

Determinants of Prenatal Nutritional Status and Adverse Birth Outcomes in HIV-Infected, Pregnant Malawian Women

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Abstract

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Pregnant Malawian Women

“(Under the direction of Dr. Linda Adair)”

This research assessed nutritional status and diet of HIV-infected pregnant women who presented for care at public antenatal clinics in Lilongwe, Malawi, and related their nutritional status to birth outcomes. We used data from a large-scaled ongoing Breastfeeding, Antiretroviral, and Nutrition (BAN) Study, a postnatal clinical trial (www.thebanstudy.org). The data used in the analysis was derived from surveys conducted to screen women for BAN study participation and to collect baseline demographic, anthropometric, dietary, and health status data; from antenatal clinic records; and from hospital records at delivery. We found a low linear increase in weight over the second and third trimesters. In addition, in our analysis of weight gain, we found weight loss in the intervals between visits. In unadjusted models, MUAC and AMA increased and AFA declined during late pregnancy. However, based on multivariable regression models, exposure to the famine season resulted in losses in AMA which increased as pregnancy progressed, while AFA losses occurred irrespective of season. In the dietary analysis, three dietary pattern clusters were derived: 1) Cluster 1: high fish, meat and oil, 2) Cluster 2: high grain and 3) Cluster 3: high leafy vegetables, nuts, and fruits. In multiple regression analysis, Cluster 2 had a 1.86 cm² higher AMA and -2.09 cm² lower than Cluster 1. Cluster 2 and Cluster 3 both had 0.3 g/dL decreased hemoglobin

compared to Cluster 1. In a longitudinal analysis of arm measures and fundal height, as fundal height increased monotonically 0.92 cm/week, AMA was positively associated with fundal height and AFA was negatively associated with fundal height, although these associations were very subtle. Although we identified significant interaction effects of AMA and AFA and week of follow up on fundal height, these effects too were very subtle. We found MUAC, AMA, and AFA collected at the baseline visit were directly associated with newborn's birth weight. MUAC and AMA were both inversely associated with LBW. Our findings contribute to the current understanding of diet, body composition and its effect on birth weight in HIV-infected sub-Saharan women. Strategies to optimize nutrition for HIV-infected women during pregnancy appear warranted.

**To my parents, Drs. Bobby and Joyce Thomas, MD, my husband, Richard Ramlal, and
my daughter, Karina Ramlal**

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LIST OF ABBREVIATIONS

AFA	arm fat area
AMA	arm muscle area
BAN	Breastfeeding, Antiretrovirals, and Nutrition
CI	confidence interval
cm	centimeters
dL	deciliters
DHS	Demographic Health Survey
FH	fundal height
g	grams
Hb	hemoglobin
HIV	human immunodeficiency virus
IUGR	intrauterine growth restriction
kg	kilogram
LBW	low birth weight
LMP	last menstrual period
MUAC	mid-upper arm circumference
OR	odds ratio
SD	standard deviation
USDA	United States Department of Agriculture
WHO	World Health Organization

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References

I. Introduction

A. Background

In sub-Saharan Africa, an estimated 13.5 million women of childbearing age are infected with HIV(1). When they become pregnant, HIV-infected women face not only the usual physiological and nutritional demands of pregnancy, but also the extra demands imposed by infection. In settings with a high level of food insecurity and high maternal work loads, women may be unable to consume sufficient high quality food to meet these heightened nutritional needs, leaving them at much greater risk of poor health, faster disease progression and increased risk of poor birth outcomes. Most research related to HIV in pregnancy has focused on antiretroviral strategies to reduce mother to child transmission (MTCT) of HIV. Little attention has been paid to maternal health and nutrition status. More research is needed on the quality and quantity of maternal diets during pregnancy, and how diet affects maternal nutrition status and infant birth outcomes, which in turn, have a major impact on infant survival and health.

B. Overall objectives and specific aims

This study focused on the nutritional status of HIV infected pregnant women in Lilongwe, Malawi, and on the relationship of nutritional status to birth outcomes. This study is a sub-study of a larger ongoing Breastfeeding, Antiretroviral, and Nutrition (BAN) Study: a postnatal clinical trial (www.thebanstudy.org). The data to be used in the analysis was derived from surveys conducted to screen women who consented to be in the BAN Study and

to collect baseline demographic, anthropometric, dietary, and health status data during antenatal care and at delivery. Specific aims are as follows:

- 1. Describe patterns and interrelationships of maternal weight gain and changes in anthropometric indicators of nutritional status during pregnancy.** We examined arm muscle area (AMA) and arm fat area (AFA) calculated from mid upper arm circumference (MUAC) and triceps skinfold thickness, among HIV-infected Malawian women. We hypothesized that AMA and AFA would decline during pregnancy. We used a longitudinal model to explore the dynamics of AMA and AFA with MUAC and weight over the course of pregnancy.
- 2. Identify dietary factors associated with nutritional status during pregnancy among HIV-infected Malawian women.** We examined dietary patterns and how they relate to maternal nutritional status, as indicated by AMA, AFA, and MUAC. We hypothesized poor diet quality in HIV-infected women is associated with low muscle and fat stores, measured by MUAC and tricep skinfold thickness in HIV-infected women. We conducted a cross-sectional analysis using cluster analysis to derive dietary patterns and linear regression analysis to examine the relationship between clusters and AMA, AFA, and MUAC.
- 3. Identify nutritional determinants, indicated by anthropometrics, of adverse birth outcomes among HIV-infected Malawian women.** We examined the associations between anthropometric indicators AMA, AFA, and MUAC and fetal growth, birth weight and low birth weight (LBW). We hypothesized low AMA and AFA in HIV-infected pregnant women are associated with poor fetal growth and are significantly associated with increased odds of low birth weight. To achieve these aims, we conducted

a longitudinal analysis using linear regression to look at fetal growth and cross-sectional analysis using linear and logistic regression to look at birth weight and LBW, respectively.

II. Literature Review

This section presents the scope of the problem, reviews the literature on specific topics covered by the aims of the study, and highlights the limitations of previous studies and key strengths of the current study.

A. Scope of the problem

Most of the world's 40 million HIV-infected people currently live in communities suffering from poverty and malnutrition. Proper nutrition with a well balanced and adequate diet, sometimes including food assistance, can play a key role in maintaining the health and well-being of affected individuals, their families, and their communities. Food and nutrition programs may minimize the effects of HIV/AIDS by helping infected individuals remain productive and by allowing their families and communities to better cope with the economic losses associated with HIV/AIDS.

The three-way interaction between human immunodeficiency virus (HIV) infection, nutrition and immune function forms a complicated pathway since all three factors are interdependent. Nutrition is a cofactor that plays an important role in the progression of HIV disease by altering its natural course (2). Viral infections, such as HIV/AIDS or other secondary infections like malaria or tuberculosis, affect nutritional status by reducing dietary intake and nutrient absorption, and by utilizing the body's protein and micronutrient stores to combat invading pathogens. The immune system's response is the release of pro-oxidant cytokines which leads to increased usage of antioxidant vitamins as well as several minerals

(3). This imbalance between pro-oxidants and antioxidants lead to further oxidative stress, inflicting damage to cells, proteins, and enzymes (4-6). HIV infection also influences the endocrine system with the production of hormones such as glucagon, insulin, epinephrine, and cortisol, which are involved in metabolism of carbohydrates, proteins, and fats. High levels of these hormones lead to weight loss and wasting syndrome seen in most adults AIDS patients (7).

Although there is little research on HIV infection and body composition, among men, weight loss results primarily from lean tissue loss, which has been shown to be a strong predictor of survival independent of CD4 counts (8-10). The difference in weight in the HIV-infected compared to uninfected was due to fat-free mass in both men and women (11). Loss of lean muscle tissue is therefore an important symptom of HIV progression which potentially has subsequent adverse effects.

In 2003, 18 percent of Malawian women aged 15-24 attending antenatal clinics tested HIV positive (UNAIDS 2007). In the 2000 Malawi DHS study, 7% of mothers had a BMI less than 18.5 kg/m², suggestive of some malnutrition. In this densely-populated, agrarian country, Malawi has one of the highest infant mortality rates, despite a decline in recent years and is one of the poorest countries in the world. It ranks 161 out of 174 on the Human Development Index in 2000 (UNDP, 2001).

Information is currently very limited on how nutrition affects birth outcomes among HIV-infected pregnant women in Africa, since more attention is given to antiretroviral medications to prevent vertical transmission. It is likely the relationships between HIV-associated factors, such as poor prenatal nutritional status, and perinatal outcomes, such as low birth weight and HIV transmission, are different in developing countries than in

developed countries because of high prevalence of malnutrition, infectious diseases, such as malaria, and poor access to care (12,13). In this study, since all women are receiving the HIVNET 012 regimen, transmission rates are expected to be very low at birth. However, data will still allow for a richer understanding of the effects of prenatal factors on fetal growth during early stages of HIV infection. Pregnant HIV-infected women can be presumed to be in the early stages of infection, since fertility is affected by those in advanced HIV stages as found in a cross-sectional study of 5000 women in Uganda (14).

B. Body composition and diet in HIV and pregnancy

B.1. Body composition patterns during pregnancy

Normally, pregnancy affects body composition due to increasing fat mass accumulation. In healthy women, almost 4 kg of fat are deposited during pregnancy to nourish the fetus (Institute of Medicine 1990). Fat is then redistributed from central stores (WHO 1995) and through increased lipogenesis, subcutaneous fat accumulates beginning in early pregnancy and through the second semester, and then declines during the third trimester with an increased energy supply to meet the demands of the growing fetus (15). While more advanced and accurate methods to determine body composition would be ideal, simple anthropometric measurements of arm circumference and triceps skinfold thickness are inexpensive, reliable, and practical in field studies. Tricep, subscapular and supra-iliac skinfold thickness measures are used to assess the overall distribution of subcutaneous fat and to monitor weight gain in pregnant women. During pregnancy, the various physiological changes combined with the effect of the HIV infection will influence fat redistribution and deposition of subcutaneous fat in the upper arms. Based on these measurements, arm muscle

area and arm fat area can be calculated and used to assess lean tissue and fat tissue. Under normal conditions, arm muscle area measures are not usually responsive to short term changes during pregnancy and tend to reflect pre-pregnancy arm muscle status. However, arm fat area changes in response to changes in diet, physical activity or other stresses due to pregnancy.

In developing countries, it is unclear whether patterns of low weight gain are due to maternal depletion (decreased accumulation of muscle or fat tissues), redistribution of fat stores during pregnancy to the growing fetus as in non-infected women with poor diets, poor fetal growth during pregnancy, or a combination of these. A study in Tanzania looked at patterns and predictors of weight gain in HIV-infected women and found rate of weight gain and MUAC decreased progressively during pregnancy. Predictors in the third trimester were low levels of education and helminthic infections in the pattern of weight gain due to HIV infection. In the second trimesters, factors that contributed to this decline in rate of weight gain were high MUAC, not contributing to income, low serum retinol and selenium concentrations, advanced clinical stage of HIV disease, and malaria infection. Low CD4 count at baseline was also related to poorer pattern of weight gain throughout pregnancy (16).

In a study conducted in Mangochi, Malawi, in a high HIV-prevalent area among women with unknown status, 20% of women lost weight between 24 and 33 weeks and 8% showed no weight gain. These women compared to women who gained weight had lower leucocyte counts and higher reductions in MUAC, subscapular and supra-iliac skinfolds, and arm muscle area. (17).

B.2. Prenatal food intake patterns

There are numerous studies that identify specific dietary patterns in developing countries, including trends in nutrient and food group intakes; but, very few studies examine determinants or maternal factors associated with these prenatal diet trends.

In a study in rural Malawi that investigated zinc deficiency among pregnant women, results related to both total nutrient intake and nutrient intake by food group showed low levels and poor availability of dietary zinc. The study analyzed mean percentages of energy, protein, and zinc by the major food groups in Malawian women at 24 and 33 weeks gestation. The five major food groups were grains, legumes, meat (goat), poultry (chicken) and fish, sweets, vegetables, and fruit. Their study found the percentage of women at risk of inadequate intakes of protein (adjusted for energy) according to the recommended intake for protein (FAO/WHO/UNU, 1985) to be high, ranging from 27% at 24 weeks to 35% at 33 weeks gestation. This region is close to Lake Malawi, so it is not surprising that fish made up more than 85% of animal protein; dry fish was more commonly consumed than fresh fish. The study was conducted in a rainy season, in which more small fish were consumed. Fish contain relatively high amounts of zinc. Chicken and goat combined were eaten least frequently. While meat, fish and poultry made up 5% of energy intake, this group was found to consume only 18-19% of the recommended intake for protein (18). Approximately 60% of the pregnant women had maize based-diets high in phytate, a potent inhibitor of zinc absorption, and low intakes of flesh foods, an important source of zinc (18). Dietary variety is relatively low in Malawi (18).

In 1994-1997, the Pune Maternal Nutrition Study in the rural villages near Pune, Maharashtra, India, studied maternal diet and neonatal outcome among women in a

prospective, observational study. Data revealed that low-income rural and urban women have low intakes of several micronutrients and micronutrient rich foods such as green leafy vegetables, fruit and dairy products, so consumption of these foods was measured in addition to macronutrient intakes (19-22). Similar to what one would expect in sub-Saharan Africa and Malawi, energy and protein intakes were low and did not increase during pregnancy (19,22).

