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Infant Responsiveness, Alertness, Hemoglobin and Growth in Rural Sidama, Ethiopia

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Abstract

Several recent studies have supported relations between infant behavior (alertness and responsiveness) and nutrition (e.g. Dempsey 2008, Wachs et al 2005) in addition to investigating infant behavior within the context of changes in iron status over time (e.g. Black et al. 2004, Murray-Kolb & Beard 2009). Existing research is typically limited to investigation of the effects of a single vitamin or mineral and no studies have been found that examined the influence that early alertness and responsiveness have on growth in early infancy, despite the fact that relations between behavior and nutritional status may be bidirectional (Hulthén 2003). The current study used a sample of Ethiopian infants and investigated anthropometrics, hemoglobin, the frequency of alertness, and the frequency of responsiveness at 6 and 9 months of age. Six-month weight-for-age predicted 9-month frequency of alertness, while 6-month hemoglobin predicted 9-month frequency of responsiveness. Compared to responsive infants, non-responsive infants at 6 months remained more non-responsive at 9 months, though weight-for-age for both groups converged at 9 months. Results support relations between nutrition and behavior (alertness and responsiveness) and provide evidence of a potentially useful tool (the Laboratory Temperament Assessment Battery [Lab-TAB]) that was adapted to evaluate these relations in Ethiopia.

Keywords

development; infant growth; hemoglobin; alertness; responsiveness; international nutrition

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Infant behavioral responsiveness and alertness are fundamental aspects of temperament related to developmentally influential constructs like attachment (Lozoff et al. 2003), parent-child conflict (Hulthén 2003), and functional isolation (Beard 2007). Alertness is thought to be a core underlying component in the control of attention and vigilance and early alertness is related to later cognitive functioning and state organization (Colombo 2001). Though studies have investigated longitudinal changes in infants' responsiveness or alertness following iron supplementation, few studies of responsiveness or alertness have reported anthropometric indicators of nutritional status and none were found that studied reciprocal effects of such behaviors on infant growth. In addition, most longitudinal studies examining nutritional and behavioral change have not focused on short-term differences (≤ 3 months) in early infancy. Therefore, the current study examined longitudinal relations among anthropometric markers of nutritional status (weight-for-age [WAZ], and length-for-age [LAZ]) and hemoglobin (Hb) and behavior (alertness and responsiveness) in infants from rural Ethiopia. The study also examined these early behaviors and their relations to growth delays from 6 to 9 months of age.

Hypotheses

Given that (a) infants in the region have poor diets (Gibson et al., 2009) and (b) breast milk becomes less adequate to meet the needs of infants from 6 to 9 months (Krebs 2000), it was expected that over time infants' nutritional status and associated behavior would worsen. Based on the literature below, we hypothesized that (1) lower WAZ, LAZ, and Hb concentration at 6 months would be related to less alertness and responsiveness at 9 months. We also hypothesized that (2) greater decreases in WAZ, LAZ, and Hb concentration from 6 to 9 months would be related to less alertness and responsiveness at 9 months. Lastly, to capture changes in WAZ, LAZ, and Hb from 6 to 9 months that may be related to behavior at 6 months, we divided the sample into groups based on 6-month alertness and responsiveness (i.e. Alert/Responder, Alert/Non-responder, Non-Alert/Responder, and Non-alert/Non-responder). Because these behaviors have been associated with nutritional status (Oski & Honig 1978, Walter et al. 1989) and infant behavior impacts feeding style and nutrition (Moore et al. 2006), we hypothesized that (3) infants in the Alert/Responder group would have the least negative change in nutritional status from 6 to 9 months.

Nutrition, Alertness, and Responsiveness

Measuring responsiveness and alertness in early infancy is of interest because during this time, researchers can capture these behaviors before environmental factors play a significant role in the developmental process (Matheny et al. 1985). However, one environmental factor related to infant alertness and responsiveness is early nutrition (Wachs 2009, Wachs et al. 2008). Poor infant alertness is associated with maternal diet during lactation, such as insufficient intake of riboflavin, niacin, vitamin B-6, thiamin, folate, iron, and zinc (Rahmanifar et al. 1993) and infant nutritional variables such as iron deficiency (Angulo-Kinzler et al. 2002, Wachs et al. 2005). Lethargy or inhibited responsiveness has also been associated with iron deficiency anemia (IDA, Dempsey 2008), zinc and copper deficiency (Prasad 1979), thiamine deficiency (Fattal-Valevski et al. 2005), chloride deficiency (Grossman et al. 1980), vitamin D deficiency (Molgaard & Michaelsen 2003), and severe malnutrition (Maitland et al. 2006). Many of these nutritional deficiencies impact anthropometric variables, with protein-energy malnutrition serving as a common etiological contributor to these relations.

Iron

Infants' responses tend to become more negative or diminished when they are iron deficient (ID). Lozoff et al. (1998) utilized a Costa Rican sample of ID infants and controls ranging from 12 to 23 months of age and found that the ID group displayed more wariness and hesitancy, was less playful and attentive, and made fewer attempts at test items (non-responsiveness). Wachs et al. (2005) examined Peruvian newborns and found that ID was associated with less alertness.

Longitudinal supplementation studies have also supported relations between infant alertness and responsiveness and changes in iron status (Black et al. 2004, Murray-Kolb & Beard 2009, Oski & Honig 1978). In one of the earliest studies of its kind, Oski and Honig (1978) randomly assigned iron deficiency anemic, US infants/toddlers ranging from 9 to 26 months of age to a control group or iron supplementation via intramuscular injection calculated to raise the child's Hb level to 12.0g/dL. Approximately one week following supplementation, researchers found significant differences in alertness and responsiveness between the control and treatment groups.

