**Association of Nutritional Status with the Response to Infection with**

*Leishmania chagasi*


*Health Sciences Post-Graduate Program, Health Science Center, and Department of Biochemistry, Bioscience Center, Federal University of Rio Grande do Norte, Natal, Brazil; Carver College of Medicine, University of Iowa, Iowa City, Iowa*

**Abstract.** Outcomes of infection with *Leishmania chagasi* range from self-resolving infection to visceral leishmaniasis (VL). Risk factors determining development of disease are not totally understood, but probably include environmental influences and host genetics. We assessed whether nutrition influenced the outcome of *Leishmania* infection by comparing relatives of children with VL with either self-resolving *Leishmania* spp. infection or apparently uninfected households. We observed a decrease in body mass index ($P < 0.0005$) and mid-upper arm circumference for age ($P = 0.022$) $z$-scores for children with VL. Levels of vitamin A were lower in active children with VL as measured by serum retinol ($P = 0.035$) and the modified-relative-dose-response test ($P = 0.009$). Higher birth weight ($P = 0.047$) and albumin concentrations ($P = 0.040$) protected against disease. Increased breastfeeding time ($P = 0.036$) was associated with asymptomatic infection. The results indicate that modifiable nutritional aspects are associated with the outcome of *Leishmania* spp. infection in humans.

**INTRODUCTION**

*Leishmania chagasi* infection shows a large spectrum of clinical outcomes, ranging from asymptomatic and self-resolving infection to progressive visceral leishmaniasis (VL), which is characterized by fever, hepatosplenomegaly, hypergammaglobulinemia, and death if not properly and timely treated. The determinants that lead from infection to disease development remain unclear. Complex interactions between the parasite and the human immune response seem to be involved, but other variables such as nutrition might influence the outcome of *Leishmania* spp. infection. Although the immunologic basis for the association between malnutrition and VL is not clear, overall malnutrition results in immunosuppression and is a risk factor for the development of infectious diseases. An animal model of *Leishmania* infection showed that BALB/c mice fed a hypocaloric diet lacking zinc and iron evolved with more *L. donovani* visceralization; this was attributed to failure of the lymph node barrier to limit the infection.

In Latin America, VL is still a disease of childhood with 60% of the cases occurring in children less than 10 years of age, an age group that has shown several other morbidities such as diarrhea. Diarrheal disease affects growth and development and is frequently associated with poor environmental health conditions and hygiene. These affects lead to more malnutrition.\(^{9-11}\) Conditions frequently found in leishmanial-endemic areas, especially in northeastern Brazil where the disease-endemic area has reached peri-urban locations. Brazil has undergone important socioeconomic changes since rampant inflation was controlled in 1994. As a result of inflation control and several protective measures for lower income families, better redistribution of income and a decrease in malnutrition prevalence have occurred. This changing pattern in the economy occurred in parallel with the shift of the Brazilian population to more urban areas and the periurbanization of VL.

Breastfeeding has been shown worldwide to protect infants against diarrheal and respiratory infections, resulting in an improved nutritional status. Its protective role is long lasting and independent of socioeconomic conditions, number of residents in the family, and educational level of the parents. Birth weight is an indicator of intrauterine development, and several studies have consistently demonstrated its association with growth, development, morbidity, and mortality. Micronutrient status is also an important variable associated with infections. Micronutrient deficiency co-exists with malnutrition in a complex cycle, especially in vulnerable populations within developing countries, such as pre-school children. Vitamin A has been considered the anti-infective vitamin since the 1920s. Studies have consistently demonstrated its effect in reducing child mortality caused by measles, pneumonia, diarrhea and also its effect in improving the prognosis in children with malaria. Animal models have shown that vitamin A deficiency is associated with a switch from a Th2 immune response, which is important for resolution of non-invasive infections, to a Th1 response, which is crucial for resolution of intracellular infections, such as those with *L. chagasi*.\(^{26-28}\)

Expression of the interferon gamma (IFN-γ) gene is down-regulated by retinoic acid, the active form of retinol in cells. A murine model has shown that vitamin A deficiency stimulated the production of Th1 cytokines IFN-γ and interleukin-12 (IL-12), but not of IL-4 and IL-10, which are associated with a type 2 response. In the same study, vitamin A supplementation increased production of IL-4, IL-5, and IL-10 and decreased production of IFN-γ. Supplementation of the diet with vitamin A either as a prophylactic or therapeutic measure promoted multiplication of *L. donovani*. However, a recent study conducted in a Bangladesh population showed low serum retinol concentrations in human VL cases.