B.3. Maternal anthropometry and birth outcomes in HIV/AIDS

In developing countries, low birth weight affects large numbers of infants and intrauterine growth retardation (IUGR) is the primary cause. It is well established that the short-term consequences of IUGR include increased risk of fetal, neonatal and infant death and impaired postnatal growth and immune function, and intellectual development ((23-25). A review of studies in Africa found that HIV-infected pregnant women are at increased risk of delivering low birth weight infants, but exact mechanisms have yet to be determined (13). This association is also likely to be different than in developed countries due to confounders as mentioned previously: malnutrition, infection, and poor access to prenatal care. Low birth weight rates across studies among HIV-infected pregnant women in sub-Saharan Africa range from 9% in Nairobi, Kenya, to 26% in Kigali, Rwanda. Most of these studies were in urban settings, but the prevalence across studies of symptomatic HIV infections were inconsistent (26,27). In a study in Kinshasa, Zaire, in a sample of more than 8,000 women, an increased incidence of low birth weight, prematurity and mortality among infants of HIV-seropositive mothers was found (28). In Kenya, Braddick et al. found a 17% LBW rate among women with HIV-related disease compared with a 6% LBW rate among women who

were asymptomatic and had generalized lymphadenopathy alone. The LBW rate was only 3% among uninfected women in the study. In Rwanda, LBW rates reported were 9.8%, 16.9%, and 32.9% among women who were HIV-uninfected, HIV-infected without AIDS, and HIV infected with AIDS, respectively. A Tanzanian cohort study which initially analyzed the association between HIV-infection and adverse pregnancy outcomes found no significant difference between HIV-infected and HIV-uninfected groups. However, when the HIV-infected group was categorized, they did find significantly higher rates of LBW among symptomatic (World Health Organization stage II or higher) HIV-infected women than among HIV-uninfected women however, they did find significantly higher rates of LBW among symptomatic (World Health Organization stage II or higher) HIV-infected women than among HIV-uninfected women (29). Another study, also in Tanzania, looked at maternal anthropometry and adverse birth outcomes among HIV-infected women. Poor anthropometric status at the first prenatal visit, weight loss and low weight gain were identified as strong risk factors for low birth weight; however, the direction and magnitude of these associations were comparable to those reported among HIV-uninfected women living in poor settings (30).

C. Summary and significance

Minimal data are available on women who experience increased nutritional demand during pregnancy in conjunction with the elevated risks of weight loss related to HIV disease progression. This explains the need for additional analysis of the factors that are related to maternal nutritional status and birth outcomes in HIV-infected pregnant women. Although the relationship between HIV progression and nutrition has been established, this relationship

and its effects in pregnancy are poorly understood. More research is needed to demonstrate the combined effects of HIV infection and nutrition in pregnant women, especially in areas where prevalence of HIV infection is so high. This study will focus on mothers and examine the factors associated with body composition during pregnancy.

A cross-sectional study in Dar es Salaam, Tanzania, found mid upper arm circumference (MUAC) less than 22 cm at the first prenatal visit to be significantly associated with HIV infection and low birth weight (30,31). However, this study did not look arm muscle and fat area in relationship to MUAC.

In Zimbabwe, a cross-sectional study among HIV-infected women looked at the relationship maternal body composition, HIV infection, and other predictors of gestation length and birth size. Significant predictors of birth weight included season of birth, maternal height, arm fat area, gravidity, twin pregnancies, and female sex and HIV viral load. Maternal age, arm muscle area, hemoglobin and malaria were neither predictors nor confounders of birth weight (32). Although Zimbabwe and Malawi share some regional similarities such as cuisine, there are also some key differences. Malawi is a much poorer country than Zimbabwe. Drought combined with low socioeconomic status in Malawi is a definite contrast to Zimbabwe. Malaria was not endemic in the Zimbabwe study area due to the high altitude, while it is common problem in Malawi. They also did not consider dietary factors in their analysis.

This study in Lilongwe, Malawi, is unique in that it used a large cohort of over 1100 HIV-infected, pregnant women to examine body composition and diet. With repeated anthropometric measures of body weight, arm fat, and arm muscle area, we explored the dynamics of body composition during pregnancy, adjusting for various cofactors identified as

confounding or predicting factors in other studies. The large number of dietary recalls collected permitted a pattern analysis of food intake to acquire a better understanding of diet quality in HIV-infected pregnant women. With repeated measures of fundal height, we were able to examine fundal height as an outcome. There is no current literature on fundal height as an outcome, although numerous studies have examined serial measures of fundal height as a predictor of low birth weight. Using cross-sectional baseline data, we were also able to look at the relationship between AMA, AFA, and MUAC and birth weight to compare with the other findings in Tanzania and Zimbabwe to Malawian women and evaluate MUAC as a screening tool for LBW in HIV-infected pregnant women. This research contributes to the growing body of scientific evidence of the importance of enhancing maternal nutrition among HIV-infected women in resource-limited countries. Results can be used to direct and improve future nutritional epidemiological studies and food aid programs targeting HIV-infected women in these settings.

III. Patterns of body composition among HIV-infected, pregnant Malawians and the effects of famine season

A. Abstract

Background: Patterns and predictors of maternal body composition throughout pregnancy among HIV-infected women remain poorly understood, despite the fact that the prevalence of HIV infection exceeds 25% in the worst afflicted countries in sub-Saharan Africa. **Methods:** We describe change in weight, mid-upper arm circumference (MUAC), arm muscle (AMA) and arm fat area (AFA) in 1130 pregnant HIV-infected, Malawian women with CD4 counts > 200 who met pre-delivery screening criteria and consented to participate in the BAN Study: a postnatal randomized, controlled clinical trial (www.thebanstudy.org). **Results:** In an adjusted analysis, we found a linear increase in weight with a mean rate of weight gain of 0.27 kg/wk, from baseline (12 to 30 wks gestation) until the last follow-up visit (32 to 38 wks). Analysis of weight gain showed that 17.1% of the intervals between visits resulted in a weight loss. In unadjusted models, MUAC and AMA increased and AFA declined during late pregnancy. In a longitudinal analysis using multivariable regression, exposure to the famine season resulted in larger losses in AMA [-0.082, 95%CI: -0.14, -0.02; p=.01] while AFA losses occurred irrespective of season [-0.556, 95%: -0.97, -0.14, p=.01]. CD4 count was directly associated with AFA [.002, 95%CI: .001, .003, p=.03]. Age was positively associated with AMA and AFA [AMA: 0.162, 95%CI: 0.07, 0.26; p<.01; AFA: 0.200, 95%CI: (0.11, 0.29), p<.01]. Wealth index was positively associated with AFA [0.830, 95%CI: 0.53, 1.13; p<.01]. **Conclusion:** While patterns between HIV-infected, pregnant

women and uninfected women are similar in sub-Saharan Africa, effects of the famine season among undernourished, Malawian women are of concern. Strategies to optimize nutrition during pregnancy for these women appear warranted.

B. Introduction

Maternal body composition and its changes during pregnancy have an impact on women's health and their birth outcomes. The interaction of HIV/AIDS disease progression and nutrition is well established and bi-directional (33,34). In sub-Saharan Africa, studies have established a link between HIV-infection and maternal wasting (mid-upper arm circumference <22cm) in pregnant women (35). However, patterns and predictors of maternal body composition throughout pregnancy among HIV-infected women remain poorly understood, despite the fact that the prevalence of HIV infection exceeds 25% in the worst afflicted countries in sub-Saharan Africa (32).

Among predominantly HIV-uninfected populations, maternal anthropometry is one of the strongest predictors of pregnancy outcome (36-43). In well-nourished pregnant women, subcutaneous fat, as measured by arm fat area, increases during the first part of pregnancy and declines in the last trimester as maternal fat stores are mobilized to increase energy supply to the growing fetus. Muscle mass, as measured by arm muscle area, increases during the third trimester and remains elevated through at least 8 weeks postpartum (44-47).

Few studies have examined maternal anthropometry and its predictors during pregnancy in sub-Saharan Africa in the context of HIV/AIDS. Among HIV-infected women in Tanzania, poor anthropometric status at the first prenatal visit and weight loss during pregnancy predicted adverse pregnancy outcomes, however the direction and magnitude of

associations were similar to reports from comparable HIV-uninfected populations (30). Another study in Zimbabwe of HIV-infected and HIV-uninfected pregnant women reported no crude differences in anthropometric measurements (48).

In resource-limited countries, exposure to inadequate dietary intake, frequent reproductive cycles, infectious disease, and demanding physical labor may alter women's body composition dynamics during pregnancy compared to what is seen among women in countries where these stresses are mostly absent (32,49-51). The importance of seasonality to reproductive health is emphasized by several studies across Africa. Among predominantly HIV uninfected populations in Africa, lower gestational weight gain and increased maternal morbidity have been associated with the rainy season which is often characterized by increased food shortage, physical labor, and malarial infection rates (52). Similarly, in The Gambia, a 200-300 g birth weight deficit was seen in the wet season (53).

Because loss of lean body mass has been shown to be a predictor of HIV survival, independent of CD4 count (Suttmann 1995, Ott 1995), it is important to describe the changes in body composition throughout pregnancy and to identify its key determinants in HIV-infected populations (9,54). We analyzed repeated anthropometrics from HIV-1 infected pregnant Malawian women to evaluate two primary objectives: 1) to describe patterns of change in maternal weight, arm muscle area, and arm fat area among HIV-infected pregnant women, and (2) to identify potential seasonal, clinical, and sociodemographic predictors of maternal anthropometrics in this population.

C. Methods

C.1. Study design and population

The current study included women who consented and met pre-delivery screening criteria between April 2004 and August 2006 for the Breastfeeding, Antiretrovirals, and Nutrition (BAN) Study, a postnatal clinical trial (www.thebanstudy.org) (55). Briefly, study participants were recruited from four sites with outreach to all pregnant women in Lilongwe, Malawi (56). By August 2006, 1745 women met initial prenatal screening criteria: ≥ 14 years of age, no prior antiretroviral medication use, ≤ 30 weeks gestation, and no serious complications of pregnancy. Of these, 1336 women returned for the second screening visit and met eligibility criteria based on blood test results: CD4 count ≥ 200 cells/ μ L (≥ 250 cells/ μ L after July 2006), hemoglobin ≥ 7 g/dL, and normal liver function tests (≤ 2.5 times the upper limit of normal). At the second antenatal visit (here to forth referred to as the baseline visit), eligible women completed a baseline interview and physical exam and provided blood specimens. Of the 1336 eligible women at the baseline visit, 168 were missing a height measurement and 38 were missing another key baseline factor. Therefore, there were 1130 women available for this analysis. We conducted a sensitivity analysis comparing women with complete data to those without ($n=206$) and found no significant differences in the key study variables at baseline. The sample size of 1130 was determined to be sufficient to detect a 1 unit difference in the main outcomes with 90% power ($\alpha=0.01$) (30). The BAN Study protocol was approved by the Malawi National Health Sciences Research Committee and the institutional review boards at the University of North Carolina at Chapel Hill and the U.S. Centers for Disease Control and Prevention (ClinicalTrials.gov identifier NCT00164762).

C.2. Key variables

Gestational ages at baseline and subsequent prenatal visits were derived from the date of last menstrual period (LMP) or, if LMP was unknown, the first available fundal height (FH). Depending upon the estimated gestational age at baseline (range: 12 to 30 weeks), women were asked to return for follow-up prenatal care at approximately 28, 32, and 36 weeks gestation. The average gestational age at each follow-up visit was 29, 33, and 36 weeks, and the number of participants at these visits was 694, 868, and 703, respectively. More than 90% of the sample had at least 2 visits. The average time between visits was 4.5 (Standard Deviation (SD) = 2.8) weeks; the average total time between the baseline and last follow-up visits was 10.4 (SD = 5.1) weeks. The time between the last antenatal visit and delivery ranged from 10 to 0 weeks because in a few cases, the last visit coincided with the delivery date. Our analyses therefore reflect changes during the later stages of pregnancy.

Weight, height, mid-upper arm circumference (MUAC) and triceps skinfold thickness were measured at each visit by trained BAN nutrition staff. Weight was measured to the nearest 100g at each visit using a Tanita Digital Scale. Height was measured once either prenatally or postnatally with a standard height board. MUAC was measured to the nearest 0.1 cm using a nonstretchable insertion tape around the midpoint between the olecranon and acromion process while the arm hung freely at the side. Triceps skinfold thickness was measured in triplicate using Lange Calipers and was derived from the mean of the three measurements. MUAC and triceps skinfold thickness were used to derive arm muscle area (AMA), an indicator of muscle mass, and arm fat area (AFA), an indicator of fat mass: $AMA = [MUAC - (triceps\ skinfold \times \pi)]^2 / 4\pi$; $AFA = (MUAC^2 / 4\pi - AMA)$.

CD4 count which was collected either before referral to BAN or during the first screening visit was measured cross-sectionally. None of the women took antenatal antiretrovirals during this or prior pregnancies. Data were collected on interim malarial infections but were not available for all participants. Certain clinical factors, such as anemia, were not evaluated as potential predictors because of little variation due to study design: all women received iron and folate supplements, screening for anemia, malaria prophylaxis, and mosquito nets. After mid-June 2006, women with CD4 counts below 500 cells/ μ L received cotrimoxazole.

Malawi has a subtropical climate characterized by four seasons: cool (May to mid-August), hot (mid-August to November), rainy (November to April), and post-rainy (April to May). Food availability, malnutrition, and infectious disease morbidity vary substantially by season due to cycles of rainfall and agricultural production (57). The famine season, locally referred to as the “Green Famine”, extends from August to March and includes the rainy season prior to the harvest. This time period is marked by limited food availability as stores of the previous year's crops are depleted and incidence of infectious diseases peaks (58). Exposure to the famine season was measured as the number of days during the month prior to each measurement that were spent in the famine season.

Basic sociodemographic information was collected during the baseline interview: age, parity, marital status, household characteristics, and the educational level and occupation of both the mother and her partner. A wealth index was derived from household characteristics: house construction (type of walls, floors, and roof), number of rooms and residents,

electricity, refrigeration, sanitation, water source and cooking fuel source (59). The different levels of wealth were represented by index quintiles.