Black and colleagues (2004) performed a double-blind, randomized, placebo-controlled trial with Bangladeshi infants (6 to 12 months of age) with four treatment conditions, including supplementation with iron (20mg as ferrous sulfate [$FeSO_4$] and 1mg riboflavin), zinc (20mg zinc as zinc acetate and 1mg riboflavin), iron and zinc (20mg iron, 20mg of zinc, and 1mg riboflavin), or a mix of 16 vitamins/minerals including iron and zinc (approximately twice the recommended dietary allowances from the World Health Organization [WHO] of thiamine, niacin, folic acid, pantothenic acid, iodine, copper, manganese, selenium, and vitamins C, D, E, B-6, and B-12 in addition to 20mg iron as $FeSO_4$, 20mg zinc as zinc acetate, and 1mg riboflavin). The control group only received riboflavin (1mg). Supplementation occurred weekly from 6 to 12 months of age. After controlling for maternal education, home behavior, breastfeeding duration, anemia, and growth, several group differences were found. Orientation and engagement scores decreased from 6 to 12 months only for the control group. At 12 months, all four treatment groups supplemented with iron and/or zinc also demonstrated significantly greater orientation and engagement relative to the riboflavin control group.

Utilizing a South African sample, Murray-Kolb and Beard (2009) conducted a randomized, double-blind, supplementation trial to investigate the influence of maternal iron status on infant responsiveness. Breastfeeding mothers with IDA were randomly assigned to receive 125mg of $FeSO_4$ or placebo daily from 10 weeks to 9 months postpartum. A control group of iron-sufficient mothers was also used. Non-structured mother-infant interactions were coded at 10 weeks and 9 months postpartum. At 10 weeks, infants in the control group were more responsive than both IDA groups. At 9 months, the control and supplemented group were significantly more responsive than infants in the placebo group. For breastfeeding mothers, better maternal iron status was related to greater infant responsiveness.

Not all supplementation studies have produced positive behavioral results. Walter et al. (1989) followed a sample of Chilean infants who were assigned to a placebo control or a supplementation group (15mg iron in $FeSO_4$ suspension administered three times/day from 12 to 15 months of age). During assessments at 10 days and 3 months after beginning supplementation at 12 months of age, those in the supplementation group demonstrated no significant improvements in responsiveness, alertness, or activity. However at 12 months of age, those with IDA demonstrated less responsiveness, goal directedness, and activity in addition to poorer attention span, as compared to infants with normative iron status (Hb ≥ 11.0g/dL, mean cell volume ≥ 70fL, Fe/iron binding capacity ≥ 10%, free erythrocyte protoporphyrin < 100μg of zinc protoporphyrin per deciliter of RBC, and serum ferritin ≥ 10ng/mL; Walter et al. 1989). Therefore, although iron status was related to alertness and responsiveness, improvements in these behaviors were not seen after 10-day and 3-month iron supplementation of 12-month-olds previously ID or anemic.

The longitudinal effects of iron deficiency on development are also persistent. Lozoff and colleagues performed a follow-up study of the above mentioned Costa-Rican infants. At approximately 5 years of age, those supplemented infants (3 months of iron therapy including two doses of 5mg/kg oral iron daily) who were chronically and severely ID in infancy were less active and vocal and displayed less mother-child reciprocity than infants who had been previously iron sufficient (Hb ≥ 12g/dL, serum ferritin > 12μg/L, free erythrocyte protoporphyrin < 100μg/dL of RBC, and serum ferritin ≥ 10ng/mL; Corapci et al. 2006). These results demonstrate the importance of measuring early nutrition and behavior because of the long-term negative consequences that nutrient deficiencies have on development.

Culturally Appropriate Assessment

Given the frequency of iron deficiency/anemia (WHO 2006) and malnutrition (WHO 2007) in Ethiopia, this study took place in the Sidama region of Southern Ethiopia. The Laboratory Temperament Assessment Battery (Lab-TAB) developed by Goldsmith and Rothbart (1988) was used to minimize difficulties with parental report (particularly with language translation) and to enhance the measure's internal validity, given greater control/ standardization associated with a structured measure.

Methods

Participants

A convenience sample of 108 infants (49 male, 59 female) and their mothers were recruited from four adjoining villages in the Sidama region of Southern Ethiopia with the help of community health workers. Recruitment occurred when infants were 6 months of age or younger and participants were tested at both 6 and 9 months of age. The study procedure was approved by Institutional Review Boards at Oklahoma State University and Hawassa University (Hawassa, Ethiopia) and participation was voluntary. Oral informed consent was given via maternal audio recording. Compensation included a t-shirt for the infant, a bottle of hair oil, and a photograph of the mother-infant dyad at the 6-month visit and another photograph and shawl at the 9-month visit.

Mothers—There was moderate to extensive sociodemographic homogeneity in the original sample of 108. In fact, 97% of the infants had heads-of-household who were self-employed farmers. Additionally, 85% of mothers had 4 or fewer years of schooling. Within this sample, no health data (i.e. nutritional status or infection rate) were obtained from mothers. However, several statistics computed within the region estimate that approximately 27% of Ethiopian women between ages 15 and 50 are anemic (WHO 2006). Almost 75% of Ethiopia's population is at risk for malarial infection and upwards of 50 million people are at risk for developing parasitic infections (Central Statistical Agency [Ethiopia] and ORC Macro 2006). Although mothers in our sample were not asked about treatment, about 10% of pregnant women in Ethiopia are treated for parasitic infections. Forty-three percent of mothers in the sample did report using bed nets in the home for malarial prevention.

Pregnancy and birth—Although mothers were not asked in detail about their pregnancies, several regional statistics may be helpful in characterizing the sample. Specifically, results of the Ethiopia Demographic and Health Survey (EDHS) estimate that about 94% of infants are born at home (Central Statistical Agency [Ethiopia] & ORC Macro 2006). Of those, 28% are assisted by a traditional birth attendant; 61% are attended by a relative or other person; and 5% are not assisted at all. Therefore birth weights are rarely measured (≈ 3% of the time), but 14% of measured infants have birth weights less than 2.5kg. In rural areas like the current study area, this percentage may be higher (approximately 23%; Central Statistical Agency [Ethiopia] & ORC Macro 2006). Within Ethiopia, 21% of mothers reported newborns to be very small and 7% rated their infants as smaller than average (Central Statistical Agency [Ethiopia] & ORC Macro 2006).

