 (622) | 0.72±0.2 (546) | 0.89±0.2 (40) | 1.51±0.3 (19) | 1.37±0.2 (17) |
| CRP (mg/L) ³ AGP (g/L) ³ | 6.0±14.8 (617) 0.62±0.3 (617) | 5.6±12.9 (541) 0.62±0.3 (541) | 7.4±20.8 (40) 0.68±0.4 (40) | 14.1 ±38.2 (19) 0.70±0.7(19) | 4.5±5.6 (17) 0.53 ±0.2 (17) |

Table 23: Mean acute phase protein concentration in women of childbearing age1.

¹=Means significant at p<0.05 except for sTfR.

²Samples analyzed at the DBS-Tech Laboratory, Willstaett, Germany

³Samples analyzed at the South Africa Medical Research Council, Tygerberg, Cape Town, South Africa

Nutrition status

Among the investigated women of childbearing age only 9.8% (n=59) of the subjects were thin or under-nourished. The majority of the women (58.6%; n=378) had BMI values in the normal range, 31.9% (n=141) were overweight, and 9.3% (n=60) were obese with BMI greater than 30 (**Table 24**). If we combine those that are overweight with the obese, approximately one in three (31.2%) of women of childbearing age are overweight or above their ideal/normal weight.

Table 24: Nutritional anthropometry of women of childbearing age.

| | | | _ |
|------------------|--------------------------|---------------------------|-------------------|
| Category | Body Mass Index (BMI) | Number of subjects (N) | Percentage (%) |
| Thin/underweight | <18.5 | 59 | 9.8 |
| Normal weight | 18.5-24.9 | 378 | 58.6 |
| Overweight | 25-29 | 141 | 21.9 |
| Obese | >30 | 60 | 9.3 |

Micronutrient status of women of childbearing age

Vitamin A Status

Vitamin A status measured by serum retinol convalescence

Serum samples from 640 subjects were processed and analyzed for serum retinol concentration. Of the 640 women, 614 (95.9%) had a serum retinol concentration of >0.7 μ mol/L indicating adequate vitamin A status. When we adjusted for inflammation using CRP and AGP in combination, 528 (82.5%) were in the healthy category, 58 (9.1%) in incubation, 22 (3.4%) in early convalescence and 15 (2.3%) in late convalescence. Only 26 (4.1%) suffer from VAD in all the subjects and 3.4% (n=21) in the healthy/reference group (**Table 25**).

Vitamin A status measured by retinol binding protein (RBP)

Six hundred and seventeen (99.2%) had adequate RBP while 5 (0.8%) had a marginal level of RBP. Among the samples with adequate level of RBP 544 (87.5%) were in the healthy, 40 (6.4%) in the incubation, 16 (2.6%) in the early and 17 (2.7%) in the late convalescence categories (**Table 25**).

Iron Status

Iron status measured by serum ferritin

A total of 622 samples were analyzed for serum ferritin concentration to determine iron deficiency using a < 30 μ g/L as a cut-off point. Only 188 (30.2%) had serum ferritin less than 30 μ g/L consistent with iron deficiency. Adjusting for inflammation using CRP and AGP alone or in combination revealed that 366 (58.8%) were healthy, 35 (5.6%) in incubation, 17 (2.6%) in early, and 16 (2.6%) in late convalescence. Of the healthy group, 181 (29.1%) suffer from ID. Prevalence of ID ranged from 0.2% in early and late convalescence to 0.8% in the incubation group (**Table 25**).

Iron status measured by serum transferrin receptor (sTfR)

As presented in **Table 25**, more than one in every three subjects (36%; n=224) had sTfR values >8.3 mg/L which is consistent with ID and 398 (64%) had sTfR concentration < 8.3mg/L i.e., not iron deficient in all the subjects. Prevalence of ID as measured by sTfR was significantly high in the healthy/reference group (57.7%; n=359) compared to the inflammation groups which had ID ranging from 1.1% (early convalescence) to 3.5% (incubation).