C.3. Statistical analysis

Since repeated measures were available for most women during pregnancy, we fitted random effects multiple linear regression models for each anthropometric outcome (MUAC, AMA, AFA and maternal weight) using XTREG in STATA version 9.0 (60). The XTREG function fits cross-sectional time-series regression models that account for the correlation among repeated measures on the same individual.

Model specification was guided by studies on nutritional outcomes among pregnant and non-pregnant HIV-infected women. Time-independent factors included maternal height, age, parity, marital status, education, wealth, working status, and CD4 count. Time-varying factors included gestational age and number of days in prior month spent in the famine season. We tested for linearity in the association of each exposure to each anthropometric outcome. Variables with linear associations were introduced into the model as continuous variables, while those with nonlinear associations were categorized. Interaction terms with the main predictors were tested and were retained in the model if significant at $p < 0.15$ (61,62).

D. Results

Most women in this sample were young and married with low parity (**Table 1**). The mean baseline CD4 count was 439 (Interquartile range: 319 - 592) cells/ μ L. Average maternal weight, MUAC, AMA, and AFA at baseline were 58.7 (SD = 8.2) Kg, 26.5 (SD =

2.7) cm, 36.7 (SD = 6.5) cm², and 19.7 (SD = 8.1) cm², respectively (**Table 1**). The prevalence of wasting (MUAC<22cm) was 1.9%, 3% of women gained no weight, 8.4% showed weight loss, and 59% had low fat stores (AFA < 20 cm²).

A crude analysis of weight change over the 2338 intervals between consecutive prenatal visits indicated that 17.3% of intervals showed weight loss. About half of intervals also had a loss in AMA (48.7%) and AFA (53.1%). Of those intervals in which muscle stores were lost, 34.3% lost both muscle and fat stores.

In bivariate linear analysis, maternal weight increased at a rate of 0.24 kg per week. There was no evidence of change in MUAC, 0.03 (95% CI: 0.001, 0.055) cm² for AMA, and -0.05 (95% CI: -0.08,-0.03) cm² for AFA.

In multivariate analysis, weight increased at a rate of 0.27 (95% CI: 0.26, 0.28) kg per week and AFA decreased at a rate of 0.06 (0.09-0.04) cm² per week (Table 2). The rate of change in MUAC and AMA during pregnancy was modified by exposure to the famine season. Women with no exposure to famine during the previous month experienced a subtle increase in MUAC (0.004 cm² per week) and a significant increase in AMA (0.06 cm² per week) (Figures 1-2). In contrast, women who spent the entire preceding month in a famine period had a significant decrease in MUAC (-0.02 cms) and AMA (-0.03 cm²) per week of gestation. Exposure to the famine season was also associated with decreased weight gain (-0.67 kg, 95% CI: -0.85, -0.49) and loss of AFA (-0.55 cm², 95% CI: -0.95, -0.14) per week of pregnancy (**Table 2**), however there was no significant modification of the pattern for weight or AFA change throughout pregnancy (Figures 3-4). There was no evidence that annual differences had any effect on anthropometric indicators.

Although there was no association between CD4 categories (200-<350, 350-<500, and ≥ 500 cell/ μ L), CD4 count, as a continuous variable, was directly associated with MUAC, AFA, and weight. Each 100 cells/ μ L increase in CD4 count was associated with an increase of 0.08 cm² (95% CI: 0.01, 0.15) cm in MUAC, 0.15 (95% CI: -0.01, 0.30) cm² in AMA, 0.21 (0.01, 0.41) cm² in AFA, and 0.24 (0.03, 0.45) kgs in weight.

Key sociodemographic factors were associated with anthropometric changes during pregnancy. Women with a higher wealth index score or who worked for income had increased MUAC and AFA; only women who worked, not wealthier women, had a significant direct positive association with AMA. Women, who completed primary education, had higher MUAC due to increased AMA than those who completed secondary or higher level education. Age and parity were positively associated with MUAC and AMA, but only age was associated with weight. AFA was unrelated to age and parity.

E. Discussion

In this relatively healthy, HIV-infected population with high CD4 counts, we observed similar patterns of increased weight and AMA with decreased AFA during late pregnancy as seen in comparable HIV-uninfected populations. However, lower CD4 counts negatively impacted the rate of change for MUAC, AFA, and weight. Furthermore, exposure to periods of famine modified the association between gestational week and MUAC and AMA, so that opposite trends in AMA change were observed in times of famine and non-famine.

The mean rate of weight gain in our population (0.27 kg/wk) was lower than the WHO recommended weight gain during the 4th month of pregnancy (.34 -.46 kg per week)

and that reported in studies among presumably HIV-uninfected adult women from the United States and Europe (63). However, the rate of weight gain in our population was similar to or higher than that of presumably mostly HIV-uninfected women from African settings: 0.20 and 0.22 kg/wk in rural Kenyan women (42), 0.20 kg/wk between weeks 14 and 40 in women from rural Tanzania (64), and 0.13 kg/wk between weeks 24 and 33 among rural Malawian women (65). Furthermore, weight gain in our population was comparable to that among a cohort of 957 HIV-infected, pregnant women in Tanzania: 0.25 kg/wk (16). Both the Tanzania study and our study found that low baseline CD4 count impaired the rate of pregnancy weight gain. In our population, each 100 cells/uL increase in CD4 count was associated with a 0.24 kg/week increase in rate of weight gain; in the Tanzania study, the adjusted rate difference between women with CD4 counts >500 cells/ uL and those with counts <200 cells/ uL was 118 g/wk at 18 weeks gestation and 93 g/wk at 37 weeks. Differences in pregnancy weight gain across African settings may be due to differences in study populations or anthropometric assessment over different gestational periods. Over two-thirds of the women in our study had some exposure to the famine season in the month prior to an anthropometric assessment. Exposure to the famine season negatively impacted weight gain in our study which is consistent with a report of highest pregnancy weight gain among Malawian women who deliver in July-September (mean gain 0.25-0.30 kg per week) and lowest gain among those who deliver in January-May (mean gain 0.10-0.20 kg/week) (57).

In our study, participants showed evidence of arm fat loss 0.6 cm^2 over a 10 week period. In contrast, low income, predominantly black U.S. pregnant women, experienced arm fat area loss of 1.26 cm^2 from 28 weeks to the postpartum period (4-6 weeks) (15). While

mobilization of fat stores may be beneficial to the fetus, it has detrimental consequences to the mother's health (Hartikainen 2005) if protein and fat stores were insufficient prior to conception or fat stores are overly depleted during pregnancy. In a similar sub-Saharan population, women with low AFA ($<20 \text{ cm}^2$) at baseline (22-35 weeks gestation) had higher risk for poor birth outcomes compared to women with higher AFA (32). Over half the women in our study had low AFA and could be at increased risk for poor birth outcomes, irrespective of rate of arm fat loss. Additional burdens, such as low CD4 count and exposure to famine periods, could accentuate the impact of rate of arm fat loss on maternal and fetal outcomes among women with low initial fat sources. As CD4 count declines, HIV-infected individuals begin to lose fat due to an increased metabolic rate to combat the worsening infection, and the release of cytokines (e.g. tumor necrosis factor) increase fat loss, malabsorption and susceptibility to opportunistic infections (66).

The rates of change in MUAC and AMA were modified by exposure to the famine season. Among women not exposed to a famine period, there was a subtle, non-significant increase in MUAC, and a notable, significant increase in AMA, during pregnancy. However, for women who were exposed to a famine period, both MUAC and AMA declined significantly during pregnancy. In well-nourished women, muscle mass, as measured by arm muscle area, increases during the third trimester and remains elevated through at least 8 weeks postpartum (44,45) In a US-based sample of HIV-uninfected, well nourished, low income, predominantly black population, pregnant women experienced an increase in arm muscle area was 2.00 cm^2 (15). A similar trend was seen in marginally nourished, HIV-uninfected, women in The Gambia, where pregnant women showed an increase in lean body mass, although less than a comparison well-nourished European population (67).

For women exposed to the famine season, the declining trend in MUAC is consistent with that among HIV-infected women in Tanzania who lost an average 1 cm (95% CI: 0.8, 1.1) MUAC between 12 and 38 weeks of gestation. AMA loss in HIV-infected women has been associated with greater risk for opportunistic infections, indirectly increasing HIV disease progression and risk of death, as well as potentially increasing risk of perinatal or postpartum HIV transmission (35,68).

With no uninfected comparison group in the BAN Study, we could not do a relative comparison to HIV-uninfected pregnant women. Several studies have already reported the increased risk of poor nutritional status in HIV-infected women compared to uninfected women in U.S. and African populations (11,33,69,70).

Our sample comes from those who consent, test positive for HIV/AIDS, and meet the primary eligibility criteria. Hence, the focus of this analysis was determinants of nutritional status within HIV-infected pregnant women with high CD4 counts, who received prenatal care. We do not have follow-up data on women with low CD4 or hemoglobin below 7 g/dL since they were considered ineligible for the BAN Study and referred to care; however, in an analysis done on 300 women who did not participate, the descriptive characteristics were similar to our sample (unpublished).

A major strength is access to and availability of such a large cohort of HIV-infected women with serial anthropometric measurements during pregnancy. Anthropometric measurements, which assess body composition change rather than only weight gain, were analyzed, which can be used to develop more detailed nutritional requirements for HIV-infected pregnant women. Secondly, this study was conducted on women who received no antiretroviral regimen prior to delivery. Such associations between CD4 count and nutrition

will be less likely to be reproduced as antiretroviral medications for pregnant women become more available.

Lastly, we have focused on overall patterns of anthropometric change during pregnancy, ignoring some of the more proximate determinants of nutrition status. For example, we have no direct measure of physical activity which could affect women's nutritional needs, and thus energy balance and body composition through specific physical tasks. We have also not included measures of dietary intake or subsequent birth outcomes. However, we found wealthier women had higher fat mass and lower muscle mass reflecting their sedentary lifestyle or improved dietary intake. In addition, women with primary education had higher MUAC and AMA than those with secondary education, most likely due to increased physical activity; however lower fat stores reflect decreased dietary intake compared to more educated women. The association between AMA and maternal work status may reflect ability to work for pay or may be a consequence to weight bearing activities associated with subsistence farming, heavy manual labor and child rearing tasks. In Tanzania, among non-pregnant women, HIV and wasting, as measured by MUAC < 22 cm, were strongly modified by socioeconomic status and education. HIV infection and wasting were higher in women with low levels of education and among HIV-infected women who were not able to contribute to the household income (71). In a linear regression model, Villamor et al reported MUAC in women with the secondary level of education was lower than those with less education, which is consistent to the pattern we found (71). Future studies need to explore and assess the relationship of factors such as wealth, physical activity and dietary intake with maternal nutritional status during pregnancy.

In conclusion, our findings suggest that while patterns between HIV-infected, pregnant women and uninfected women are similar in sub-Saharan Africa, effects of the famine season among undernourished, HIV-infected Malawian women are of concern. Strategies to optimize nutrition during pregnancy for these women appear warranted.

Table 1. Obstetric history, seasonal, socioeconomic, HIV and anthropometric variables of HIV-infected pregnant women in Malawi (n=1130)

Characteristic	Mean (SD)
Obstetric history	
Gestation age (weeks)	24.83 (5.1)
Age (y)	25.93 (5.0)
Parity	1.66 (1.4)
Experienced famine season [n (%)]	
None	424 (37.52)
Some	222 (19.65)
All	484 (42.83)
Education [n (%)]	
No school	140(12.39)
Primary	591(52.30)
Secondary or higher	399 (35.31)
Occupation Status [n (%)]	
Mother works	218 (19.29)
Partner works	992 (87.79)
CD4 count (cells/uL)*	439 (IQR:319-539)
Anthropometry	
MUAC (cm)	26.47 (2.7)
AMA (cm ²)	36.69 (6.5)
AFA (cm ²)	19.66 (8.1)
Height (cm)	155.77 (5.5)
Weight (kg)	58.72 (8.2)

* CD4 count displayed as median and interquartile range (IQR).