Infants—Infants were tested for malaria at 9 months using a drop of blood examined microscopically for the presence of Plasmodium falciparum and four tested positive. Two of these infants had complete data and were included in the dataset because they appeared to be otherwise healthy. Given the high prevalence of malarial infection in the country, this number is surprisingly low. Reasons may include the relatively high use of bed nets, the habit of cooking indoors in the Sidama region (smoke may deter mosquitoes), and the collection of data prior to the high-risk wet season. Mothers were not queried about infant treatment for infection or malnutrition, but infants who were anemic (i.e. Hb < 11.5g/dL) were referred to a nearby clinic. Infants who appeared ill (e.g., extremely lethargic, continued whimpering, coughing, or other apparent signs of illness) were excluded.

Infants' dietary intake was somewhat uniform in that at 9 months, all infants were breastfeeding with most receiving only one type of complementary food (68% received corn bread; Teshome 2008). Cornmeal was unfortified and lacked essential micronutrients and did not provide adequate diet diversity when coupled with breast milk (Teshome 2008). Due to the rate of breastfeeding within the sample, infant health and nutritional status were thought to be highly dependent on mothers milk. Fifty-seven percent of infants received complementary foods at 6 months of age, while 89% received complementary foods by 9 months of age (recommended by 6 months of age). However, studies have shown that staple foods in the region lack important micronutrients like zinc and iodine and are too bulky to adequately nourish the infant (Abebe et al. 2008).

Of the 108 infants who appeared healthy and completed the study at both ages, 89 had usable video data for the purposes of the present study. Nineteen 6-month-old infants did not have complete data for the following reasons: examiner error (4), technical problems (1), premature termination of taping because infants were too fussy (8), infant illness (1), and the inability of the infant to sit independently (3). Two additional infants were excluded because it was the consensus opinion of the on-site research staff that they were quite small for 6-month-olds (they were the smallest of the entire sample of 108 infants) and thus were most likely born preterm. Twelve 9-month-old infants did not have complete data for the following reasons: examiner error (4), technical problems (3), and premature termination of taping because infant were too fussy (5). All infants with usable data were included in analyses. The 6-month-old infants had a mean age of 24.9 weeks (n = 89, range = 21.4-30.0 weeks). The 9-month-old infants had a mean age 40.6 weeks (n = 85, range = 39.3-42.1 weeks). Immunization records were used to estimate infant birth date and thus age.

Materials

Demographics—Mothers were asked questions including occupational and educational information. They were also asked about typical regional sociodemographic indicators such as the composition of building materials of their houses, the number of livestock they owned, and accessibility to water. Mothers were further asked about infants' breastfeeding, complementary feeding, vaccinations, and use of bed nets.

Hemoglobin—Two drops of blood were taken from the infants' heels after the Lab-TAB measures to determine Hb. Hb was analyzed in the field by an experienced laboratory technician using the HemoCue® (Ängelholm, Sweden). Infants were considered to have low Hb if the concentration was less than 11.5 g/dL (adjusted from 11.0 g/dL for altitude, Nestel

2002). At 6 months, 35 infants were classified as having low Hb while at 9 months, 36 infants were thus classified (Table 1).

Anthropometry—The infants' weight and length were assessed by trained project staff with senior research staff providing oversight for protocol adherence. A mother-infant scale was calibrated regularly and used to measure infants' weight with an accuracy of ± 0.1kg. Length was measured with a portable length board that had inlaid tape with an accuracy of ± 0.1cm (Shorr Productions, Olney, MD). Length measurements were taken three times for each infant, and the mean was calculated. Standardized anthropometric indices (i.e. WAZ and LAZ) were derived using WHO Anthro software (v3.0.1, de Onis et al. 2009) based on international growth standards (Table 1).

Alertness and responsiveness—The frequency of alertness and frequency of responsiveness variables were both coded from videotapes of the Lab-TAB. A pilot study of 14 infants (6-12 months of age) from rural Ethiopia was completed to improve the cross-cultural validity of the measure (Grant 2008). Modifications to the paradigm improved the cultural appropriateness of the original Lab-TAB (e.g. sitting infants on their mother's laps instead of in high chairs and removing mechanical and electronic toys to which infants are unaccustomed). The former modification was not thought to interfere with infants' ability to attend to stimuli because mothers swaddle and carry infants all day long. This Ethiopian version of the Lab-TAB was used with the current sample. All 10 episodes (2 of each: joy/pleasure, fear, interest/persistence, anger/frustration, and activity level) used with the current sample were 3-5 minutes long and had adequate to excellent internal consistency reliability (α for all 10 episodes ranged from 0.78-0.94).

All coding teams were comprised of two people for each video. The team of researchers from the US recoded a percentage of videos for reliability purposes, while the Ethiopian team coded all of the videos. For the files that were coded by both teams, high levels of disagreements between coders were flagged and each team reviewed the file again independently and then collaborated between coding teams as to how to score the data for its final use. When data were altered in this way, the agreed upon data were used for final analyses but not for interrater purposes. Coders were trained using a standardized protocol described in detail by Grant (2008).

Infants' baseline states were recorded prior to each of the 10 episodes and used to assess frequency of alertness. Baseline states were coded on the following 5-point scale: 1= drowsy, 2= alert/calm, 3= alert/active, 4= fussy (upset but not crying), 5= crying. Given that the scale is not continuous, no mean baseline state was computed. Instead, the frequency of alertness (states of 2 or 3) was computed for each subject (ranged from 0-10 because of the 10 episodes) at each age. Calculations of agreement between coders for the frequency of alertness variable were 93% at 6 months and 85% at 9 months.