Published data in human and animal models of *Leishmania* spp. infection linking the role of vitamin A in the outcome of *Leishmania* spp. infections are controversial, and few studies have focused on nutritional variables associated with the outcome of *L. chagasi* infection. We believe that the response to *Leishmania* spp. infection is dependent on individual and environmental factors that persist throughout years, including...
exposure to infection and nutritional status. In this study, we investigated nutritional status, breastfeeding history, birth weight, and vitamin A status with respect to the outcome of *L. chagasi* infection.

**MATERIALS AND METHODS**

**Ethical considerations.** This protocol and informed consent were revised and approved by the Federal University Ethical Committee (CEP:UFRN 088/06). The certificate of ethical approval (CAA 0079.0.051.000-06) is available at www.sisnep.gov.br. The consent form was signed by the parents or legal guardian of the participants.

**Study groups and inclusion and exclusion criteria.** Ongoing VL cases were enrolled after VL diagnosis at the Pediatrics Hospital in Natal, Brazil, and patients who recovered from VL and their relatives were chosen from an open cohort from a *L. chagasi*-endemic area, as described. In the cohort study, VL patients and their relatives were recruited with the objective to study the genetic determinants of *L. chagasi* infection. For this study, 149 children were enrolled and divided into four groups: 1) children with active VL (n = 20), 2) children with a previous history of VL (n = 33), 3) children with a positive delayed-type hypersensitivity (DTH) response to *Leishmania* spp. antigens (n = 40), and 4) children with no apparent signs of *L. chagasi* infection, but residing in the area that has VL and having a relative with VL (n = 56). Patients with active VL were enrolled in the hospital during treatment. Other groups were enrolled at their households during a follow-up of a patient who recovered from VL.

The four groups were analyzed according to their outcome of *Leishmania* spp. infection. Because all children had an apparently equal chance of exposure to *Leishmania* spp., in addition to having similar socioeconomic status, the nutrition variables were retrospectively analyzed and considered the outcome of *Leishmania* spp. infection. The active VL group contained symptomatic persons undergoing specific treatment. The recovered VL group contained persons whose disease was resolved after successful treatment. Children with asymptomatic (DTH+) infection were persons who showed a protective response to *Leishmania* spp. infection. The no *L. chagasi* infection group (DTH-) contained children with no apparent signs of *L. chagasi* infection, but who resided in a disease-endemic area and had a sibling with either VL or DTH+.

Inclusion criteria for each group were as follows. Active VL was defined as current, symptomatic VL with a diagnosis of VL confirmed by positive results for bone marrow aspirates or positive results for antibody to rK39 antigen. Symptomatic disease was defined as a history of intermittent fever for more than three weeks, hepatomegaly and/or splenomegaly, hypergammaglobulinemia, and low hematocrit and hemoglobin levels. Previous history of VL was defined as recovery from VL a year post-treatment. Asymptomatic infection with *L. chagasi* was defined as a positive DTH result in the Montenegro skin test (% 5 mm in duration at 48–72 hours) and antibody against soluble *L. chagasi* antigen (SLA) with no history of disease. No apparent signs of *L. chagasi* infection was defined as a negative DTH result in the Montenegro skin test and a negative result for antibody against SLA.

The exclusion criteria for all groups were children with fever, with the exception of the confirmed VL cases, children who were physically or mentally impaired, children with chronic diseases, children more than 14 years of age, and children who refused to participate and/or parents/guardians did not allow their children to participate in the study.

**Sample collection.** A sample of approximately 10 mL of venous blood was collected from each child. Antibodies to SLA and rK39, and levels of vitamin A, albumin, total protein, globulins, C-reactive protein, and alpha-1-acid glycoprotein were determined. Blood samples were protected from light exposure.