TABLE 2

Longitudinal predictive model of midupper arm circumference, arm muscle area, arm fat area, weight (n=1130)

Independent variable	MUAC (cm)			AMA (cm ²)			AFA (cm ²)			Weight (kg)		
	B	(95% CI)	P	B	(95% CI)	P	B	(95% CI)	P	B	(95% CI)	P
Gestation (wks)	0.004	(-0.01, 0.01)	0.36	0.06	(0.02, 0.10)	0.01	-0.06	(-0.09, -0.04)	<0.01	0.27	(0.26, 0.28)	<0.01
Season (in past month)	0.52	(0.06, 0.98)	0.03	1.61	(-0.29, 3.51)	0.1	-0.55	(-0.95, -0.14)	0.01	-0.67	(-0.85, -0.49)	<0.01
Gestation & Season	-0.03	(-0.04, -0.01)	<0.01	-0.08	(-0.14, 0.02)	0.01						
Cd4 count x 10 ⁻² (cells/mm ³)	0.08	(0.01, 0.15)	0.04	0.15	(-0.01, 0.30)	0.06	0.21	(0.01, 0.41)	0.04	0.24	(0.03, 0.45)	0.03
Wealth Index (quintiles)	0.25	(0.13, 0.36)	<0.01	0.21	(-0.04, 0.46)	0.21	0.91	(0.58, 1.24)	<0.01	0.70	(0.36, 1.04)	<0.01
No Education	0.30	(-0.22, 0.82)	0.26	1.04	(-0.10, 2.17)	0.07	0.19	(-1.31, 1.69)	0.8	-0.27	(-1.82, 1.27)	0.73
Primary education	0.40	(0.05, 0.75)	0.02	1.04	(0.27, 1.80)	<0.01	0.70	(-0.30, 1.71)	0.17	-0.08	(-1.12, 0.97)	0.89
Secondary education	Reference											
Mother works	0.47	(0.08, 0.85)	0.02	0.90	(0.06, 1.73)	0.04	1.18	(0.07, 2.28)	0.04	2.37	(1.27, 3.48)	<0.01
Partner works	0.46	(0.01, 0.91)	0.05	0.64	(-0.34, 1.62)	0.2	1.30	(0.01, 2.59)	0.05	1.53	(0.19, 2.86)	0.03
Height	0.02	(-0.01, 0.05)	0.13	0.07	(0.01, 0.13)	0.03	0.03	(-0.05, 0.10)	0.45	0.37	(0.30, 0.43)	<0.01
Age	0.07	(0.02, 0.11)	<0.01	0.18	(0.08, 0.27)	<0.01	0.097	(-0.03, 0.22)	0.13	0.17	(0.04, 0.30)	0.01
Parity	0.22	(0.05, 0.38)	0.01	0.55	(0.18, 0.91)	<0.01	0.43	(-0.05, 0.91)	0.08	0.11	(-0.38, 0.60)	0.66

Figure 1: Predicted values of midupper arm circumference in HIV-infected pregnant Malawian women

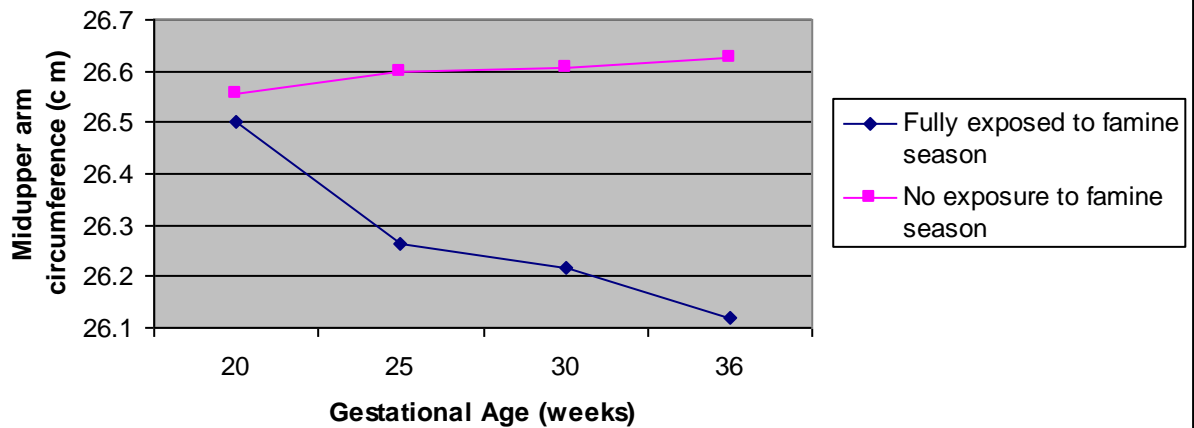


Figure 2: Predicted values of arm muscle area in HIV-infected pregnant Malawian women

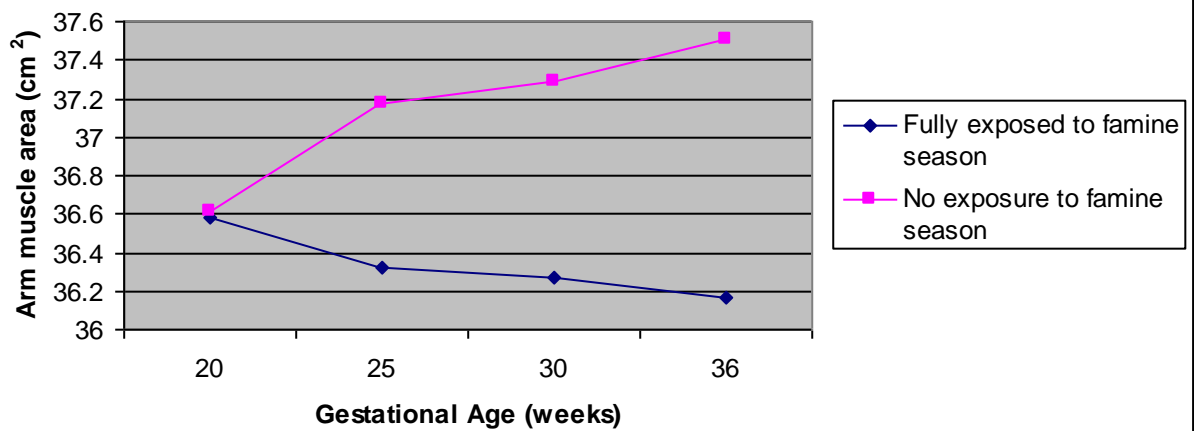


Figure 3: Predicted values of arm fat area in HIV-infected pregnant Malawian women

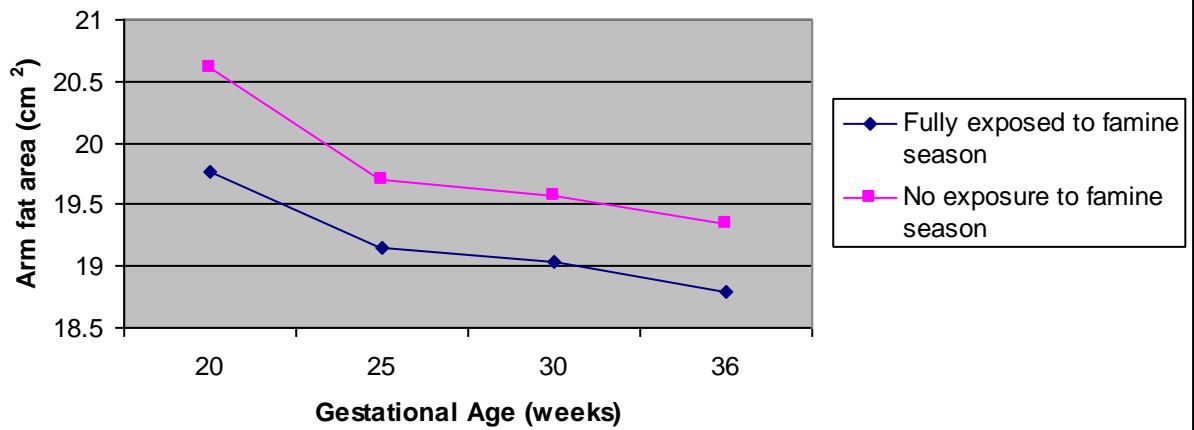
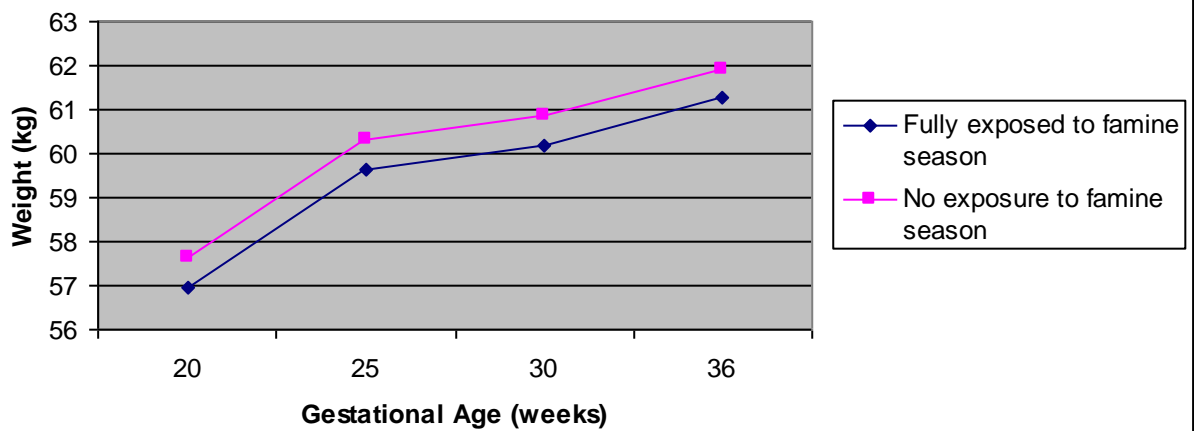


Figure 4: Predicted values of weight for HIV-infected pregnant Malawian women



IV. Dietary patterns and maternal anthropometry in HIV-infected, pregnant Malawian women

A. Abstract

Maternal diet is a potentially modifiable factor that can contribute to the health of pregnant women. In a sample of 577 HIV-positive pregnant women who completed baseline interviews for the BAN Study (www.thebanstudy.org) in Lilongwe, Malawi, cluster analysis was used to derive dietary patterns. Multiple regression analysis was used to look at associations between the dietary patterns and MUAC, AMA, AFA, and hemoglobin at baseline. Cluster 1 had significantly higher mean intakes of fats and oils, dairy (milk products), fish, meat, poultry and eggs than did the other 2 clusters, representing a diet high in animal products and thus good sources of micronutrients. Cluster 2 represents mostly a plant-based diet of grains, such as maize, rice, rice flour, and millet. This cluster also is the lowest in consumption of tubers, fruits, protein-rich, and micronutrient-rich foods. Cluster 3 had significantly higher mean intake of leafy vegetables, beans, legumes, tubers, nuts, and fruits compared to the other 2 clusters. This cluster had the lowest mean intake of grains and relatively low mean intake of fats, oils, dairy, fish and meat compared to cluster 1. Of these three clusters, cluster 2 represents women with the poorest quality diet. In multiple regression analysis, Cluster 2 had a 1.86 cm² higher AMA and -2.09 cm² lower than Cluster 1. Cluster 2 and Cluster 3 both had a 0.3 g/dL decreased hemoglobin compared to Cluster 1. Few studies in the literature have examined diets among HIV-infected pregnant sub-Saharan African women. Our findings describe the

typical diets of HIV-infected, pregnant women and the quality of maternal diets which need improvements to meet their increased demands due to HIV, malnutrition and pregnancy.

B. Introduction

In sub-Saharan Africa, women account for approximately 60% of estimated HIV infections, and almost 48% of new infections in 2008 were among women (UNAIDS 2008; Garcia-Calleja, Gouws, Ghys, 2006). A growing body of evidence supports the integral role of nutrition in HIV disease. The presence of infection increases nutrient needs and may limit dietary intake and reduce nutrient absorption, thereby impairing nutritional status. At the same time, nutritional status can influence the progression of disease (72). Wasting during pregnancy is more common among HIV-infected than –uninfected women living under the same conditions, as suggested by a study of women in Dar es Salaam, Tanzania (71). HIV-infected pregnant and lactating women are particularly at risk since they face increased nutrient demands to support fetal growth or milk production as well as their infection. Although others have shown how anthropometric indicators of nutritional status or micronutrient supplementation relate to adverse pregnancy outcomes among sub-Saharan, HIV-infected women (73) the role of diet in pregnancy has received less attention.

Maternal diet is a potentially modifiable factor that can contribute to the health of pregnant women and thus to birth outcomes. Inadequate diets can compromise women's nutritional status during pregnancy, when energy and nutrient dense foods are required to meet the needs of fetal growth and development and prepare for lactation (74). Our study uses cluster analysis to derive dietary patterns in a sample of 577 HIV-positive pregnant women in Lilongwe, Malawi.

C. Methods

C.1. Study design and population

Women were participants in the Breastfeeding, Antiretrovirals, and Nutrition (BAN) Study. Complete descriptions of the study have been reported previously (55). Briefly, study participants were recruited from four sites with outreach to all pregnant women in Lilongwe (56). At the first antenatal visit, women were consented and screened for pre-delivery criteria. Eligibility criteria included: 1) ≤ 30 weeks gestation based on last menstrual period or fundal height, 2) ≥ 14 years of age, 3) hemoglobin ≥ 7 g/dL, 4) CD4 count ≥ 200 cells/ μ L, 5) no prior antiretroviral medication use, 6) normal liver function tests (<2.5 upper limit of normal), 7) no serious complications of pregnancy, and 8) not previously enrolled in the BAN study. At the second antenatal visit (referred to as the baseline visit), a baseline interview, physical exam, and blood specimens. The BAN Study protocol was approved by the Malawi National Health Sciences Research Committee and the institutional review boards at the University of North Carolina at Chapel Hill and the U.S. Centers for Disease Control and Prevention (ClinicalTrials.gov identifier NCT00164762).

This analysis sample included BAN participants recruited between April 2004 and March 2006. During this period, 738 women had complete baseline data and live singleton births. After dietary recalls were reviewed for plausible portion sizes and nutrient intakes, 577 recalls were selected for this analysis and evaluated for selection bias using sample t-tests. There were no significant differences ($p < 0.05$) in anthropometric, clinical, or seasonal indicators in those that were excluded.

BAN Study participants were recruited from both urban and peri-urban areas of Lilongwe. These women typically acquire their food from local markets, corner stores, small

grocery stores, food aid programs, or their own small gardens. If families have any extra from their gardens, they take it to the market to sell or trade for other necessities. The typical Malawian diet includes the main staple is *Nsima*, which is a thick maize porridge that is molded into patties. Other staples include rice, potatoes, and cassava. One of these staples typically comprises the main dish and is served with beans, meat or vegetables (*Ndiwo*) or a relish to give added flavor. Plant-source, protein- and micronutrient-rich foods grown locally include soya and groundnuts. These are often made into flour and mixed into the main dish or relish. Most animal-source foods rich in protein and micronutrients are expensive and scarce. Fish from the local Lake Malawi is sold in the local markets in Lilongwe and more abundant and affordable than meat, although it is still limited due to depletion of Lake Malawi's supply.