To compute the frequency of responsiveness, for all 10 of the original episodes data were collapsed across trials, stages, intervals, and epochs. More specifically, Lab-TAB episodes include several different variables and these variables are broken down into smaller units with the same scales of measurement for ease of coding (see online supplemental material for an example). This included trials, stages, intervals, and epochs. In this study, intervals are defined as smaller temporal units, whereas trials are created based on time or by different experimenter behavior. A stage is defined by a progressive escalation of the experimental manipulation in each episode (e.g. the stranger will become progressively closer to the infant in different stages of the fear episode of stranger approach) and epochs are defined as smaller units of time. An average was computed of all trials, stages, intervals, and epochs for each variable in each episode. Then, the number of non-responses to each variable (across these averaged trials, stages, intervals, and epochs) was calculated for each participant. Finally, participants were labeled as "non-responders" for each episode if they failed to show a response (i.e. had coding of 555 [did not happen] for each coded level of the variable or summed scores of 0) to at least 50% of the variables within that episode. The frequency of responsiveness was then calculated for each infant and could range from 0-10 (because of the ten episodes). Calculations of agreement between coders for the frequency of responsiveness variable were 86% at 6 months and 73% at 9 months.

Procedure

The Ethiopian version of Lab-TAB, general demographic data, anthropometric measures, and levels of Hb were gathered from Ethiopian infants within rural communities at both 6 and 9 months of age. Researchers from Hawassa University in Ethiopia collected data from infants and mothers outdoors in portable booths that were transported between villages. All Lab-TAB sessions were video recorded using a Panasonic camcorder and coded at a later time by a trained research team in Ethiopia.

Statistical Analyses

All statistical analyses were performed using the Predictive Analytics SoftWare (PASW; formerly Statistical Package for the Social Sciences or SPSS), version 18. Descriptive statistics were computed for the three nutritional variables at both ages (see Table 1). A series of dependent samples *t*-tests were completed to investigate age differences in each of the nutritional variables. Descriptive statistics were also computed for the behavioral variables at both ages (Table 1). Two dependent samples *t*-tests were completed to investigate age differences in the frequencies of alertness and responsiveness.

To test the first hypothesis that nutritional status at 6 months would significantly predict behavior at 9 months, a correlation matrix of all nutritional and behavioral variables at 6 and 9 months of age was computed (see Table 2) to investigate bivariate relations among these variables and to examine the potential mulitcollinearity among 6-month nutritional predictors. Because of multicollinearity issues and the fact that the LAZ_6 and WAZ_6 variables reflect different underlying nutritional deficits, four regression analyses were performed. LAZ_6 and Hb_6 were used to predict frequency of $alertness_9$ and frequency of $responsiveness_9$. Additionally, separate regression analyses were conducted using WAZ_6 and Hb_6 as predictors of frequency of $alertness_9$ and frequency of $responsiveness_9$.

Based on the previous findings of multicollinearity, hypothesis 2 was tested via two hierarchical regression analyses for each behavioral outcome variable. For all models, the 6-month nutritional variables were covariates in the first step to control for the impacts of these values on the change scores. In other words, the hierarchical regression analyses were selected so that infants who were stunted or had low Hb at 6 months would not have disproportionately high change scores from 6 to 9 months of age. The four models were as follows:

1. Step 1 frequency of $alertness_9$ (y) = WAZ_6 (x_1) + Hb_6 (x_2)

   Step 2 frequency of $alertness_9$ (y) = WAZ_6 (x_1) + Hb_6 (x_2) + $WAZ_{6\text{-}9}$ (x_3) + $Hb_{6\text{-}9}$ (x_4)

2. Step 1 frequency of $responsiveness_9$ (y) = WAZ_6 (x_1) + Hb_6 (x_2)

   Step 2 frequency of $responsiveness_9$ (y) = WAZ_6 (x_1) + Hb_6 (x_2) + $WAZ_{6\text{-}9}$ (x_3) + $Hb_{6\text{-}9}$ (x_4)

3. Step 1 frequency of $alertness_9$ (y) = LAZ_6 (x_1) + Hb_6 (x_2)

Step 2 frequency of alertness$_9$ (y) = LAZ$_6$ (x_1) + Hb$_6$ (x_2) + LAZ$_{6\text{-}9}$ (x_3) + Hb$_{6\text{-}9}$ (x_4)

4. Step 1 frequency of responsiveness$_9$ (y) = LAZ$_6$ (x_1) + Hb$_6$ (x_2)

Step 2 frequency of responsiveness$_9$ (y) = LAZ$_6$ (x_1) + Hb$_6$ (x_2) + LAZ$_{6\text{-}9}$ (x_3) + Hb$_{6\text{-}9}$ (x_4)

To test hypothesis 3, using frequency of alertness$_6$ and frequency of responsiveness$_6$, subjects were categorized as Alert/Responder (frequency of alertness [f_a] greater than 5 and frequency of responsiveness [f_r] greater than 5), Alert/Non-responder ($f_a > 5$ and $f_r \leq 5$), Non-alert/Responder ($f_a \leq 5$ and $f_r > 5$), and Non-alert/Non-responder ($f_a \leq 5$ and $f_r \leq 5$). Then, group differences in anthropometry and Hb at 6 and 9 months were tested using *t*-tests. *T*-tests were also used to determine whether there were group differences in anthropometric and Hb change scores from 6 to 9 months of age.

Results

For purposes of each analysis, only infants with complete data relevant to the respective analyses were used. Sample sizes for each variable are reported in Table 1. To examine the possibility that infants with missing Lab-TAB data (n = 26 at 6 months and n = 17 at 9 months) differed in nutritional status compared to those with complete sets of data, independent sample *t*-tests were utilized. While no significant differences at the alpha = 0.05 level were found between infants missing Lab-TAB data at 9 months and those who had complete data, significant differences were found at 6 months between 6-month WAZ (t[105] = 2.41, p = 0.018) and 6-month LAZ (t[103] = 2.35, p = 0.021), with infants missing Lab-TAB data at 6 months having lower WAZ (M = −0.73, SD = 1.19) and LAZ measurements (M = −1.53, SD = 1.22) than those not missing data (M_{WAZ} = −0.14, SD_{WAZ} = 1.10; M_{LAZ} = −0.84, SD_{LAZ} = 1.35). No other significant differences were found.