**Identification of Leishmania spp. infection.** Two enzyme-linked immunosorbent assays (ELISAs) using SLA and rK39 protein as source of antigens were used, as described. Briefly, wells of ELISA plates (Costar, Corning Inc., Corning, NY) were coated with 200 ng of *L. chagasi* promastigote antigen or 50 ng of rK39. Each serum sample was assayed in triplicate. Each plate included negative control sera from unexposed Brazilians and positive control sera from patients with documented VL. The absorbance at 405 nm was determined using a Titertek Multiskan plate reader (ICN Biomedical Inc., Costa Mesa, CA). The cut-off was the mean absorbance plus three standard deviation values of negative control sera. The cut-off values were 0.117 for antibodies to SLA and 0.093 for antibodies to rK39.

The Montenegro skin test was performed using 25 μg of *Leishmania* spp. proteins (Centro de Produção e Pesquisa de Imunobiológicos, Secretaria de Saúde, Paraná, Brazil) injected intradermally. Skin tests were read after 48–72 hours by measuring in two perpendicular directions using the ball-point pen method. A positive test result was defined if the mean of the two induration measurements was greater than 5 mm.

**Modified relative dose response test and serum retinol identification.** The modified relative dose response test (MRDR) was used to indirectly determine liver storage of vitamin A because serum retinol concentrations decrease with acute-phase infections. One dose of 3,4-didehydroretinyl acetate (3,4-DHRA), a vitamin A analog not produced in humans, was given to each child dissolved in corn oil. A retinol-free snack containing approximately 10 grams of fat was given after 3,4-DHRA was administered to improve its absorption. The test uses the ratio of 3,4-didehydroretinol (3,4-DHR) to retinol and when liver concentrations of retinol are low, more 3,4-DHR binds to retinol-binding protein and circulates in the blood. Standard doses were given according to age as follows: 5.3 μmol to children less than 6 years of age, 7.0 μmol to children 6–12 years of age, and 8.8 μmol to children more than 12 years of age. A MRDR value ≥ 0.060 was considered indicative of inadequate vitamin A status, one between 0.030 and 0.060 was uncertain, and a value ≤ 0.030 showed adequate vitamin A status. Serum retinol was considered low or possibly responsive to greater intake when < 30 μg/dL, inadequate when < 20 μg/dL, and deficient when < 10 μg/dL.

**Acute-phase proteins.** C-reactive protein and alpha 1-acid glycoprotein were measured by immune turbidimetry. C-reactive protein was used to assess the presence of acute inflammation caused by *L. chagasi* in children with active VL or other infections in children from leishmaniasis-endemic areas. The alpha-1-acid glycoprotein was used to measure convales-
of Health at time of birth. This record was used to assess the child’s mothers or guardians. In Brazil, birth weight is determined by the Pediatrician for Disease Control and Prevention). The weight was obtained from references from Epi-Info software, version 3.4.1 (Centers for Disease Control and Prevention (Atlanta, GA) 2000 references from Epi-Info software, version 3.4.1 (Centers for Disease Control and Prevention).

History of breastfeeding was collected from interviewing the children’s mothers or guardians. In Brazil, birth weight is recorded in children’s vaccine cards provided by the Ministry of Health at time of birth. This record was used to assess nutritional status at birth.

Statistical analysis. Sample size calculations were performed using serum retinol concentration as parameter from a pilot study of 15 children with active VL and 60 children residing in the area endemic for VL (20 with asymptomatic infection, 20 with history of VL, and 20 with no infection). The sample size was 20 for each group using a power of 90% and a two-sided significance level of 5%.

To detect differences between groups by sex, and vitamin A deficiency, the chi-square test was used. The Kolmogorov-Smirnov test was used to test the normality of continuous variables. Levene’s test was used to determine the variance and linearity of continuous variables. Because the variables age, birth weight, breastfeeding time, exclusive breastfeeding time, retinol, MRDR, C-reactive protein, alpha-1-acid glycoprotein, albumin, and globulin had normal distribution and were linear, one-way analysis of variance (ANOVA) was used to test for significant variables. Tukey’s post hoc test was used to compare means between each group. For Tukey’s post hoc test, only P values < 0.05 are shown.

To evaluate the means for birth weight and total and exclusive breastfeeding time, children with active VL and a history of VL were considered as the same group because these parameters are not associated with acute-phase responses. Pearson linear correlation was used to test the correlation between antibodies to SLA, body mass index, MUAC-for-height, serum vitamin A, and MRDR. Two multivariable logistic regression models were used to assess nutritional and biochemical factors associated with VL and asymptomatic infection, respectively. In the first model, the risk for the outcome VL was assessed, and children with VL are compared with other healthy groups. In the second model, factors associated with the risk for an asymptomatic infection are assessed, children with asymptomatic infection are compared with children with active VL and a history of VL. In these models, values for β ± SD are presented to show the direction of the relationship between the variable and the outcome analyzed (whether positive or negative). The odds ratios with 95% confidence intervals are shown to assess for the risk found between a variable and the outcome analyzed in the model.