Anemia

Hemoglobin levels were determined in 622 mothers; the mean hemoglobin level was 11.6 ± 1.6 g/dL. Among all the mothers 281 (45.2%) had hemoglobin > 12g/dL) while 341 (54.8%) had hemoglobin <12g/dL i.e., anemia. Among the healthy/reference group (CRP<10 mg/L and AGP<1.2 g/L), 302 (48.6%) were anemic. In the inflammation groups, anemia ranged from 1.0% in late convalescence to 3.5% in incubation (**Table 25**).

| Table 25: Micronutrient status of women of childbearing age. |
|--|
|--|

| Variable | All subjects | Healthy (CRP < 10 mg/L and AGP < 1.2 g/L) | Incubation (CRP > 10 mg/L) | Early Convalesce (CRP > 10 mg/L and AGP > 1.2 g/L) | Late Convalesce (CRP < 10 mg/ Land AGP > 1.2 g/L) |
|--|-----------------|---|----------------------------------|--|---|
| | | N (%) | | | |
| Subjects with serum retinol > 0.7 µmol/L | 593 (95.8) | 522 (84.3) | 38 (6.1) | 16 (2.6) | 17 (2.7) |
| Subjects with serum retinol <0.7 to ≥0.35 µmol/L | 25 (4.0) | 21 (3.4) | 2 (0.3) | 2 (0.3) | 0 |
| Subjects with serum retinol <0.35 µmol/L | 1 (0.7) | 0 | 0 | 1 (0.2) | 0 |
| Total | 619 (100) | 543 (87.7) | 40 (6.5) | 19 (3.1) | 17 (2.7) |
| P value=0.000 | | | | | |
| Subjects with RBP > 0.7 µmol/L | 617 (99.2) | 543 (87.3) | 40 (6.4) | 17 (2.7) | 17 (2.7) |
| Subjects with RBP <0.7 to ≥0.35 µmol/L | 5 (0.8) | 3 (0.5) | 0 | 2 (0.3) | 0 |
| Subjects with RBP <0.35 µmol/L | 0 | 0 | 0 | 0 | 0 |
| Total | 622 (100) | 546 (87.8) | 40 (6.4) | 19 (3.1) | 17 (2.7) |
| P value=0.023 | | | | | |
| Subject with serum ferritin> 30 | 434 (70.0) | 365 (58.9) | 35 (5.6) | 18 (2.9) | 16 (2.6) |
| Subject with serum ferritin <30 µg/L | 186 (30.0) | 179 (28.9) | 5 (0.8) | 1 (0.2) | 1 (0.2) |
| Total | 620 (100) | 544 (87.7) | 40 (6.5) | 19 (3.1) | 17 (2.7) |
| P value=0.000 | | | | | |
| Subjects with sTfR> 8.3 mg/L | 397 (63.9) | 357 (57.5) | 23 (3.7) | 7 (1.1) | 10 (1.6) |
| Subjects with sTfR< 8.3mg/L | 224 (36.1) | 188 (30.3) | 17 (2.71) | 12 (1.9) | 7 (1.1) |
| Total | 621 (100) | 545 (87.8) | 40 (6.4) | 19 (3.1) | 17 (2.7) |
| P value=0.057 | | | | | |
| Subjects with hemoglobin > 12 g/dl | 282 (45.3) | 245 (39.4) | 18 (2.9) | 8 (1.3) | 11 (1.8) |
| Subjects with hemoglobin <12 g/dl | 340 (54.7) | 301 (48.4) | 22 (3.5) | 11 (1.8) | 6 (1.0) |
| Total | 622 (100) | 456 (87.8) | 40 (6.4) | 19 (3.1) | 17 (2.7) |
| P value=0.440 | | | | | |

Hemoglobin and indices of iron deficiency

We were also interested in knowing what percentage of the women who were anemic (Hb<12g/dl) also had elevated ferritin or elevated sTfR. Results of this analysis are presented in **Table 26**. Only 40% of the women (n=257) were anemic and had elevated (> 30μ g/L) serum ferritin; 20.8% of anemic women (n=134) had elevated sTfR.

Table 26: Hemoglobin and indices of iron deficiency.

| | Serum F | Serum Ferritin | | ferrin receptor |
|--------------------|------------------------|---------------------|------------------------|----------------------|
| Hemoglobin | Adequate (>30 µg/L) | Deficient (<30µg/L) | Adequate (<8.3mg/L) | Deficient (>8.3mg/L) |
| Adequate (>12g/dL) | 200 (31.1%) | 90 (14.0%) | 178 (27.6%) | 113 (17.5%) |
| Anemic (<12g/dL) | 257 (40%) | 96 (14.9%) | 219 (34%) | 134 (20.8%) |
| Total | 457 (71.1%) | 186 (28.9%) | 397 (61.6%) | 247 (38.4%) |