Covariates

Maternal health (use of bed nets, use of treated water, source of health care), maternal education, income, occupation of head of household, length of breastfeeding, and infant health (question to mothers about infant health, iron supplementation, immunizations) were unrelated to predictor and outcome variables (infant WAZ, LAZ, Hb, alertness and responsiveness) at both ages. LAZ differed by gender at both ages. In particular, female infants ($M_{6\ Months}$ = −0.69, $SD_{6\ Months}$ = 1.28; $M_{9\ Months}$ = −1.29, $SD_{9\ Months}$ = 1.11) had greater LAZ than male infants ($M_{6\ Months}$ = −1.42, $SD_{6\ Months}$ = 1.42; $M_{9\ Months}$ = −1.94, $SD_{9\ Months}$ = 1.34) at 6 (t[93] = 2.65, p = 0.009) and 9 months of age (t[94] = 2.56, p = 0.012). Given the largely null findings and the fact that gender was unrelated to outcome variables, none of these variables were used as covariates in the following analyses.

Nutritional Status

WAZ significantly decreased from 6 (M_6 = −0.35, SD_6 = 1.15) to 9 months of age (M_9 = −0.81, SD_9 = 1.13; t[84] = 5.72, p < 0.001), as did LAZ (M_6 = −1.03, SD_6 = 1.39; M_9 = −1.58, SD_9 = 1.25; t[83] = 7.82, p < 0.001). No significant age differences in Hb concentration were found, but Hb did decrease over time. At 6 months, 31% of infants were anemic (i.e. Hb < 11.5g/dL) whereas at 9 months, 37% of infants were anemic. Twenty infants were anemic at both ages.

Alertness and Responsiveness

Results indicated that the frequency of alertness significantly decreased from 6 (M_6 = 9.31, SD_6 = 1.48) to 9 months of age (M_9 = 7.88, SD_9 = 2.03; t[66] = 4.46, p < 0.001), while the frequency of responsiveness significantly increased from 6 (M_6 = 5.84, SD_6 = 1.42) to 9 months of age (M_9 = 6.52, SD_9 = 1.22; t[73] = −3.11, p = 0.003). No significant differences in alertness or responsiveness were found among those that had low Hb (< 11.5 g/dL) at 6 months, 9 months, or at both ages. This may have been due to small group sizes, but given these results, the Hb variable was not trichotomized (i.e. low Hb at 6 months, low Hb at 9 months, or low Hb at both ages) prior to completion of data analyses. Rather, it was used as a continuous predictor at both ages.

Hypothesis 1: Prediction of 9-Month Behavior from 6-Month Nutritional Status

Each of the nutritional variables was significantly related across age. Moreover, WAZ_6 was positively correlated with frequency of $alertness_6$ (r = 0.29, p = 0.016) and frequency of $alertness_9$ (r = 0.28, p = 0.014). Hb_6 was positively correlated with the frequency of $alertness_6$ (r = 0.32, p = 0.008) and frequency of $responsiveness_9$ (r = 0.27, p = 0.017). Lastly, Hb_9 was positively correlated with frequency of $responsiveness_9$ (r = 0.23, p = 0.047).

Investigation of the matrix also indicated significant relations between LAZ_6 and both WAZ_6 (r = 0.63, p < 0.001) and Hb_6 (r = 0.35, p < 0.001). The relation between WAZ_6 and Hb_6 was also significant (r = 0.23, p = 0.026). Multicollinearity diagnostics were performed and revealed that for all four models, variance inflation factors were below 1.5.

Weight-for-age—The overall model for predicting frequency of $alertness_9$ (F[2, 73] = 3.85, p = 0.026) accounted for 10% of variance (see Table 3). WAZ_6 was the significant predictor, (β = 0.31, p = 0.008), where greater WAZ_6 predicted a greater frequency of $alertness_9$. The overall model for predicting the frequency of $responsiveness_9$ was also significant, (F[2, 77] = 3.24, p = 0.045), and accounted for 8% of variance (see Table 3). Hb_6 was the significant predictor, (β = 0.29, p = 0.013), where greater Hb_6 predicted a greater frequency of $responsiveness_9$.

Length-for-age—The overall model for predicting the frequency of $alertness_9$ was not significant. Conversely, the overall model for predicting the frequency of $responsiveness_9$ was significant, (F[2, 74] = 3.26, p = 0.044), and accounted for 8% of variance, though neither predictor was significant at the α = 0.05 level.

Hypothesis 2: Prediction of 9-Month Behavior from 6 to 9-Month Nutritional Change

Based on the previous findings of multicollinearity, two hierarchical regression analyses were computed for each behavioral outcome variable. None of these analyses produced significant results.

Hypothesis 3: Relations between 6-Month Behavioral Groups and Nutritional Change

There were no infants categorized as Non-alert/Responder. This was expected because if infants were not alert during the baseline state, it is unlikely that they would be responsive in the subsequent episode. Examination of the plots of the anthropometric and Hb change from 6 to 9 months for each of the three groups (Alert/Responder, Alert/Non-responder, and Non-alert/Non-responder) revealed that the Alert/Non-responders and Non-alert/Non-responders had very similar anthropometric and Hb values at both ages. Therefore, t-tests were completed to investigate whether there were significant differences in anthropometry and Hb between these groups. No significant differences were found, so the groups were combined into Responders versus Non-responders. Changes in anthropometry and Hb from 6 to 9 months were then investigated for Responders and Non-responders.

An analysis of differences in change scores ($WAZ_{6\text{-}9}$, $LAZ_{6\text{-}9}$ [retained because multicollinearity was irrelevant], and $Hb_{6\text{-}9}$) between Responders and Non-responders demonstrated a significant difference in $WAZ_{6\text{-}9}$ change (Figure 1). Although WAZ decreased for both groups, Responders showed a greater decrease from 6 to 9 months of age ($M_{change} = -0.73$, $SD_{change} = 0.75$) than Non-responders ($M_{change} = -0.36$, $SD_{change} = 0.60$; $t[77] = 2.42$, $p = 0.018$). Although Non-responders had a noticeably lower WAZ_6 than Responders (group difference of 0.37 z-score), there was no between-group difference in WAZ_9 (see Figure 2). It should be noted that although the WAZ_6 difference of 0.37 z-score is large enough to be practically meaningful, a follow-up *t*-test revealed no statistically significant differences in WAZ_6 for Responders versus Non-Responders (consistent with correlation analyses). Both of these null findings are likely due to the large amount of variance in WAZ_6 in the sample. Additionally, even though Non-responders' WAZ decreased over time, Responders' rate of negative change was significantly greater.