C.2. Key variables

24-hour dietary recalls were collected by trained study staff during the antenatal screening follow-up visits. Recipes and ingredients of mixed dishes were recorded. Women were asked if the diet recall indicated “typical intake” and explanation if no. Food models and utensils were used to help respondents recall portion sizes and proportion of mixed dishes consumed. A Malawi nutrient food composition database (FCT) (75) was used to estimate nutrient intakes. Additional nutrient information was added from a FCT obtained from Malawi's Ministry of Health, a Tanzanian FCT (76), and the USDA nutrient database (77). The resulting data base had nutrient content of individual food items as well as mixed dishes based on standard recipes.

We categorized all foods into 12 food groups (Fats and oils; dairy; leafy green vegetables; nuts, beans, seeds, legumes, soy; tubers; fruits; fish; meat, poultry, eggs; grains; sugars, candy, soft drinks; hot beverages; miscellaneous). For cluster analysis, food group intake may be measured in absolute weight (in grams), percentage of energy, or frequency (number of servings) (78). Nutrient densities (grams/total calories) of each food group for daily intake were calculated and used in the cluster analysis. Standardization by energy contribution helps to remove dietary variations due to differences in sex age, body size, and physical activity and to retain the proportionally base food intake patterns (78,79). Values were transformed into sample-specific Z-scores so that food intake differences between clusters could be illustrated and compared.

Weight, height, mid-upper arm circumference (MUAC) and tricep skinfold thickness were measured by trained BAN nutrition staff. Weight was measured to the nearest 100g at each visit using the same beam-balance. Height was measured with a standard height rod. At each visit, MUAC was measured at the midpoint between the olecranon and acromion process, to the nearest 0.1 cm using an insertion tape, while the arm hung freely at the side. Triceps skinfold thickness was measured at each visit using Lange Calipers. The mean of the three separate determinations was used with MUAC to derive arm fat and muscle areas.

Arm muscle area (AMA), an indicator of muscle mass, was derived from MUAC and triceps skinfold measures as follows: $AMA = [MUAC - (triceps\ skinfold \times \pi)]^2 / 4\pi$. Arm fat area (AFA), an indicator of fat mass, was derived from MUAC and AMA: $AFA = (MUAC^2 / 4\pi - AMA)$.

CD4 count, which provides a measure of HIV progression, and hemoglobin, an indicator of iron status, were measured before referral to BAN or in the antenatal clinic as

part of BAN's screening process. Iron tablets were provided pre-delivery for all participants screened and consented for BAN.

Malawi has a subtropical climate characterized by four seasons: cool (May to mid-August), hot (mid-August to November), rainy (November to April), and post-rainy (April to May). Food availability, malnutrition, and infectious disease morbidity vary substantially by season due to cycles of rainfall and agricultural production (57). The famine season, locally referred to as the "Green Famine", extends from August to March and includes the rainy season prior to the harvest. This time period is marked by limited food availability as stores of the previous year's crops are depleted and incidence of infectious diseases peaks (58). Exposure to the famine season was measured as the number of days during the month prior to each measurement that were spent in the famine season.

Basic sociodemographic information was collected during the baseline interview: age, parity, marital status, household characteristics, and the educational level and occupation of both the mother and her partner. A wealth index was derived from household characteristics: house construction (type of walls, floors, and roof), number of rooms and residents, electricity, refrigeration, sanitation, water source and cooking fuel source (59). The different levels of wealth were represented by index quintiles.

C.3. Statistical analysis

We used the STATA (60) kmeans cluster method to group women according to their percentage of energy contributed from each of the 12 food groups. To find the most reasonable number of clusters, we ran a series of cluster analyses with predefined cluster numbers from 2 to 5. So that we would have reasonable sample sizes for further regression

analysis, we chose a 3- cluster solution. Using substantial knowledge of typical dietary patterns of Malawian women, we chose a cluster set, which best represented the dietary patterns and was nutritionally meaningful.

In univariate analyses, we compared dietary intakes (mean food group intakes, micronutrient intake) as well as sociodemographic and anthropometric characteristics across the 3 clusters, using one-factor ANOVA with Bonferroni correction for multiple pair-wise comparisons ($p < 0.017$).

We also performed multivariate linear regression to examine associations between dietary patterns and maternal nutritional indicators: MUAC, AMA, AFA, and Hb. Women were also asked if the one 24-hour dietary recall reflected their typical intake or not. All models were adjusted for “typical intake.” We also explored interactions between diet and maternal anthropometric indicators in relation to birth weight. Interactions were considered significant if at $p \leq 0.20$ (62).

D. Results

The women in this sample represent a young, low parous, unemployed, poorly educated group with high CD4 count (**Table 3**). Daily energy intake was very low with median 1235 (Interquartile range: 778, 1813). The majority of our sample was mildly anemic, according to the Malawi 2004 Demographic Health Survey (DHS), which defines women with 7.0-9.9 g/dL as having “moderate” anemia and pregnant women with 10.0-10.9 g/dL as having “mild” anemia.

Three dietary pattern clusters were derived, which we descriptively labeled 1) Cluster 1: high fish, meat and oil; low grain, 2) Cluster 2: high grain; low fruits and vegetables and

meat; and 3) Cluster 3: high leafy vegetables, nuts, and fruits; low grains and meats (**Figure 5**). Nutrient densities for each food group are presented and were compared between clusters in **Table 4**. Cluster 1 had significantly higher mean intakes of fats and oils, dairy (milk products), fish, meat, poultry and eggs than did the other 2 clusters, representing a diet high in animal products and thus good sources of micronutrients. Cluster 2 represents mostly a plant-based diet of grains, such as maize, rice, rice flour, and millet. Maize is the most common grain consumed since it is used to make the main staple food (nsima). This cluster also is the lowest in consumption of tubers, fruits, protein-rich, and micronutrient-rich foods. Cluster 3 had significantly higher mean intake of leafy vegetables, beans, legumes, tubers, nuts, and fruits compared to the other 2 clusters. This cluster had the lowest mean intake of grains and relatively low mean intake of fats, oils, dairy, fish and meat compared to cluster 1.

Cluster 2 also had the significantly lowest daily energy, protein, fat and iron intake compared to the other groups (**Table 5**). Cluster 1 had the significantly highest protein and fat intake. Furthermore, Cluster 3 had significantly higher carbohydrate intake compared to the other two clusters, which may be attributed to carbohydrate rich tubers, fresh fruits, and sugary drinks. Of these three clusters, cluster 2 represents women with the poorest quality diet.

Cluster 2 had the highest proportion of women in the lowest quintile of the wealth index, highest proportion of women with more than two live births, and highest proportion exposed to the famine season which extends from August to March (**Table 5**). Subjects in the 3 clusters did not differ significantly by age, CD4 count, or working status outside the home for income (predominantly farming).

MUAC did not vary across clusters in the unadjusted or adjusted analysis. In the unadjusted analysis, AMA in Cluster 2 was significantly higher than in Cluster 1 (**Table 5**). AMA did not appear to differ between Cluster 2 and 3 (**Tables 5**). AFA was significantly lower in Cluster 2 than Cluster 3 (**Table 5**).

After adjusting for age, education, wealth index, parity, maternal work status, seasonal exposure, annual differences, total energy intake, and CD4 count, Cluster 2 had 1.68 cm² higher mean AMA than Cluster 1 and -2.09 cm² and -2.47 cm² lower in mean AFA compared to Cluster 1 and Cluster 3, respectively (**Table 6**).

In the unadjusted analysis, Cluster 2 was significantly lower in hemoglobin compared to only Cluster 1, although there is little difference in mean hemoglobin levels in Cluster 2 and 3 (**Table 6**). This may be may explained by the slightly higher proportion of women with “moderate” anemia women and slightly lower proportion of women with normal hemoglobin in Cluster 2 (compared to cluster 3). In the adjusted analysis, hemoglobin was significantly lower (0.3 g/dL) in both Cluster 2 and 3 compared to Cluster 1, although the differences remain very subtle (**Table 6**).

E. Discussion

In this analysis, HIV-infected, pregnant Malawian women with high CD4 counts and mild anemia had had similar baseline MUAC, AMA, and AFA to other findings among HIV-uninfected and HIV-infected sub-Saharan women (18,32,48). We identified 3 distinct dietary patterns using cluster analysis and their relationships to maternal anthropometry measurements and hemoglobin levels.

In our study, we identified 3 clusters, each representing a food pattern typically found in diets of the HIV-infected, pregnant women in the BAN Study. By looking at nutrient density of intake per day of food groups in each cluster, we are able to describe, compare, and contrast the nutrient quality of each dietary pattern within our sample. Women in Cluster 1 consumed the most energy-dense foods, as reflected by their relatively high caloric intake compared to the others in our sample. Oil is a high commodity since it is typically used to prepare vegetables (frying or sautéing), or to fry or stew fish, poultry or other meats. Women consume more small fish (fresh or dried) in the rainy season when physical demands for harvesting are diminished (Huddle et al 1998). Therefore, these diets did not depend on the harvest season. These diets are rich in animal- and plant-based protein, fat, and micronutrients, particularly iron.

Women in Cluster 2 (high grain) represented the group with the poorest nutrient quality diet. Other studies of Malawian diets have found their diets are predominately cereal-based; intake of flesh food, a rich source of readily available iron and zinc are generally low (Ferguson et al 1995). Although Cluster 2 had the lowest iron intake, iron intake and mean hemoglobin values, reflective of iron deficiency anemia, were low for the majority of women. Clusters 2 and 3 patterns appear to reflect higher phytate, lower iron foods which may decrease iron bioavailability (75,80-82). Cluster 2 had lower consumption of fish than Cluster 1, a food relatively high in iron content and density. Consumption of fish has also been found to be associated with improved zinc absorption and higher calcium intake at levels that did not exacerbate any phytate-induced inhibition of zinc absorption (18). Cluster 2 also appear to have lower diet variation, an indicator of poor diet quality. In contrast, Cluster 3 consumed carbohydrate-rich tubers in addition to grain-based foods. Higher levels

of the plant-based protein-rich food group in Cluster 3 indicate consumption of soya-based foods, groundnut, kidney beans, split peas and other lentils. Lower levels of iron intake in Cluster 2 suggest a need for iron supplements or more iron-rich foods. Cluster 3 contained plant-based protein and micronutrient rich foods, yet women in Cluster 3 may require even higher levels of iron intake given their HIV and pregnant status.

Women in Cluster 2 with higher AMA consumed lower protein than Cluster 1. This is consistent with our comparison analysis between groups which found Cluster 2 had lower income and higher parity. Therefore, higher lean muscle mass may be a result of child rearing activities and manual labor. Famine season also appeared to impact Cluster 2 worse than the other clusters, which could have also contributed to increased physical demands. In contrast, Cluster 1 had lower AMA and higher AFA than Cluster 2. This most likely reflects women with less access to fruits and vegetables and more access to dried fish and poultry at the markets. Thus, this cluster may also represent higher income, more education, and more sedentary lifestyles than women in clusters 2 and 3.

In countries with high prevalence of HIV-infection, anemia due to underlying nutritional deficiencies may be compounded by parasitic infections, compromised immunity, and the hematological consequences of chronic and systemic inflammation. According to DHS definitions, 23.7 % of our sample had moderate anemia and 32.1% had mild anemia (DHS 2004). A cross-sectional study in Tanzania in 1064 HIV-infected pregnant women with median CD4 count of 407 found mean Hb level to be 9.4 g/dL. Twenty-eight percent was the prevalence of severe anemia; 83% of the women had Hb <11.0 g/dL (12). Among the HIV-infected women in our study, less than 3% had severe anemia and 56% had Hb < 110 g/L.

While multiple 24-hour recalls would be ideal for analyzing the determining factors of maternal body composition and birth weight (83), only one 24-hour recall was collected in the BAN Study. This is a limitation; however, unlike in Western diet, African diets among low-income women tend to vary little. A previous study in similar population in a group of sixty pregnant Malawian women living in three villages in Mangochi, Malawi, showed that the median daily intake was not significantly different (75). In addition, the high quantity of recalls still allowed for the examination of dietary patterns.

With over 500 dietary recalls from HIV-infected, pregnant Malawian women, we were able to derive dietary patterns typical of Malawians with cluster analysis. To our knowledge, this is the first study that used cluster analysis to examine prenatal dietary patterns in among HIV-infected women in sub-Saharan Africa. This method enabled us to examine diet quality of each pattern by looking across clusters. The use of dietary patterns rather than individual nutrients in assessing diet and health relationships has emerged as a method of assessing total food consumption (84-87). This type of evaluation allows for the examination of effects of group of dietary components on the outcome of interest (78). Utilizing foods versus nutrients in terms of food group intakes makes it easier to translate into intervention messages. Furthermore, seasonal and cultural factors influence diet patterns, which in turn affect nutrient intakes. It is also important to note there is a lack of studies on dietary patterns and health outcomes from developing countries, including sub-Saharan Africa. To our knowledge, this is the first study to look at dietary patterns among HIV-infected, pregnant women living in sub-Saharan Africa.

Although the use of patterning methods to analyze dietary data is still being explored, there are many publications on the subject (88). Some limitations of the cluster analysis

method include inherent subjectivity, which occurs throughout the pattern analysis because investigators must decide how to collapse the data (typically into food groups) and how to quantify the variables (as weight, frequency, or percentage of energy) (84,89,90). In addition, naming the derived patterns involves subjectivity, which may be described quantitatively based on the predominant food group consumed (fruit, vegetable), or qualitatively (healthy pattern) . However, we used substantial knowledge of the typical Malawian diets to select the food groups and appropriate clusters.

Our results suggest that prenatal women living with HIV/AIDS in sub-Saharan Africa could benefit from nutritional counseling on the importance of consuming protein, fat and iron rich diets. Sustainable strategies to increase intake of these foods should be explored.