Table 27: Indicators of iron deficiency and hemoglobin among *Healthy group

| | Serum Ferritin | | Serum Transferr | Serum Transferrin Receptor | | |
|--------------------|------------------------|------------------------|------------------------|----------------------------|--|--|
| Hemoglobin | Adequate (>30 µg/L) | Deficient (<30µg/L) | Adequate (<8.3mg/L) | Deficient (>8.3mg/L) | | |
| Adequate (>12g/dL) | 158 (29%) | 86 (15.8%) | 163 (29.9%) | 82 (15.0%) | | |
| Anemic (<12g/dL) | 208 (38.2%) | 93 (17.1%) | 195 (35.7%) | 106 (19.4%) | | |
| Total | 366 (67.2%) | 179 (32.8%) | 358 (65.6%) | 188 (34.4%) | | |

*Healthy group (CRP<10 mg/L and AGP<1.2 g/L).

Presented in **Table 27** are the indicators of iron deficiency (ferritin and sTfR) and anemia (hemoglobin) amongst women in the healthy/reference group. Almost two in five women (38.2%; n=208) of the anemic women also had elevated serum ferritin (> 30μ g/L) while almost one in five anemic women (19.4%; n=106) also had elevated sTfR. These were not significantly different (p>0.05).

Malaria

Prevalence of malaria parasites was 9.8% (n=624) in women of childbearing age (Table 28).

| | All subjects | Healthy (CRP < 10 and AGP < 1.2) | Incubation (CRP > 10) | Early Convalescence (CRP > 10 and AGP > 1.2) | Late Convalesce (CRP < 10 and AGP > 1.2) |
|--------------------------|--------------|--|--------------------------|---|--|
| | | ٦ | N (%) | | |
| N | 624 (100) | 526 (84.3) | 61 (9.8) | 21 (3.4) | 16 (2.3) |
| Negative (Mp0) | 90.2 | 91.3 | 82.0 | 95.2 | 81.3 |
| Mild/Moderate (Mp1-3) | 9.6 | 8.6 | 18.97 | 4.8 | 18.8 |
| Severe (Mp4)) | 0.2 | 0.2 | 0.0 | 0.0 | 0.0 |

Table 28: Malaria parasite load in women of childbearing age.

Five hundred and eighty-four (584) women were analyzed to examine if there is any difference in malaria parasite load and level of acute phase proteins (**Table 29**). There is no significant difference in either low or high CRP (p=0.620) or AGP (p=0.929). No relationship also exists between the malaria parasite load and the four groups based solely on acute phase proteins, i.e., healthy/reference, incubation, and convalescence groups (p=0.988).

| | С | RP | AGP | | |
|---------------|--------------|--------------|--------------|-------------------|--|
| Malaria | CRP <10 mg/L | CRP >10 mg/L | AGP <1.2 g/L | High AGP >1.2 g/L | |
| Negative | 427 (81.5) | 50 (8.6) | 496 (84.9) | 30 (5.1) | |
| Mild/Moderate | 103 (17.6) | 3 (0.5) | 55 (9.4) | 2 (0.4) | |
| Severe | 1 (0.2) | 0 | 1 (0.2) | 0 | |
| Total (584) | 531 (90.9) | 53 (9.1) | 552 (94.5) | 32 (5.5) | |
| | P=(| 0.620 | | P=0.929 | |

Of the 598 subjects whose results were examined for a relationship between vitamin A level and malaria parasite load, 516 (86%) had adequate serum retinol and were negative for malaria parasite load, 9.4% had mild/moderate malaria parasite load. This difference is significant (p=0.002). This significant relationship persists (p=0.000) when only the healthy group was compared (**Table 30**).