Follow-up analyses were computed to examine whether the frequency of responsiveness in both groups converged to a similar pattern as the WAZ over time (see Figure 2). Similar to the pattern seen for $WAZ_{6\text{-}9}$, infants' frequency of responsiveness became more similar for the Responders and Non-responders at 9 months of age. This may be due to regression to the mean, given that groups were categorized based on 6-month responsiveness. However, unlike $WAZ_{6\text{-}9}$, differences in the frequency of responsiveness between groups remained significant at 9 months: Infants classified as Responders at 6 months had a significantly greater frequency of $response_9$ ($M_9 = 6.70$, $SD_9 = 1.40$) than the Non-responders ($M_9 = 6.00$, $SD_9 = 1.11$; $t[61] = -2.17$, $p = 0.034$) despite the fact that the Responders' WAZ_9 matched the Non-responders'.

Discussion

The primary purpose of the present study was to investigate longitudinal changes in nutritional status and behavior from 6 to 9 months of age in a group of Ethiopian infants in the rural Sidama region suspected of dietary protein-energy and micronutrient deficits. Unlike previous studies, common maternal (i.e. education, health, and income) and infant characteristics (i.e. health) were not systematically related to outcome variables, likely because of the sociodemographic homogeneity of the sample. Though female infants had greater LAZ at 6 and 9 months, gender differences in alertness and responsiveness were null.

Longitudinal Changes in Anthropometric and Behavioral Variables

As predicted, WAZ and LAZ significantly declined over time. Hb concentrations decreased but not significantly. Furthermore, infants' mean frequency of alertness also decreased over time, though their average frequency of responsiveness increased over time. Regarding the former, normally developing infants that are adequately nourished are expected to have increased frequencies of alertness over time (i.e. increased percentage of time in alert/active stage, longer duration of wakefulness, more prolonged alertness after induced visual pursuit movements, enhanced multi-task performance abilities, and sustained alertness; Wolff 1987). Therefore, the opposite pattern of changes in alertness in this sample is thought to be indicative of worsening nutritional status over time.

Colombo (2001) reviews the literature regarding brainstem pathways related to alertness and highlights that four pathways have been consistently linked to vigilance/attention. This includes noradrenergic projections from the locus coeruleus related to anticipation of external stimuli, the cholinergic system for sustained attention, serotonergic projections that may be related to behavioral inhibition, and dopaminergic projections that may activate behavior (Colombo 2001). Although mechanisms of relations among these neurobiological pathways, alertness, and nutrition are not well understood, it is feasible that these systems are regulated (e.g. via neurotransmitters synthesis or binding) by compounds derived from nutrients.

The finding that the frequency of responsiveness increased in the sample from 6 to 9 months of age may have been due to several developmentally appropriate changes over time. Enhanced mobility from 6 to 9 months of age may have led to coding of greater responsiveness. Additionally, in a recent review of infant responsiveness (Field et al. 2009), the failure of infants to respond was attributed to greater infant arousal, slower processing speed, and less attentiveness. As infants mature, they are more able to regulate their emotions and endogenously control their attention in addition to processing information more quickly (Colombo 2001). Their behavioral expression of emotion is also increased. An example of this is an increase in negative affect and withdrawal related to the development of stranger anxiety during this time (Mangelsdorf 1992). Therefore, it is not surprising that the frequency of responsiveness increased, especially given that the measure of responsiveness requires minimal levels of behavior from the infant (e.g. withdrawal in response to a stranger, or gazing at a toy).

Hypothesis 1: Prediction of 9-Month Behavior from 6-Month Nutritional Status

The study further sought to examine relations between early (6 month) infant nutritional variables, including WAZ, LAZ, and Hb concentration and later (9 month) behavioral variables including the frequencies of alertness and responsiveness. As hypothesized, 6-month WAZ significantly predicted the frequency of alertness at 9 months, such that a greater WAZ was associated with more frequent alertness. Baker-Henningham and colleagues (2009) similarly found that WAZ was related to attentiveness. Additionally, 6-month Hb concentrations significantly predicted 9-month frequency of responsiveness. These relations between Hb and responsiveness are consistent with Lozoff and colleagues (1998) and additional studies that found that iron is related to alertness (Oski & Honig 1978, Wachs et al. 2005). Variability in findings regarding relations between nutritional variables and alertness may reflect how alertness was defined and coded. Therefore, future studies should use multimodal assessment of alertness and responsiveness behavior examining several different operational definitions of both constructs.

The null results regarding LAZ and differential significance of beta weights for nutritional variables depending on the type of behavioral outcome variable used suggest that WAZ may be more robustly related to alertness than LAZ and Hb. Then again, it should be noted that WAZ is significantly related to LAZ and Hb. This may mean that there is overlapping variance among these nutritional variables in predicting behavior. But as noted before, multicollinearity was limited in analyses. Furthermore, Hb may be more robustly related to responsiveness than LAZ and WAZ. Whereas Hb is an indicator of anemia, which may result from iron deficiency, the two anthropometric variables reflect different underlying nutritional etiology. In particular, decreased WAZ is more reflective of energy deficiencies that are relatively recent, whereas LAZ may be influenced by genetic factors, may reflect deficiencies in particular minerals such as zinc (Castillo-Duran & Cassorla 1999), or may be indicative of cumulative nutritional deficiencies. Therefore, follow-up studies should examine the hypothesis that alertness and responsiveness may be related to different specific indicators of nutritional status. Furthermore, subsequent testing of the neurobiological mechanisms related to these differential relations is also warranted. Overall, these results support the relations between early nutrition and later behavior, which may interact (Wachs 2009) leading to prolonged deficits in the motor, cognitive, physical, and temperament development of malnourished infants/children.

Hypothesis 2: Prediction of 9-Month Behavior from 6 to 9-Month Nutritional Change

None of the models from hypothesis 2 were significant. Thus, early nutritional status was more predictive of later behavior than change in that status from 6 to 9 months of age.