Table 3. Nutritional status, caloric intake, and clinical characteristics among 577 pregnant women participating in the BAN Study		
Characteristic	Mean	Std. Dev.
Age (y)	25.9	4.9
Parity	1.7	1.3
Experienced famine season [n (%)]		
None	229	39.7
Some	84	14.6
All	264	45.7
Education [n (%)]		
No school	65	11.3
Primary	305	52.9
Secondary or higher	207	35.9
Occupation Status [n (%)]		
No employment	469	81.3
Gestational age (weeks)	25.2	5.4
Midupper arm circumference (cm)	26.4	2.6
AMA (cm ²)	36.6	6.4
AFA (cm ²)	19.5	7.8
CD4 count (cells/uL)	442	325-601
Daily energy total intake (kcal)	1378	821
Hemoglobin (g/dL)	10.8	1.2

***CD4 count displayed as median and interquartile range (IQR).**

Table 4. Food patterns showing nutrient densities (g/total calories per 100 kcal) across the 3 clusters among 577 pregnant women participating in the BAN Study ⁷							
	Cluster 1 ¹	Cluster 2 ²	Cluster 3 ³	Global P	Pairwise P		
	(n=160)	(n=254)	(n=163)		Cluster 1 compared with cluster 2	Cluster 1 compared with cluster 3	Cluster 2 compared with cluster 3
Fats and oils	2.71	0.52	0.54	<0.001	<0.001	<0.001	-
Dairy	2.20	0.68	0.43	<0.001	<0.001	<0.001	-
Leafy greens	3.58	4.59	11.10	<0.001	-	<0.001	<0.001
Nuts, etc	1.39	0.86	3.80	<0.001	-	<0.001	<0.001
Tubers	13.36	9.16	23.68	<0.001	<0.001	-	<0.001
Fruits	11.31	5.89	30.75	<0.001	<0.001	<0.001	<0.001
Fish	4.55	1.48	2.09	<0.001	<0.001	<0.001	-
Meat	5.53	0.46	0.62	<0.001	<0.001	<0.001	-
Grains	29.18	68.84	34.45	<0.001	<0.001	-	<0.001
Sugars, drinks	2.94	2.27	9.65	<0.001	-	-	<0.001

Figure 5.

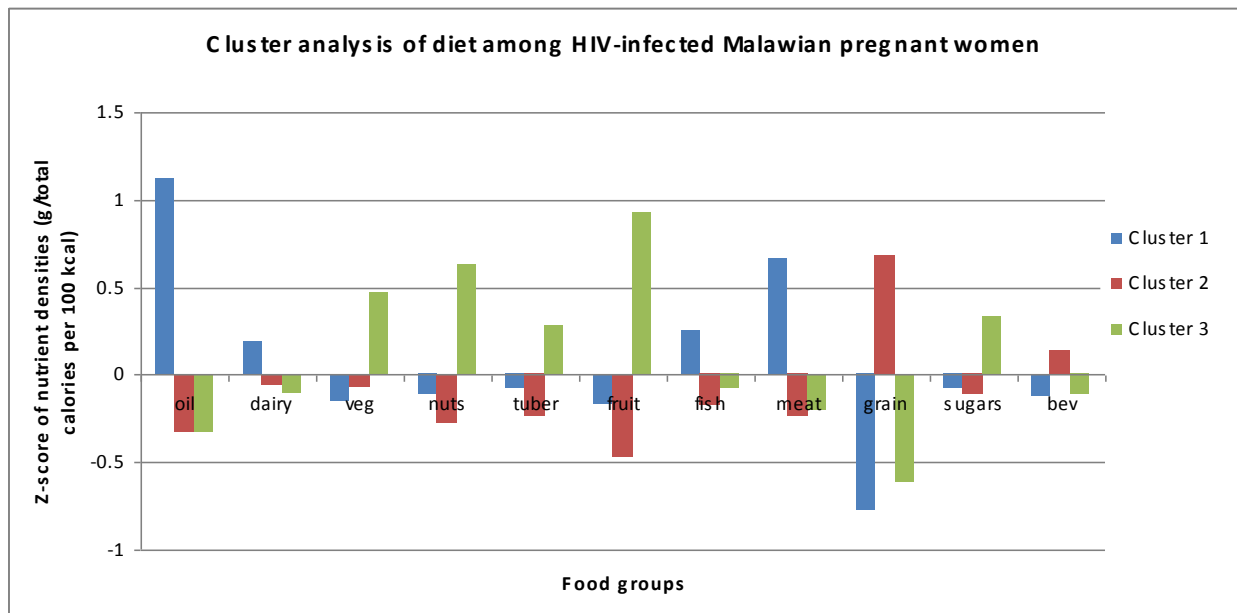


Table 5. Demographic, nutrient, and clinical indicators by the 3 clusters among 577 pregnant women participating in the BAN Study ⁷

	Cluster 1 (n=160)	Cluster 2 ⁵ (n=254)	Cluster 3 (n=163)	Pairwise P ⁴		
				Cluster 1 compared with cluster 2	Cluster 1 compared with cluster 3	Cluster 2 compared with cluster 3
Sample characteristics						
Age (y) (mean \pm SD)	25.0 \pm 4.4	26.2 \pm 5.1	26.2 \pm 4.9	-	-	-
No school (n=65) [n (%)]	11 (6.9)	37 (14.5)	17(10.4)	-	-	-
Wealth index=1 (n=108) [n(%)]	17 (15.7)	63 (58.3)	28 (25.9)	0.001	-	-
Parity>2 (n=133) [n(%)]	23(14.3)	67 (26.4)	43 (26.3)	0.014	-	-
Famine exposed (n=348) [n(%)]	83 (51.9)	180 (70.9)	85 (51.2)	<0.001	-	<0.001
Nutrition status						
MUAC (cm)	26.3 \pm 2.7	26.3 \pm 2.6	26.8 \pm 2.6	-	-	-
AMA (cm ²)	35.3 \pm 6.2	37.3 \pm 6.5	37.0 \pm 6.3	0.007	-	-
AFA (cm ²)	20.2 \pm 8.0	18.7 \pm 7.0	20.7 \pm 8.5	-	-	0.003
Birth weight (g)	3058.6 \pm 431.4	2976.9 \pm 434.4	3053.9 \pm 395.2	-	-	-
Hemoglobin (g/dL)	11.0 \pm 1.1	10.7 \pm 1.2	10.7 \pm 1.1	0.017	-	-
Daily energy total intake (kcal)	1776.8 \pm 859.5	1083.5 \pm 672.2	1445.4 \pm 818.5	<0.001	<0.001	<0.001
Carbohydrates (g/d)	195.8 \pm 116.3	201.1 \pm 128.9	237.4 \pm 135.3	-	0.010	0.014
Protein (g/d)	69.3 \pm 57.1	32.6 \pm 28.3	47.1 \pm 35.9	<0.001	<0.001	0.001
Total fat (g/d)	82.9 \pm 51.2	19.9 \pm 18.5	41.2 \pm 43.8	<0.001	<0.001	<0.001
Iron (g/d)	10.1 \pm 9.9	6.9 \pm 5.9	11.3 \pm 8.4	<0.001	-	<0.001

¹ Food pattern of high fish, meat and oil.

² Food pattern of high grain and grain-derived foods.

³ Food pattern of high leafy vegetables, nuts, tubers, fruits.

⁴ Determined by *t* test (values *P* <0.017, Bonferroni adjustment for multiple comparisons)

⁵ Food or food group contributed the relatively lowest mean intake across the 3 clusters.

Table 6. Multivariate linear regression models showing adjusted associations between clusters and AMA, AFA, iron status in 577 HIV-infected, pregnant Malawian women participating in the BAN Study

Outcome					
AMA ¹ (cm ²)	B	SD	P	95 % CI	
Cluster 2	1.86	0.68	0.01	0.53	3.19
Cluster 3	1.23	0.70	0.08	-0.14	2.60
Cluster 3 (referent Cluster 2)	-0.63	0.64	0.33	-1.89	0.63
AFA ¹ (cm ²)					
Cluster 2	-2.09	0.84	0.01	-3.75	-0.44
Cluster 3	0.38	0.87	0.67	-1.33	2.08
Cluster 3 (referent Cluster 2)	2.47	0.80	<0.01	0.90	4.03
Hb ¹ (g/dL)					
Cluster 2	-0.27	0.13	0.04	-0.52	-0.01
Cluster 3	-0.32	0.13	0.01	-0.59	-0.07
Cluster 3 (referent Cluster 2)	-0.06	0.12	0.62	-0.30	0.18

¹Adjusted for total energy intake, season, age, parity, education, SES, maternal work status, height and CD4 count

V. Maternal mid-upper arm circumference is associated with birth weight among HIV-infected Malawians

A. Abstract

We examined the relationship of maternal anthropometry to fetal growth and birth weight among 1005 HIV-infected women in Lilongwe, Malawi, who consented to enrollment in the Breastfeeding, Antiretrovirals, and Nutrition (BAN) Study (www.thebanstudy.org). Anthropometric assessments of mid-upper arm circumference (MUAC), arm muscle area (AMA), and arm fat area (AFA) were collected at the baseline visit between 12 and 30 weeks gestation and up to 4 follow-up prenatal visits. In longitudinal analysis, fundal height increased monotonically at an estimated rate of 0.92 cm/week and was positively and negatively associated with AMA and AFA, respectively. These latter relationships varied over weeks of follow-up. Baseline MUAC, AMA, and AFA were positively associated with birth weight [MUAC: 31.84 grams per cm increment, 95% CI: 22.18, 41.49 ($p < 0.01$); AMA: 6.88 g/cm², 95% CI: 2.51, 11.26 ($p < 0.01$); AFA: 6.97 g/cm², 95% CI: 3.53, 10.41 ($p < 0.01$)]. In addition, MUAC and AMA were both associated with decreased odds for LBW (<2500 g) [MUAC: OR=0.85, 95% CI: 0.77, 0.94 ($p < 0.01$); AMA: OR=0.95, 95% CI: 0.91, 0.99 ($p < 0.05$)]. These findings support the use of MUAC as an efficient, cost effective screening tool for LBW in HIV-infected women, as in HIV-uninfected women.

B. Introduction

In resource-limited settings, low birth weight (LBW) is common. Research comparing data from resource-limited countries to that from resource-rich countries shows that most of the differences in LBW rates may be attributed to an increased prevalence of intrauterine growth restriction (IUGR), rather than preterm birth, with relative risks of 6.6 and 2.0, respectively (91). It is well established that the short-term consequences of IUGR include increased risk of fetal, neonatal, and infant death and impaired postnatal growth, immune function, and intellectual development (23-25).

Maternal nutritional status, as estimated by anthropometrics, is an important contributor to fetal growth and infant birth weight{WHO, 1991 #79; WHO, 1995 #209; WHO, 1995 #171}. In pregnant women, weight alone may not be the best indicator of maternal muscle and fat stores, since it is a measure of both the mother and the fetus. Therefore, simple and inexpensive anthropometric measurements, such as mid-upper arm circumference (MUAC) and skinfold thickness measurements, are used in large-scaled epidemiological studies to derive estimates of lean muscle mass and adiposity (30,32,48). In resource-limited settings, intrauterine growth assessments rely on serial fundal height measurements during antenatal care and determination of intrauterine growth restriction is based on low birth weight for estimated gestational age (92).

The relationship between MUAC and LBW has been studied extensively in HIV-uninfected populations (42,93,94). However, there is limited data on the utility of maternal anthropometrics as predictors for fetal growth and birth weight among cohorts of pregnant HIV-infected women (8-10). HIV-infected individuals may lose fat mass quicker than HIV-uninfected individuals due to an increased metabolic rate to combat infection and

malabsorption of nutrients (66). In addition, women in sub-Saharan Africa are vulnerable to factors such as high parity and short birth intervals prevalent infectious disease and seasonal variations in food insecurity. These combined factors place an added burden on pregnant women and therefore may contribute to poor birth outcomes, such as LBW (de Onis et al. 1998). We hypothesized that low maternal nutritional stores, measured by low arm muscle area (AMA) and low arm fat area (AFA) would relate to slower intrauterine growth and increased risk of LBW.

C. Methods

C.1. Study design and population

The current study includes 1005 HIV-infected women who delivered live singleton births between June 2004 and December 2006 and consented to enrollment in the Breastfeeding, Antiretrovirals, and Nutrition (BAN) Study, a postnatal clinical trial (www.thebanstudy.org) (55). These study participants were recruited from four sites with outreach to all pregnant women in Lilongwe, Malawi and met prenatal screening criteria: ≥ 14 years of age, no prior antiretroviral medication use, ≤ 30 weeks gestation, and no serious complications of pregnancy, CD4 count ≥ 200 cells/ μ L, hemoglobin ≥ 7 g/dL, and normal liver function tests (≤ 2.5 times the upper limit of normal). At the second antenatal visit (here to forth referred to as the baseline visit), 1130 eligible women completed a baseline interview, physical exam and provided blood specimens. Of these, 125 women were excluded from the analysis sample owing to fetal loss, still birth, twins, home deliveries or late presentation after delivery. Out of 1005 women who had live singleton births, no fundal

height was available for 92 women. Of 913 women who had at least one fundal height, 20 women had no baseline measurement.

C.2. Key variables

Mid-upper arm circumference (MUAC), triceps skinfold thickness, and height were measured by trained BAN nutrition staff. Height was measured once either prenatally or postnatally with a standard height board. At each visit, MUAC was measured at the midpoint between the olecranon and acromion process, to the nearest 0.1 cm using an insertion tape, while the arm hung freely at the side. Triceps skinfold thickness was measured at each visit using Lange Calipers. The mean of three separate determinations was used with MUAC to derive arm fat and muscle areas. Arm muscle area (AMA), an indicator of total muscle mass, was derived from MUAC and triceps skinfold measures as follows: $AMA = [MUAC - (triceps\ skinfold \times \pi)]^2 / 4 \pi$. Arm fat area (AFA), an indicator of total fat mass, was derived from MUAC and AMA: $AFA = (MUAC^2 / 4 \pi - AMA)$.