Table 30: Malaria and adjusted vitamin A in mothers.

| Malaria | Adequate (serum retinol >0.7 µmol/L) | Marginal VAD (serum retinol <7 to >0.35 μmol/L) | Severe VAD (serum retinol <0.35 µmol/L) | All subjects |
|---------------|--|---|---|--------------|
| | | N (%) | | |
| Negative | 516 (86.3) | 22 (3.7) | 1 (0.2) | 539 (90.1) |
| Mild/Moderate | 56 (9.4) | 2 (0.4) | 0 | 58 (9.7) |
| Severe | 1 (0.2) | 0 | 0 | 1 (0.2) |
| Total | 573 (95.8) | 24 (4.0) | 1 (1.2) | 598 (100) |

From this analysis there is no significant difference between mothers who had adequate ferritin level and malaria parasite load (p=0.763). This relationship persists when the different groups (healthy/ reference, incubation, convalescence) were examined for malaria load and ferritin level (**Table 31**).

| Malaria Load | Adequate/ serum ferritin > 30 µg/L | lron deficient/serum ferritin <30 μg/L | All subjects |
|---------------|---------------------------------------|---|--------------|
| | N (% | 6) | |
| Negative | 381 (63.4) | 161 (26.8) | 542 (90.2) |
| Mild/Moderate | 44 (7.3) | 14 (2.3) | 58 (9.6) |
| Severe | 1 (0.2) | 0 | 1 (0.2) |
| Total | 426 (70.9) | 175 (29.1) | 601 (100) |

Table 31: Malaria parasite load and adjusted iron deficiency in women of childbearing age.

Correlations (Relationship among biochemical characteristics) for women of childbearing age.

Presented in **Table 32** are the observed relationships among the different biochemical measurements of women of childbearing age. The results obtained show a relatively strong and significant relationship (p<0.05) between iron store and ferritin (r=0.749); AGP and CRP (r=0.523), iron store and sTfR (r=-0.641). Other positive but weak correlations were observed between hemoglobin and retinol (r=0.239); CRP and ferritin (r=0.255); and AGP and ferritin (r=0.312)

| | Retinol | RBP | Ferritin | STFR | Iron store | Hemoglobin | CRP | AGP |
|------------|-------------|--------------|--------------|--------------|-------------|------------|-------------|-----|
| Retinol | - | | | | | | | |
| RBP | - | - | | | | | | |
| Ferritin | - | 0.189 (622) | - | | | | | |
| STfR | - | - | -0.137 (622) | - | | | | |
| Iron Store | - | -0.126 (622) | 0.749 (622) | -0.641 (622) | - | | | |
| Hemoglobin | 0.239 (640) | - | - | - | - | - | | |
| CRP | - | -0.197 (622) | 0.255 (622) | - | 0.140 (622) | - | - | |
| AGP | - | - | 0.312 (622) | 0.187 (622) | 0.139 (622) | - | 0.523 (622) | - |
| | | | | | | | | |

Table 32: Correlation coefficients (r) serum retinol, ferritin, and acute phase proteins in women of childbearing age.

All correlations were significant at <0.05.

Conclusions

The foregoing results of the Nutrition survey in Akwa Ibom indicate the need to take appropriate action to ameliorate the observed levels of malnutrition and micronutrient deficiencies among children under-5 and their mothers (women of childbearing age). Several clear deductions can be made, based on the findings in this survey.

Children 6-59 months

(1). Apparently healthy children and their mothers were recruited for this survey. However, with adjustment for infection/inflammation using acute phase proteins, about 48.2% of the children were in one or other stages of sub-clinical infection/inflammation. The implication of this is that even among the children who were regarded as healthy in the community, many are harboring sub-clinical conditions.

(2). Sixty percent of children (n=581) recruited had an adequate level of vitamin A, based on the assessment of their serum retinol. Another 36.7% had marginal VAD and 2.8% had severe VAD. However, since we know that infections, inflammations, and febrile conditions deplete vitamin A, their influence was removed; after adjustment for infection/inflammation, using acute phase proteins 32.9% of the children were adequate as regards vitamin A, VAD was marginal in 17.4% and 1.5%, and severe in 2.5%.

(3). As vitamin A may sometimes be determined by serum retinol-binding protein (RBP), 75% (n=542) of the children had adequate vitamin A, while VAD was marginal in 23% and severe in 1.3%. When influence of sub-clinical infection/inflammation was removed however, 47% of these children had adequate vitamin A; VAD was marginal in 11.4% and severe in 0.6%

(4). Iron status is best measured by serum ferritin since it reflects body iron than other parameters. However ferritin rises/increases in response to infection and inflammation as it is also an acute phase protein. Serum ferritin in children showed that 78% (n=548) had adequate iron status while for 22% it was inadequate. When the influence of sub-clinical infection/inflammation was removed, however, about 40% had adequate and about 18% had inadequate iron status and were thus iron deficient.