Hypothesis 3: Relations between 6-Month Behavioral Groups and Nutritional Change

Lastly, it was hypothesized that Alert/Responders would have less negative change in nutrition from 6 to 9 months of age than the other groups. Contrary to this hypothesis, responders at 6 months had a greater decline in WAZ than non-responders. This may be due to the poverty level in the region such that despite the protective effects of alertness/ responsiveness, extreme poverty and lack of resources still leads to growth stunting. Perhaps more interesting, however, was the finding that despite this steeper decline and the convergence of WAZ of both groups at 9 months, there were still significant differences in the frequency of responsiveness between groups. In other words, the responders at 6 months stayed significantly more responsive at 9 months, even though their WAZ scores fell to equal those of the non-responders.

This may be related to an early sensitive period. In particular, Rosales and colleagues (2009) suggested that there may be a sensitive period in early development related to responsiveness behavior. Several reviews have supported the existence of sensitive periods (Lucas 1998, Thomas et al. 2009, Zeisel 2006), in which nutritional factors may be particularly influential on the development of specific neuronal structures and functions, which impact behavior. Within the current study, despite later nutritional changes, the difference in behavior between the two groups was retained. Several long-term (3-month) supplementation studies have similarly displayed group differences in behavior despite significant improvements in iron status for previously anemic or ID groups (Lozoff et al. 1998, Walter et al. 1989). In other words, adequate nutrition during early infancy (i.e. the first 6 months) may be important in the establishment and maintenance of alertness and responsiveness behavior, which positively influence learning and the development of cognitive skills (Colombo 2001) in addition to social interaction and social-emotional development (Beard 2007, Lozoff et al. 2003).

However, the 9-month frequency of responsiveness scores were not adjusted for age and the measure assesses whether or not the infant responded, as opposed to how the infant responded. Therefore, it is possible that if accurately adjusted for age or if a measure of qualitative differences in response were used, the pattern of changes in the frequency of responsiveness from 6 to 9 months of age would be similar to the pattern of WAZ changes over this time. Future studies are needed that investigate sensitive periods related to behavioral responsiveness (e.g. reciprocal interaction that may lead to cognitive or social competence in later ages) and alertness development, given that studies have largely focused on cognitive and motor development. These studies should use measures appropriate for both ages and correct for motor and other forms of development at 9 months of age. Moreover, future studies may want to continue examining responsiveness past 9 months of age to determine whether group differences in responsiveness will be retained for longer periods of time.

Study findings also highlight the importance of investigating group differences between infants and young children that have codeable and non-codeable data. Specifically, infants that do not respond to manipulations are typically removed from analyses. However, these differences may be related to nutritional status, as in the current study, and may be important to examine. The “responsiveness” variable may prove to be of interest for future analyses and can be easily derived from the Lab-TAB measure, as described above. As noted previously, this variable does not require a great deal of behavior from infants to code them as responsive and thus older infants, or younger infants with adequate nutrition, may be too motoric for the paradigm. However, the measure may be useful in populations with substantial suspected or known malnutrition. The level of responsiveness in the current sample (M_6 = 5.91 and M_9 = 6.37 out of a possible 10) was markedly low at both ages. Adequately nourished infants at 6 months of age would be expected to respond to most, if not all, of the episodes (e.g. mean frequencies of 9-10) and ceiling effects at both ages in well-nourished infants would likely be a concern.

The present study adds to the growing literature that suggests that nutritional factors play a significant and early role in development (e.g. Wachs 2000) and that relations between behavior and nutrition may be particularly influential in early development, when sensitive periods may exist. For example, Hulthén (2003) presented a multivariate model suggesting relations between iron deficiency and associated brain disruptions that impact behavior. This behavior may lead to functional impairment limiting learning experiences and positive interactions with parents, which then lead to poor feeding practices and result in even worse nutritional deficiencies.

Moreover, the current study highlights the importance of measuring such variables in developing countries where nutritional deficits are more prevalent, despite the potential need to modify measures for cultural appropriateness and to seek creative ways to collect data in field settings. The study also suggests that a culturally modifiable laboratory assessment (Lab-TAB) may be useful in elucidating relations between these variables in less developed countries, where risk for malnourishment or nutritional deficiencies may be prevalent. Because the methodological environment was challenging and not all potential mediators were measured or controlled for (e.g. infant birth weight), further studies are required to replicate and extend these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Messages

- In infancy, early anthropometric growth variables are differentially predictive of later behavior. In particular, 6-month weight-for-age is predictive of 9-month alertness, while 6-month Hb is predictive of 9-month responsiveness.
- Early behavior is also related to later behavior, but not anthropometry. Specifically, responsive infants at 6 months were more responsive at 9 months, despite the fact that weight-for-age for both groups was similarly stunted at 9 months.
- This study supports relations between behavior (alertness and responsiveness) and nutrition/growth.
- Findings support the use of the Laboratory Temperament Assessment Battery (Lab-TAB) for assessing behavior and temperament in Ethiopia.

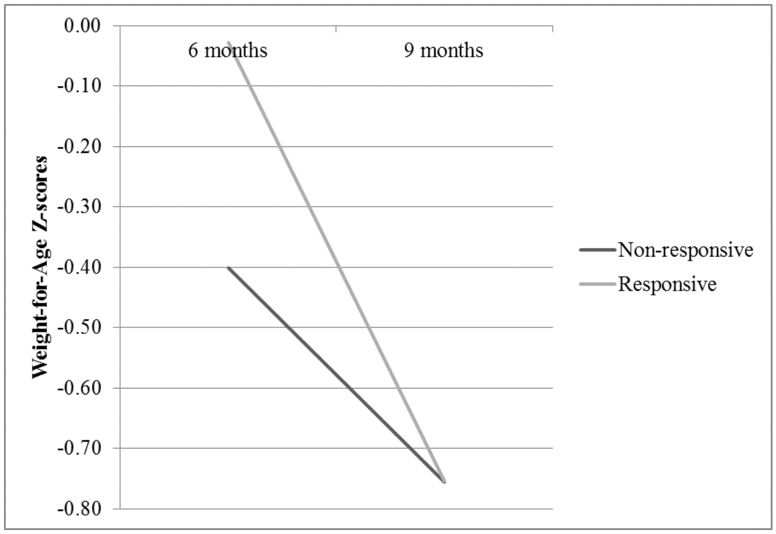

Figure 1.
Weight-for-Age Z-scores of Ethiopian Infants from 6 to 9 Months of Age
Illustration of changes in weight-for-age from 6 to 9 months for responsive and non-responsive Ethiopian infants at 6 months of age.