Gestational age at baseline and subsequent prenatal visits were derived from the date of last menstrual period (LMP) or, if LMP was unknown, first available fundal height. Depending upon the estimated gestational age at baseline, women were asked to return for follow-up prenatal care at approximately 28, 32, and 36 weeks gestation and fundal height was used to estimate intrauterine growth. In the BAN Study, fundal height was ascertained by measuring the distance between the upper edge of the pubic symphysis and the top of the uterine fundus using a tape measure. Beginning in the second trimester, fundal height +/- 1 to 3 cm should estimate gestational age in weeks (i.e., a pregnant woman's uterus at 26 weeks

should measure 23 to 29 cm) (63,95,96). Birth weight was measured using a Tanita Digital Baby Scale to the nearest 0.1 kilogram immediately after delivery in the hospital or within 48 hours of delivery, upon arrival to the hospital, for home deliveries. LBW was defined according to the WHO definition of less than 2500 g (97).

Basic sociodemographic information was collected during the baseline interview: age, parity, marital status, household characteristics, and the educational level and occupation of both the mother and her partner. A wealth index was derived from household characteristics: house construction (type of walls, floors, and roof), number of rooms and residents, electricity, refrigeration, sanitation, and cooking fuel source (59). CD4 count was measured cross-sectionally either before referral to BAN or during the first screening visit. None of the women took antenatal antiretrovirals during this or prior pregnancies. All participants received iron and folate supplements, malaria prophylaxis and treatment, and mosquito nets; after mid-June 2006, women with CD4 counts below 500 cells/ μ L received cotrimoxazole. At onset of labor, all participants received the HIVNET 012 regimen and a 7-day postnatal “tail” of zidovudine and lamivudine to prevent perinatal HIV transmission. The BAN Study protocol was approved by the Malawi National Health Sciences Research Committee and the institutional review boards at the University of North Carolina at Chapel Hill and the U.S. Centers for Disease Control and Prevention (ClinicalTrials.gov identifier NCT00164762).

C.3. Statistical Analysis

We used STATA version 9.0 for this analysis (60). First, we evaluated the potential for selection bias by first comparing women who had anthropometric measurements

collected at baseline (n=1005) to those who were excluded from the analysis sample owing to fetal loss, still birth, home deliveries or late presentation after delivery (N=125). Two-sample t-tests and chi-square tests were used to compare means and proportions, respectively, between the samples, and inverse probability weighting was used to evaluate selection bias in our statistical models (98). The likelihood of being in the analysis sample (with complete data) was estimated from a logistic regression model including baseline socioeconomic and demographic variables, and the inverse predicted probabilities were included as sample weights in regression models for birth outcomes.

Fundal height at each antenatal visit was modeled using longitudinal random effects regression. Time-varying anthropometric indicators measured concurrently with fundal height (e.g. AMA and AFA) were included in the model, along with time-independent baseline characteristics described above. Since food availability, malnutrition, and infectious disease morbidity vary substantially by season due to cycles of rainfall and agricultural production (57), the number of days spent in the famine season (August to March) in the month prior to anthropometric assessment was included as a time-varying cofactor. In addition, due to reported annual variations in the maize production, year at baseline was also included in the models (99). The time between the baseline visit and each subsequent visit was included in the model for fundal height. To anchor the timing of measurements relative to total gestational duration, the time between the last antenatal visit and delivery was included in the model.

Linear regression models were used to estimate birth weight and logistic regression models were used to estimate odds ratios for LBW associated with each risk factor after adjustment of covariates. Model specification was guided by studies on nutritional outcomes

among pregnant HIV-infected women. Two multivariable models were constructed for each outcome. One included AMA and AFA together as the main exposures; a second model included MUAC as the main exposure. We tested for linear trends between the independent variables and study outcomes, and if non-linear, categorical variables were included in the models. Interaction terms were estimated and retained in the models if their p-value was <0.15 (62).

D. Results

The median CD4 count was 439 cells/ μ l (IQR: 319-592), and about a third of women fell into each of three CD4 categories: 200 to 350 cells/ μ l (31%), 350 to 500 (31%), and >500 (38%) (**Table 7**). Almost two-thirds (62%) of the women were exposed to the famine season in the month prior to their baseline visit. The mean mid-upper arm circumference (MUAC) was 26.5 cm, with an arm muscle area of 36.8 cm² and arm fat area of 19.7 cm², at baseline. The average birth weight was 2998 g, and the prevalence of low birth weight was 8.7% (**Table 7**).

Women with complete baseline data (n=1005) were significantly younger (p<0.01), commenced prenatal care earlier (p=0.03), and were more likely to be primiparous (p<0.01) than women without complete baseline data (n=125); they did not differ significantly on CD4 count, maternal anthropometrics, or fundal height. Weighted and unweighted models were not appreciably different, suggesting that selection bias was not a problem. Thus, we present unweighted results.

Fundal height increased monotonically at a rate of about 0.92 cm/week (**Table 8**). In the longitudinal analysis, AMA was associated with a subtle, yet significant increase in

fundal height over the course of the latter part of pregnancy; in contrast, AFA was associated with a subtle, yet significant decrease in fundal height. MUAC was not significantly associated ($\beta = 0.01$ (95% CI: -0.06, 0.07; $p=0.85$). The effects of AMA [$\beta = 0.03$ (95% CI: 0.01, 0.06)] and AFA [$\beta = -0.03$ (95% CI: -0.05, -0.002)] were significantly modified by weeks of follow up [AMA x week: $\beta = -0.003$ (95% CI: -0.006, -0.001); AFA x week: $\beta = 0.003$ (95% CI: 0.001, 0.006)]. An important covariate in the model for fundal height was infant HIV status [-1.42 cm, 95% CI: -2.61, -0.23 ($p = 0.02$)]. However, there was no association between fundal height and maternal CD4 count. Furthermore, there was no evidence famine season or year of enrollment impacted fundal height. Age, parity and wealth index had weak associations with fundal height.

Maternal MUAC, AMA, and AFA were associated with infant birth weight. MUAC was the strongest predictor. For each 1 cm increase in MUAC, there was a 31.8 g (95%CI: 22.2, 41.5) increase in birth weight (**Table 9, Model 2**). Each 1 cm² increase in AMA and AFA was associated with a 6.88 g and 6.97 g increase in birth weight, respectively (**Table 9, Model 1**). In models with low birth weight as the outcome, maternal MUAC was associated with a decreased odds of low birth weight [OR=0.85, 95% CI: 0.77, 0.94 ($p<0.01$)] (**Table 9, model 2**). Similarly, the risk of having a LBW infant decreased with increasing AMA (OR: 0.95, 95% CI: 0.91, 0.99); however, the relationship between AFA and LBW was not statistically significant ($p=0.08$) (**Table 9, Model 1**).

E. Discussion

Birth weight is an important predictor of infant survival and future development. In our study of HIV-infected pregnant women with high CD4 counts, mean MUAC (26.5), fetal

growth (0.92 cm/ week) and birth weight (2998 g) were similar to other sub-Saharan populations (100,101). In addition, maternal MUAC was a strong predictor of birth weight and LBW in this HIV-infected population, as in HIV-uninfected women.

With repeated measures, we observed similar patterns of increasing fundal height with increasing muscle mass and decreasing fat mass during late pregnancy, as seen in comparable HIV-uninfected populations (15). However, the small increase in AMA coincided with an equally small decrease in AFA, thus explaining why MUAC did not show any relationship to fetal growth (44,46,47). Currently, there is no comparative literature that examines maternal factors and their associations with fundal height. The use of fundal height to estimate gestational age has been criticized in resource-limited contexts where intrauterine growth restriction (IUGR) is prevalent because fundal height can misclassify a growth-restricted infant as having an earlier week of gestation (102). Rather than using fundal height as an indicator of gestation week, we modeled repeated measurements of fundal height as an outcome and examined how it related longitudinally to maternal anthropometry and other maternal characteristics. Serial measurements of fundal height are being increasingly used in resource-limited settings for the prediction of birth weight and diagnosis of IUGR (103-105).

While AMA and AFA minimally affected fetal growth, and MUAC not at all, AMA, AFA, and MUAC were directly related to birth weight. MUAC was the strongest predictor for birth weight, both as a continuous measurement and dichotomized to indicate low birth weight. This finding is consistent with reports from European and African women. In pregnant European women, pregravid lean body mass was found to be a significant predictor of birth weight (106). Among a cohort of 110 Kenyan women, increased odds for low birth weight were reported for mothers with smaller MUAC during the 2nd and 3rd trimesters (42).

Among 1002 HIV-infected women in Tanzania, mothers with the highest quartile of MUAC distribution had higher infant birth weights relative to mothers in the lowest quartile of MUAC (30). In contrast, the latter study found no association between MUAC and LBW.

While arm muscle area (AMA) was associated with both birth weight and low birth weight, arm fat area (AFA) was significantly associated only with birth weight. The direct association between AFA and birth weight may appear contradictory to the inverse association between AFA and fundal height; however, the association between AFA and fundal height was evaluated in a longitudinal model which includes serial measures during pregnancy over which AFA tends to decline while fundal height increases. In contrast the association between AFA and birth weight was evaluated in a cross-sectional model utilizing a baseline AFA measurement, which reflects fat storage earlier in pregnancy. The cross-sectional association between AFA and birth weight is consistent with a study in Zimbabwe among 1669 HIV-infected women that found low AFA ($<20 \text{ cm}^2$) measured once during late pregnancy was associated with lower birth weight (32). However, unlike our study, they found no relationship between AMA and LBW.

A major strength of our study is serial anthropometric measurements during pregnancy on a large cohort of HIV-infected women. However, with no HIV-uninfected comparison group in the BAN Study, we could not do a relative comparison to HIV-uninfected pregnant women. Hence, the focus of this analysis was of the effects of nutritional status among HIV-infected pregnant women who received prenatal care on intrauterine growth and birth weight. Our sample comes from those who consent, test positive for HIV/AIDS, and meet the primary eligibility criteria. We do not have follow-up data on women with low CD4 counts or hemoglobin levels below 7 since they were considered

ineligible for the BAN Study and referred to care; however, in an analysis done on 300 women who did not participate, the descriptive characteristics were similar to our sample (unpublished). Higher CD4 counts and low LBW prevalence in our sample may explain why we were unable to detect an association between maternal CD4 count or infant HIV status and LBW, as reported by other studies (107,108). Additionally, regular antenatal care and prevention or monitoring and treatment for opportunistic infections may have attenuated any possible effects of HIV status on birth outcomes in this population.

Our study supports the use of MUAC as an efficient, cost-effective screening tool for LBW in HIV-infected women, as with HIV-uninfected women, without concern for potential risk factors, such as seasonality, infectious disease and poor SES conditions, often associated with adverse birth outcomes.

Table 7

Characteristics of HIV-infected pregnant women and their infants in Malawi (n=1005)

Characteristic	Value
Maternal characteristics at baseline	
Age (%)	
<20 y	6.76
20-24 y	35.88
25-29 y	34.39
>=30 y	22.96
Education Level (%)	
None	12.46
Primary level	52.74
Secondary level	34.8
Marital Status (%)	
Married or Cohabiting	92.82
Single, divorced, or widowed	7.18
Employment (%)	
No	80.46
Yes	19.54
Exposure to famine season in month prior to baseline visit (%)	
Total	42.83
Partial	19.56
None	37.61
Parity (%)	
0	18.21
1 to 2	58.7
>=3	23.09
Height (cm)	155.9± 5.3
Midupper arm circumference	26.5 ± 2.7
Arm muscle area	36.8 ± 6.6
Arm fat area	19.7 ± 7.8
Fundal Height*	24.9 ± 4.8
CD4 count (X 10 ⁶ cells/L) **	439 (IQR: 319-592)
Infant characteristics at birth	
Birthweight (g) (mean ± SD)	2998±440
Low birth weight (%)	8.7
Infant sex (% male)	50.8
In utero transmission (% positive at birth)	3.8

*Sample size reduced to 893; 92 women had no FH and 20 women had FH missing at baseline.

**CD4 count displayed as median and interquartile range (IQR).

Table 8. Longitudinal analysis of fundal height as main outcome and maternal arm measurements as main predictors in 913 HIV-infected Malawian women.

Main predictor ¹	Fundal height (cm)			
	β	<i>P</i>	95% CI	
Arm muscle area (cm ²)	0.03	<0.01	0.01	0.06
Arm fat area (cm ²)	-0.03	<0.01	-0.05	-0.002
Weeks ²	0.92	<0.01	0.83	1.02
AMA x weeks	-0.003	<0.01	-0.006	-0.001
AFA x weeks	0.003	<0.01	0.001	0.006
Weeks between last visit and delivery	-0.32	<0.01	-0.39	-0.18
Age				
<20 y	0.52	0.27	-0.41	1.45
20-24	Reference	-	-	-
25-29	0.78	<0.01	0.22	1.34
30+	0.75	0.01	-0.01	1.51
Parity	0.31	<0.01	0.08	0.54
Wealth index	0.25	0.01	0.07	0.4
Famine	-0.19	0.16	-0.46	0.07
Year of enrollment				
2004	-0.59	0.09	-1.27	0.09
2005	-0.15	0.55	-0.63	0.34
2006	Reference	-	-	-
CD4 count				
200-350 cells/uL	-0.36	0.24	-0.95	0.24
350-499 cells/uL	-0.33	0.24	-0.88	0.22
≥ 500 cells/uL	Reference	-	-	-
Infant Male sex	0.48	0.03	0.04	0.93
Infant HIV infected at birth	-1.69	<0.01	-2.91	-0.48

¹ Number of weeks since baseline visit; baseline visit = 0 weeks

² Sample size reduced to 913, because 92 had no FH available.

Table 9. Multiple variable regression models with birth weight and LBW as main outcomes and maternal arm measurements as main predictors in 1005 HIV-infected Malawian women.