Statistical analysis was performed using the Statistical Package for Social Sciences version 11.5 (SPSS Inc., Chicago, IL). P values ≤ 0.05 were considered significant.

RESULTS

A total of 149 children were evaluated, 20 were active VL cases, 33 had recovered successfully from VL, 40 had asymptomatic infections, and 56 had no apparent signs of L. chagasi infection. Children with asymptomatic Leishmania infection had a positive DTH response (n = 40); among these children, 7 also had antibodies to SLA. The mean ± SD induration in the Montenegro skin test size in the group with asymptomatic L. chagasi infections and the group with no apparent signs of the leishmanial infection was 7.6 ± 3.5 mm and 1.8 ± 1.8 mm, respectively. Children with a history of VL had a mean ± SD induration in the Montenegro skin test of 11.4 ± 4.0 mm and 3 (9.1%) had antibodies to SLA. All children with active VL had antibodies to SLA (Table 1).

The mean ± SD age of children was 8.9 ± 3.8 years and age varied significantly among the groups with different infection status (P < 0.0005, by ANOVA). Children with VL had the lowest mean ± SD age (4.7 ± 4.0 years), followed by the group with no apparent signs of infection (8.1 ± 3.4 years), children with a history of VL (10.1 ± 3.3 years), and the asymptomatic infection group (11.2 ± 2.4 years). No difference between the number of children and sex distribution was observed (P > 0.05, by chi-square test) (Table 2).

Nutritional z-scores for height-for-age, weight-for-age, and weight-for-height did not differ between the groups, with mean ± SD values of −1.06 ± 0.84, −0.72 ± 0.92, and 0.17 ± 0.92 z-scores, respectively. Body mass index was lower for children with active VL (P < 0.0005, by ANOVA), and MUAC-for-height was also lower in this group (P = 0.022, by ANOVA) (Table 2).

Data for breastfeeding history was available for 122 children. Birth weight was available and collated from vaccine cards for 87 children. Nutritional history of breastfeeding, exclusive breastfeeding, and birth weight were not different between groups, although there was a trend for children with VL to have a lower breastfeeding time (Table 3).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Montenegro skin test size in mm</th>
<th>Antibodies to Leishmania spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>response, mm of induration ± SD</td>
<td>No. (%) positive</td>
</tr>
<tr>
<td>Active VL</td>
<td>ND</td>
<td>20 (100.0)</td>
</tr>
<tr>
<td>History of VL</td>
<td>11.4 ± 4.0</td>
<td>3 (9.1)</td>
</tr>
<tr>
<td>DTH</td>
<td>7.6 ± 3.5</td>
<td>7 (17.5)</td>
</tr>
<tr>
<td>No L. chagasi</td>
<td>infection</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*VL = visceral leishmaniasis, ND = not done, DTH = delayed-type hypersensitivity.
Blood samples for biochemical indicators were available for 145 children (20 had symptomatic VL, 32 had a history of the disease, 39 had asymptomatic infections, and 54 had no infections). The group with active VL had the highest C-reactive protein and alpha-1-acid glycoprotein levels. The mean ± SD C-reactive protein (5.7 ± 5.9 mg/dL) and alpha-1-acid glycoprotein (144.9 ± 43.6 mg/dL) levels indicated inflammation and early convalescence from infection, respectively (Table 4).

Vitamin A status was significantly lower in the group with active VL (Table 4). The mean ± SD serum vitamin A level was 23.3 ± 13.2 μg/dL in the group with active VL, 30.9 ± 9.7 μg/dL in the children with a history of disease, 32.0 ± 11.6 μg/dL in children with asymptomatic infections, and 31.7 ± 12.3 μg/dL in the group with no apparent signs of L. chagasi infection (Table 4).