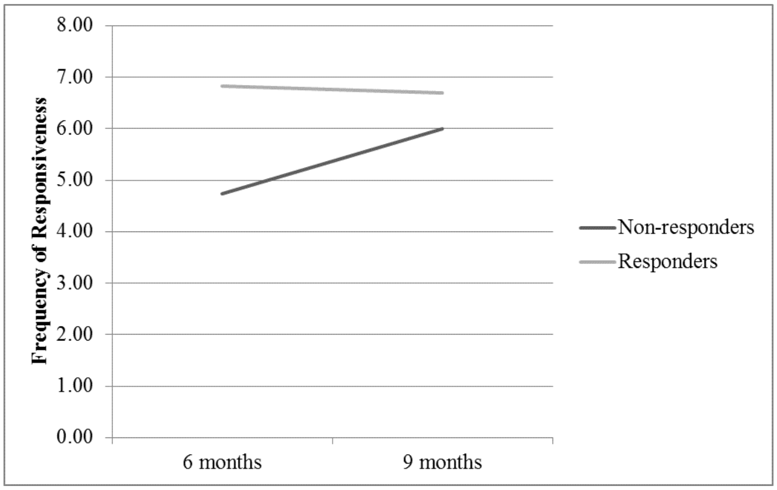

Figure 2.

Frequency of Responsiveness (Number of Episodes the Infant Responded to 50% or More of Variables) of Ethiopian Infants from 6 to 9 Months of Age

The investigation of changes in the frequency of responsiveness from 6 to 9 months for responsive and non-responsive Ethiopian infants at 6 months of age.

Table 1

Descriptive Statistics for Hemoglobin Concentrations, Anthropometric Variables, and Frequency of Alertness and Responsiveness Variables

	N	M	SD
Hb at 6 months (g/dL)	*87*	11.74	1.31
WAZ at 6 months	*89*	−0.35	1.15
LAZ at 6 months	*88*	−1.03	1.39
Frequency of Alertness at 6 months	*67*	9.31	1.48
Frequency of Responsiveness at 6 months	*74*	5.84	1.42
Hb at 9 months (g/dL)	*83*	11.62	1.48
WAZ at 9 months	*85*	−0.81	1.13
LAZ at 9 months	*84*	−1.58	1.25
Frequency of Alertness at 9 months	*76*	7.88	2.03
Frequency of Responsiveness at 9 months	*78*	6.52	1.22

Note. The following acronyms are included in the table: Hb (hemoglobin), WAZ (weight-for-age z-score), and LAZ (length-for-age z-score)

Table 2

Correlations between Nutritional and Behavioral Variables at 6 and 9 Months of Age

		WAZ 6	LAZ6	Hb 6	WAZ 9	LAZ 9	Hb 9
Alertness	*r*	.292*	0.16	.324**	0.135	0.122	0.232
Frequency	*p*	0.016	0.197	0.008	0.276	0.324	0.059
(6 mos.)	*N*	67	67	67	67	67	67
Alertness	*r*	.282*	0.034	−0.062	0.169	0.07	−0.148
Frequency	*p*	0.014	0.77	0.593	0.144	0.548	0.204
(9 mos.)	*N*	76	75	76	76	76	75
Responsive	*r*	0.15	0.161	0.091	0.04	0.136	0.076
Frequency	*p*	0.201	0.17	0.44	0.735	0.248	0.525
(6 mos.)	*N*	74	74	74	74	74	72
Responsive	*r*	−0.024	0.193	.271*	0.057	0.15	.228*
Frequency	*p*	0.838	0.093	0.017	0.622	0.189	0.047
(9 mos.)	*N*	78	77	78	78	78	77
WAZ 6	*r*		0.629***	0.227*	0.768***	0.695***	0.178
	p		< 0.001	0.026	< 0.001	< 0.001	0.089
	N		95	96	96	96	92
LAZ6	*r*			0.353***	0.503***	0.871***	0.198
	p			< 0.001	< 0.001	< 0.001	0.060
	N			95	95	95	91
Hb 6	*r*				0.233*	0.342**	0.456***
	p				0.022	0.001	< 0.001
	N				96	96	92

* $p < 0.05$ (two-tailed)

** $p < 0.01$ (two-tailed)

*** $p < 0.001$ (two-tailed)

Note. The following acronyms are included in the table: Hb (hemoglobin), WAZ (weight-for-age z-score), and LAZ (length-for-age z-score).

Table 3

Coefficient Summary Table for the Prediction of 9-Month Frequencies of Alertness and Responsiveness from 6-Month Weight-for-Age and Hemoglobin Concentrations

Dependent Variable: Frequency of alertness at 9 months	**Unstandardized Coefficients**		**Standardized Coefficients**		
	B	**Std. Error**	**Beta**	**t**	**Sig.**
(Constant)	10.244	2.173		4.714	.000
Hemoglobin at 6 months (g/dL)	−0.210	0.183	−0.131	−1.144	0.256
Weight-for-age at 6 months	0.573	0.211	0.310**	2.720	0.008
Dependent Variable: Frequency of responsiveness at 9 months	Unstandardized Coefficients		Standardized Coefficients		
	B	Std. Error	Beta	t	Sig.
(Constant)	3.226	1.245		2.591	.012
Hemoglobin at 6 months (g/dL)	0.265	0.105	0.287*	2.535	0.013
Weight-for-age at 6 months	−0.085	0.119	−0.080	−0.711	0.479

* $p < 0.05$ (two-tailed)

** $p < 0.01$ (two-tailed)