(5). The concentration of Serum Transferrin receptor (sTfR) has been suggested as another method of determining the adequacy or otherwise of iron status. sTfR indicated that about 40% of the children (n=547) had adequate iron, while for about 60% it was inadequate. Although it has been suggested that sTfR is not markedly affected by infection/inflammation process, the level of adequate iron status in children changed to 27.1% and inadequate to 32% when sub-clinical infection/inflammation was removed.

(6). Although iron is needed to synthesize hemoglobin, the hemoglobin level does not begin to fall until total iron store depletion, so it is not a good measure/reflection of iron status. Anemia (hemoglobin < 11g/dL) was found in 75% (n=637) of the children. Although it was not expected that sub-clinical infection/inflammation would directly affect hemoglobin level, anemia remained in about 33% of the children when the data were adjusted to eliminate sub-clinical infection/inflammation.

(7). Malaria infection as determined by malaria parasite load on microscopy. When categorized into three groups 65% (n=584) had no malaria parasite on their slides; 30% had a mild/moderate malaria parasite load, and only about 5% had a severe malaria parasite load as shown by their slides. Surprisingly, a majority (44.2%) of these children negative in malaria were in the group with low acute phase proteins and about 17% had a mild/moderate malaria parasite load.

(8). It is believed that nutritional anthropometry is a measure of outcome between dietary intake, nutrient utilization, and infection/inflammation. Among the children in this survey: 43% were stunted (n=580), 17% underweight (n=586), and 12% wasted (n=544). It is sufficient to note that the prevalence of stunting found in this survey is similar to the 42% in the Nutrition and Food Consumption Survey of 2001 and the 41% reported in the 2008 NDHS survey.

Women of childbearing age (mothers)

(1). Of the 645 apparently healthy mothers recruited into this survey, 58 (9%) were in the incubation group, 22 (3.5%) were in early convalescence and 15 (2.4%) were in the late convalescence. This scenario agrees with the fact that over 80% of these women had low acute phase proteins, indicating low or minimal level of sub-clinical infection/inflammation.

(2). Six hundred and nineteen women had results for serum retinol; of these 593 (95.8%) had an adequate level, 25 (4.0%) had marginal VAD, and only one person had severe VAD. When vitamin A is determined by retinol binding protein (RBP), 617 (99.2%) had adequate serum vitamin A and 5 (0.8%) had marginal VAD. Adjusting for infection/inflammation resulted in only 3.4% of women having VAD. It is therefore conclusive that VAD is not a problem among women of childbearing age encountered in this survey.

(3). Iron status as measured by serum ferritin among these women showed that 434 (70%) had adequate ferritin and therefore iron while 186 (30%) had low serum ferritin indicating ID. When the effect of sub-clinical infection/inflammation was removed, about 59% had adequate iron while about 29% had ID. When ID is determined by concentration of serum transferrin receptor (sTfR), 224 (36.1%) had adequate iron while 63.9% were iron deficient. The values became 188 (30.3%) with adequate iron and 357 (57.5%) with ID when adjustment for sub-clinical infection/inflammation was made. Iron deficiency is a problem in these women of childbearing age.

(4). Anemia was high in women of childbearing age; 54.7% in all subjects (n=622), but this was reduced to 48.4% among women who had no evidence of sub-clinical infection/inflammation. Although the level of anemia is high and almost like the level of ID, a causal relationship cannot be established.

(5). An overwhelming majority (90.2%) had negative malaria parasite load with only 9.8% in all the subjects having the mild/moderate category of malaria parasite load and 8.8% after adjustment for infection/inflammation. It is also sufficient to summarize that malaria was not a serious problem among these women. At the level of these analyses serious relationships have not been found in terms of malaria parasite load and vitamin A or iron status.

Recommendations

Results of the 2011 Akwa Ibom State Nutrition Survey indicate that VAD is a serious public health problem among children and anemia among women and children, and that the situation has not changed and may be getting worse especially for children under-5. In addition, stunting and wasting are also prevalent. The assessment of micronutrient and nutritional status reveals ID in women of reproductive age, and a low prevalence of VAD compared to their children and comparable to the vitamin A status of women in industrialized countries. Based on these findings, urgent action is needed both to strengthen current programs and expand efforts in the prevention and control of micronutrient deficiencies, especially of vitamin A, iron in children under-5 and iron and overweight in women of childbearing age.