Statistical analysis was performed to exclude children with high C-reactive protein (> 5 mg/dL) or high alpha-1-acid glycoprotein (> 100 mg/dL) levels within the healthy groups (four in the group with a history of VL, two in the group with asymptomatic infections, and eight in the group with no L. chagasi infection). The mean serum retinol level and result of the MRDR test did not change after this analysis for any of the groups, and significance between groups remained the same.

The prevalence of low serum retinol (< 30 μg/dL) levels was high in all groups (Table 5), especially in children with active VL: 63% had low retinol levels, compared with 43.8% in children who recovered from VL, 46% in the asymptomatic infection group, and 50% in the children with no apparent signs of L. chagasi infection (P = 0.001, by chi-square test). Prevalence of vitamin A inadequate status measured by an MRDR test value ≥ 0.060 did not differ between groups, although 15% of children with active VL showed inadequate vitamin A status (Table 5).

Results of the ELISA for antibodies to SLA showed a negative correlation with body mass index (r = −0.359, P < 0.0005), and MUAC-for-height (r = −0.205, P = 0.030), and a positive correlation with the MRDR test result (r = 0.169, P = 0.043), as shown in Figure 1. No correlation was found with serum retinol (r = −0.098, P = 0.243).

The multivariable logistic regression model for VL in Table 6 showed that birth weight was inversely associated with the outcome of Leishmania spp. infection, with each increase in 100 grams in birth weight reducing the risk of being in the VL group by 15%. Serum albumin was also inversely associated with the disease, with each increase in 1 g/dL representing a decrease in the risk of being in the VL group by 90%. In contrast, for the asymptomatic infection group, the multivariable logistic regression model showed that albumin concentrations were positively associated with a positive Montenegro skin test result. As shown in previous studies,36 a higher age increased the likelihood of having a positive Montenegro skin test result, with each increase in one year representing an increase of 90% in the likelihood of a positive Montenegro skin test result. Breastfeeding time was also associated with an increased likelihood of a positive Montenegro skin test result, with each month of breastfeeding increasing the risk of a positive Montenegro skin test result by 16%. The higher height-for-age z-scores were inversely associated with a positive Montenegro skin test result. This finding might be explained by the fact that a positive Montenegro skin test result indicates previous infection, which might have led to compromised linear growth.43

**DISCUSSION**

Nutritional and micronutrient status have long been known to influence the risk of infectious diseases.44,45 Although it is

### Table 2

Characteristics of the study groups by age, sex, and nutritional status*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total, n = 149</th>
<th>Active VL, n = 20</th>
<th>History of VL, n = 53</th>
<th>DTH+, n = 46</th>
<th>No Leishman</th>
<th>a chagasi infection, n = 56</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years, mean ± SD</td>
<td>8.9 ± 3.8</td>
<td>4.7 ± 3.9</td>
<td>10.1 ± 3.3</td>
<td>11.2 ± 2.4</td>
<td>8.1 ± 3.4</td>
<td>&lt; 0.0005</td>
<td></td>
</tr>
<tr>
<td>Males, no. (%)</td>
<td>68 (45.0)</td>
<td>9 (45.0)</td>
<td>14 (42.4)</td>
<td>20 (50.0)</td>
<td>25 (44.6)</td>
<td>0.925</td>
<td></td>
</tr>
<tr>
<td>Z-scores, mean ± SD</td>
<td>−1.06 ± 0.84</td>
<td>−0.86 ± 1.02</td>
<td>−1.09 ± 0.74</td>
<td>−1.13 ± 0.81</td>
<td>−1.04 ± 0.87</td>
<td>0.765</td>
<td></td>
</tr>
<tr>
<td>Height-for-age</td>
<td>−0.72 ± 0.92</td>
<td>−0.52 ± 1.01</td>
<td>−0.85 ± 0.91</td>
<td>−0.92 ± 0.83</td>
<td>−0.60 ± 0.94</td>
<td>0.259</td>
<td></td>
</tr>
<tr>
<td>Weight-for-age</td>
<td>0.17 ± 0.92</td>
<td>0.22 ± 1.56</td>
<td>0.87 ± 0.38</td>
<td>−0.49 ± 0.96</td>
<td>0.24 ± 0.77</td>
<td>0.219</td>
<td></td>
</tr>
<tr>
<td>Weight-for-height#</td>
<td>−0.87 ± 1.02</td>
<td>−1.53 ± 1.10</td>
<td>−0.89 ± 1.18</td>
<td>−0.99 ± 0.93</td>
<td>−0.62 ± 0.89</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>MUAC-for-height</td>
<td>−0.30 ± 1.07</td>
<td>−1.48 ± 1.28</td>
<td>−0.29 ± 1.11</td>
<td>−0.36 ± 0.77</td>
<td>0.11 ± 0.89</td>
<td>&lt; 0.0005</td>
<td></td>
</tr>
<tr>
<td>Body mass index**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* VL = visceral leishmaniasis, DTH = delayed-type hypersensitivity, MUAC = mid-upper arm circumference.
† For sex, the chi-square test was used. For other variables, analysis of variance was used.
‡ Tukey’s post hoc test indicated a significant difference between active VL and all other studied groups (P < 0.0005).
§ Tukey’s post hoc test indicated a significant difference between active VL and no L. chagasi infection group (P < 0.005).
§ Tukey’s post hoc test indicated a significant difference between DTH+ and no L. chagasi infection group (P < 0.0005).
* For weight-for-height, 49 children were evaluated (13 with active VL, 2 with a history of VL, 6 with DTH+, and 28 with no L. chagasi infection). Centers for Disease Control and Prevention (Atlanta, GA). 2000 growth charts only allows calculation of this indicator for children with a height range of 45–121 cm.
** Body mass index was calculated for children > 2 years of age. Five children with acute cases of VL were excluded from the analysis.