Main predictor	Birth weight ¹ (g)				LBW ² (<2500 g)			
	β	<i>P</i>	95% CI		Odds Ratio	<i>P</i>	95% CI	
Model 1:								
Arm muscle area (cm ²)	6.88	<0.01	2.51	11.26	0.95	0.03	0.91	0.99
Arm fat area (cm ²)	6.97	<0.01	3.53	10.41	0.97	0.08	0.94	1
Model 2:								
MUAC (cm)	31.84	<0.01	22.18	41.49	0.85	<0.01	0.77	0.94

¹ For multiple linear regression models with birth weight as the outcome variable and predictors that included continuous variables for anthropometric indicator plus the following covariates: maternal height, age, parity, education, place of delivery, CD4 cell count, male sex, HIV-infected at birth.

² OR and CI are from logistic regression models with LBW as the outcome and covariates described in footnote 1.

VI. Synthesis

A. Overview of findings

This research assessed nutritional status and diet of HIV-infected pregnant women who presented for care at public antenatal clinics in Lilongwe, Malawi, and related their nutritional status to birth outcomes. We used data from a large-scaled ongoing Breastfeeding, Antiretroviral, and Nutrition (BAN) Study, a postnatal clinical trial (www.thebanstudy.org). The data used in the analysis was derived from surveys conducted to screen women for BAN study participation and to collect baseline demographic, anthropometric, dietary, and health status data; from antenatal clinic records; and from hospital records at delivery. This offered a unique opportunity to increase our current understanding of the nutritional health of HIV-infected, pregnant mothers in Malawi by examining dietary behavior and maternal nutrition status during pregnancy. First, we explored the patterns and predictors of change in weight, midupper arm circumference (MUAC), arm muscle (AMA) and arm fat area (AFA) in the second and third trimesters. Second, we used cluster analysis to derive dietary patterns and examined associations between these patterns and maternal anthropometric measures during pregnancy. Finally, we examined the relationship of maternal nutritional status during pregnancy and fetal growth (birth weight, low birth weight (LBW), and fundal height (FH)). This section provides a summary and synthesis of our key findings.

A.1. Body composition and effects of famine on HIV-infected, pregnant Malawians.

We aimed to describe patterns and interrelationships of maternal weight gain and changes in anthropometric indicators of nutritional status during pregnancy, including arm muscle area (AMA), an indicator of protein stores, and arm fat area (AFA), an indicator of fat stores (calculated from mid upper arm circumference (MUAC) and triceps skinfold thickness) among HIV-infected Malawian women. We also aimed to identify predictors of these patterns. Among HIV-infected women, we hypothesized that with decreasing MUAC, both arm muscle and fat mass would decrease, as weight increased over the course of pregnancy, after adjusting for covariates. We explored these trends in an unadjusted and then adjusted longitudinal analysis using multiple regression.

We found a low linear increase in weight over the second and third trimesters. In addition, in our analysis of weight gain, we found weight loss in the intervals between visits. In unadjusted models, MUAC and AMA increased and AFA declined during late pregnancy. However, based on multivariable regression models, exposure to the famine season resulted in losses in AMA which increased as pregnancy progressed, while AFA losses occurred irrespective of season. Therefore, low rate of weight gain, seasonal effects, and low AFA accompanied by further AFA loss during pregnancy among HIV-infected, undernourished women are of concern. If maternal diet is insufficient, fat loss during pregnancy indicates mobilization of fat stores, which can be beneficial to the fetus; however, it could also have detrimental consequences to maternal health if overly depleted fat stores lead to muscle wasting. Our finding of low fat stores, accompanied by muscle and fat loss, supports our hypothesis that HIV-infected women are at risk of wasting during pregnancy, as a result of both muscle and fat loss. In addition to seasonal effects, other predictors we identified were

CD4 count, primary education level, wealth, maternal work status, partner work status, height, age, and parity. Strategies to optimize nutrition during pregnancy appear warranted. Our findings contribute to the current understanding of body composition dynamics of HIV-infected women during pregnancy. These findings also suggest further investigation into dietary determinants of poor maternal body composition, as well as the effects of poor maternal body composition on birth outcomes in HIV-infected, undernourished Malawian women.

A.2. Dietary patterns were derived and found to be associated with AMA, AFA, and hemoglobin levels in HIV-infected, pregnant Malawians

We aimed to describe the dietary patterns of pregnant women and identify which aspects of maternal diet predict anthropometric indicators of nutritional status during pregnancy. Our main focus was on total energy intake and diet quality. We hypothesized that low protein intake was associated with low AMA and AFA. We used cluster analysis to derive dietary patterns typical of Malawian women and examined how these patterns were associated with anthropometric measures of women's nutritional status during pregnancy, and with birth weight of their offspring.

Three dietary pattern clusters were derived, which we descriptively labeled 1) Cluster 1: high fish, meat and oil, 2) Cluster 2: high grain and 3) Cluster 3: high leafy vegetables, nuts, and fruits. Cluster 2 represents mostly a plant-based diet, high in intake of the micronutrient-poor staple food (nsima) and a low intake of protein and micronutrients. Participants in this cluster were significantly more likely to have lower energy intake, lower

iron intake, lower hemoglobin, be less educated, low on the wealth index, multiparous, and exposed to the famine season which extends from August to March.

Our findings suggest that HIV-infected pregnant women who consume poor quality diets, lack protein-rich and iron-rich foods. To our knowledge, this is the first study that used cluster analysis to examine dietary patterns in an HIV-infected population in sub-Saharan Africa. Hence, similar studies need to be replicated among HIV-infected women with HIV-uninfected comparison groups to better compare and contrast the influence of HIV infection on these dietary patterns. In addition, other factors that affect nutrition among HIV-infected pregnant women such as physical activity (or disability) and psychosocial support also need to be explored to acquire a clearer understanding of how these factors impact dietary behavior in HIV-infected women particularly during pregnancy in the sub-Saharan context.

A.3. Mid-upper arm circumference is associated with birth weight in HIV-infected, pregnant Malawians

We aimed to identify nutritional determinants, indicated by anthropometrics, of fetal growth (birth weight, low birth weight, and fundal height). We hypothesized low AMA and AFA in HIV-infected pregnant women are significantly associated with decreased fundal height and with increased odds of low birth weight. We conducted a longitudinal analysis to examine trends in MUAC, AMA, and AFA in relation to fundal height during pregnancy. We also conducted a cross-sectional analysis adjusting for covariates using multiple regression to examine the relationship between baseline MUAC, AMA, and AFA values and birth weight and LBW.

In a longitudinal analysis, as fundal height increased monotonically 0.92 cm/week, AMA was positively associated with fundal height and AFA was negatively associated with fundal height. These associations were very subtle. Although we identified significant interaction effects of AMA and AFA and week of follow up on fundal height, these effects too were very subtle. We found MUAC, AMA, and AFA collected at the baseline visit were directly associated with newborn's birth weight. MUAC and AMA were both inversely associated with LBW. Our study supports the use of MUAC as a screening tool in the nutritional care for HIV-infected women as part of antenatal care.

B. Limitations and Strengths

This study has certain limitations. Since this data came from an ongoing study of HIV-infected women with no uninfected comparison group, we could not do a relative comparison to HIV-uninfected pregnant women or HIV-infected pregnant women who receive minimal or no prenatal care. The low prevalence of LBW and higher average CD4 count may be due to earlier stages of the disease, as indicated by a successful pregnancy, but it may also reflect sample selection criteria and study protocols. First, women with low counts (≤ 250) were excluded from the study and referred for treatment. Second, regular, high quality antenatal care and monitoring and prevention or early treatment of opportunistic infections may have attenuated any possible effects of HIV status on birth outcomes in this population. Low LBW prevalence and higher CD4 count may be some of the reasons we were unable to detect associations between CD4 count and LBW or fundal height; or an association between HIV status of infant at birth and LBW, as reported by other studies. Hence, the focus of this analysis was determinants of nutritional status within HIV-infected

pregnant women who received high quality prenatal care. Still, our findings are valuable and unique since it fills some of the gap in understanding nutrition among women living with HIV/AIDS. Several other studies have already reported the increased risk of poor nutritional status in HIV-infected women compared to uninfected women. In addition, BAN study participants were a representative sample since they were recruited from four sites with outreach to all pregnant women in Lilongwe, Malawi.

We also had limitations which affected our analysis of diet and its effects on nutritional status and birth outcome. One limitation was only one 24-hour recall was collected in the BAN Study. While one study showed that Malawian diets among low-income women tend to vary little, another study noted that seasonal difference affects the intraindividual variance ratios and even slight differences can influence interindividual variance ratio. So therefore, many days are required for estimating usual intake. Although multiple 24-hour recalls would have been ideal, the high quantity of recalls still allowed for the examination of dietary patterns.

Another limitation was the inability to find any associations between nutrient intake and the anthropometric measures. Nutrient intake may have been grossly underestimated, though estimates are plausible. This may have been due poor estimation of portion sizes, quantities of ingredients included in mixed dishes, or data entry errors. However, the large number of recalls still allowed for a food pattern analysis of diet quality.

Estimating gestational age also proved to be a challenge, since last menstrual period was not collected on all women. Estimating gestation age based on fundal height is prone to bias in populations where malnutrition and intrauterine growth retardation (IUGR) is

prevalent. The gold standard would be an ultrasound scan, which is not practical in limited resource settings. Other practical methods used in resource-limited settings to assess gestational age at birth, such as Dubowitz or Ballard scoring, would have improved gestational age estimation and allowed for examination of preterm or small-for-gestational age as birth outcomes.

Our study also has notable strengths. Such studies are needed to validate the few previous studies that have examined the associations between maternal anthropometry and adverse birth outcomes among HIV-infected women in sub-Saharan Africa. Findings can also provide insight for future HIV programs and intervention studies. A major strength is access to and availability of such a large cohort of HIV-infected women with serial anthropometric measurements during pregnancy. Anthropometric measurements, which assess body composition change rather than only weight gain, were analyzed, which can be used to develop more detailed nutritional requirements for HIV-infected pregnant women. With repeated measures of AMA and AFA, we are able to examine the pattern of AMA and AFA change in relationship to fundal height increase during the latter part of pregnancy. Secondly, with both MUAC and tricep skinfold measures, we were able to compare MUAC with AMA and AFA measurements both longitudinally and as predictors of LBW. Another strength was this sub-study was conducted on a sample of women who received no antiretroviral regimen prior to delivery. We found fundal height was 1.42 cms lower in those mothers whose babies were HIV- infected at birth, which indicates a significant association between in utero HIV transmission and fetal growth. Such associations will be less likely to be reproduced as ARVs for pregnant women become more available. Lastly, unique to our study, with over 500 dietary recalls from HIV-infected, pregnant Malawian women, we were

able to derive dietary patterns typical of Malawians with cluster analysis. This method enabled us to describe typical Malawian diets and examine diet quality of each pattern and its associations with SES, anthropometric, clinical and fetal indicators.

C. Public Health Significance

Most of the world's 40 million HIV-infected people currently live in communities suffering from poverty and malnutrition. Proper nutrition with a well balanced and adequate diet, sometimes including food assistance, can play a key role in maintaining the health and well-being of affected individuals, their families, and their communities. Food and nutrition programs may minimize the effects of HIV/AIDS by helping infected individuals remain productive and by allowing their families and communities to better cope with the economic losses associated with HIV/AIDS.

In sub-Saharan Africa, an estimated 13.5 million women of childbearing age are infected with HIV(1). When they become pregnant, HIV-infected woman face not only the usual physiological and nutritional demands of pregnancy, but also the extra demands imposed by infection. In settings with a high level of food insecurity and high maternal work loads, women may be unable to consume sufficient high quality food to meet these heightened nutritional needs, leaving them at much greater risk of poor health, faster disease progression and increased risk of poor birth outcomes. Most research related to HIV in pregnancy has focused on antiretroviral strategies to reduce mother to child transmission (MTCT) of HIV. Little attention has been paid to maternal health and nutrition status.

Our findings suggest a large proportion of women had weight gains that were far below the WHO standards. Low rate of weight gain, seasonal effects, and low AFA stores accompanied by AFA loss during pregnancy among HIV-infected, undernourished women are of concern. Our study supports the use of MUAC as an efficient, cost effective screening tool for LBW in HIV-infected women, as done with HIV-uninfected women. Our findings describe the typical diets of HIV-infected, pregnant women and highlight poor quality of maternal diets which need to be enhanced to meet the demands of this particular group of pregnant women, vulnerable to both HIV and malnutrition. Therefore, nutritional interventions and food aid programs tailored to the needs of HIV-infected pregnant women are of great importance, particularly those with low SES and those with limited food intake during the rainy seasons when food insecurity is at its peak.

D. Direction for future research

This research comprised relatively healthy HIV-infected women with high CD4 count and high quality antenatal care. Future research should also examine women with lower CD4 or high viral load to see how the relationship between nutrition and HIV in women who have more HIV progression. With a broader population of HIV-infected pregnant with low CD4 counts and a comparison group of HIV-infected women, studies can look for cut points that can be used to screen and identify HIV-infected pregnant women who are more “at risk” in settings where HIV testing is not practical.

Studies also must continue to look at nutritional predictors of small-for-gestational age and preterm delivery. For such studies, accurate estimate of gestational age is essential. More validation studies of estimation of gestational age using last menstrual period or fundal

height are needed in sub-Saharan Africa in well-nourished, non-HIV-infected women.

While much work in the U.S. has been done to improve the dietary recall method of estimating usual intake, future studies examine diet in resource-limited settings should focus on dietary collections methods that are both efficient and improve the accuracy and quality of dietary recalls. In addition to diet, other proximate measures of nutritional status include physical activity, disabilities, and psychosocial support would be useful in analysis of nutrition in resource-limited settings.

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