Further analysis of the survey data can help to provide information for strategies and make more specific recommendations regarding malaria control, and child feeding practices during the first two years of life. (Specific recommendations should be on issues involved directly in the research to which you have data that quantifies or describes it.)

The following areas of additional analysis are recommended:

- Developing a proxy indicator of socio-economic status (low, medium, and high level) so that the data can be re-analyzed using this factor
- Linking the biochemical data with the food consumption dataset for the investigated subjects to determine the relationship between foods consumed, nutritional status, and micronutrient deficiencies
- Linear associations to identify any pattern of association (not causal effect) among the variety of variables observed
- Linear explorations involving dependence models

Additional research could also be considered such as the following.

- Assessing micronutrient status in pre-school children, especially of zinc and iodine to determine the extent of deficiency in this vulnerable group.
- Formative research looking at awareness and knowledge of micronutrient issues and food practices in vulnerable groups. Results of this investigation could serve as a basis for developing a strategic approach to communication and nutrition education.
- Operational research to explore best strategies for increasing vitamin A and iron intake of children under-5 and women of childbearing age.

Advocacy and Coordination

Micronutrient deficiencies are recognized as a serious health problem within the nutrition and health sector. However, the extent of these deficiencies in the State and what is now known of the damaging consequences and threat to overall development may not be realized. Strategically planned dissemination of survey results, packaged for specific target audiences within the health sector, as well as for other government sectors such as agriculture and finance and to NGOs, can help to increase overall awareness of the problem and solicit support for program action.

Wide-spread dissemination of survey results is recommended. The *PROFILES* package provides
a good framework for targeting policymakers and senior-level management to ensure adequate
support for intervention programs. Representatives from Nigeria have already been trained in
the use of this package, and findings from this assessment can be included in the package to
produce a powerful tool for advocacy to policy and decision-makers.

- Activities which will improve the nutrition and micronutrient status of the population tend to cut across multiple government sectors and programs. Countries which have been successful in combating this problem have had two key organizational elements: a) a prevention and control program housed in one government department with a mandate to oversee program implementation, and b) strong leadership of a multi-sectoral committee for coordination.
- Given the extent of malnutrition and micronutrient problems in the State, it may be time to strengthen the State Committee on Food and Nutrition. This Committee could be tasked with (a) overseeing a situation assessment of programs and opportunities to address malnutrition and micronutrient deficiencies, (b) acting on findings to develop a State plan of action, c) ensuring coordination between various partners, and d) overseeing monitoring of the program.

The WHO recommends that in countries where prevalence rates for anemia are more than 40, iron supplementation should be considered. According to survey results, prevalence of anemia was greater than 40% in women and slightly less than 40% (32.8%) in children under-5. Preventive iron supplementation for these groups can be considered. According to the INACG, it is now considered more cost-effective to prevent iron deficiency prior to pregnancy so that women enter pregnancy with good iron stores.

Links with the reproductive health program can be further strengthened since delayed first pregnancy, child spacing, early pre-natal care and post-natal follow-up, and good breastfeeding practices will all improve the micronutrient status of both mother and child. Other public health programs (immunization, sanitation, and hygiene) also play an important role, especially for children.

Agricultural production, food processing and the diversification of cropping systems are of critical importance for food security. There is a need to revitalize the national food production program and implement policy to provide an easily produced source of animal protein. Additionally, production of value-added products should be increased to ensure availability throughout the year.

Existing programs for micronutrient deficiency control need to be supported. Micronutrient supplementation, food fortification, and dietary diversification should be vigorously promoted. Increased research and public education are needed to address the issue of iodine nutrition, including the monitoring of iodine levels. Alongside strengthened existing programs for nutrition education, a nation-wide community-level training program should be implemented to educate health workers on the symptoms of malnutrition.

To make significant improvement in child health, there should be increased support for the State Government's ongoing malaria project, increased promotion of ORT to control diarrhea, and strengthening of existing child survival programs, including childhood immunization and supplementation.

There is a need for improved information, education, and communication in matters of health and nutrition, particularly in the areas of child health, infant feeding, and hygienic practices. It is recommended that a more focused, State coordinated Behavioral Change Communication strategy be formulated and implemented. The radio is one of the widest forms of communication; therefore, social marketing strategies should be used to create demand-driven intervention services that will address the deficiencies found among the under-5 children and their mothers. Low-cost high-impact effective interventions may be put together to create not only synergy but also to manage resources.

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