### Table 3

Nutritional history of breastfeeding and birth weight in children by study groups*

<table>
<thead>
<tr>
<th>Nutritional history</th>
<th>Total, mean ± SD, n = 122</th>
<th>VL, mean ± SD, n = 46</th>
<th>DTH+, mean ± SD, n = 20</th>
<th>No Leishman</th>
<th>a chagasi infection, mean ± SD, n = 47</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breastfeeding time (months)</td>
<td>11.4 ± 12.2</td>
<td>9.4 ± 12.1</td>
<td>12.9 ± 14.1</td>
<td>12.5 ± 11.2</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Exclusive breastfeeding time (months)</td>
<td>3.6 ± 3.7</td>
<td>3.5 ± 3.9</td>
<td>4.0 ± 4.4</td>
<td>3.5 ± 2.9</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>3,404 ± 619</td>
<td>3,430 ± 747</td>
<td>3,408 ± 402</td>
<td>3,379 ± 607</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

* VL = visceral leishmaniasis, DTH = delayed-type hypersensitivity.
† By analysis of variance.
‡ Total children evaluated = 107 (75 with VL, 19 with DTH+, and 13 with no L. chagasi infection).
known that nutritional status might influence the outcome of different infections, there are few data available on how nutrition influence the outcome of *Leishmania* spp. infection. The interaction of nutritional history and basic health care measures, such as breastfeeding time and decreased birth weight, might be long-term determinants that result in increased risk of disease development. In this study, increased breastfeeding time was associated with an increased chance of being in the asymptomatic infection group, whereas decreased birth weight was associated with a higher chance of being in the VL group. These results reinforce the hypothesis that malnutrition is likely to occur before infection with *Leishmania* spp. Previous studies have shown that children who develop VL have worse anthropometric measures before infection than healthy children.46

In this study, the presence of antibodies to *Leishmania* spp. was correlated with lower anthropometric indexes and worse vitamin A status, as indicated by a higher MRDR test result. However, we were not able to determine whether the low anthropometric measures seen in the VL group were caused by infection *per se* or were a contributing factor. These questions could be better answered by a prospective cohort study of children exposed to *Leishmania* spp. infection. However, VL occurs focally in a large geographic area of Rio Grande do Norte in Brazil, and the sample size of children needed to be followed to reach significance would be large and difficult to obtain. This is a limitation of this type of study. However, because a shorter history of breastfeeding and lower birth weight are indicative of a more tenuous prior nutritional status and a greater chance of being in the VL group, we believe that malnutrition precedes *L. chagasi* infection in this group and might contribute to the outcome of this infection.

Children who recovered from VL continue to have lower levels of vitamin A, even after a year of treatment, when inflammation has disappeared, which was shown by average C-reactive protein and alpha-1-acid glycoprotein concentrations. Although the serum retinol and the MRDR test results did not differ between the group with a history of VL and the healthy groups, they also did not differ from the group with active VL. This shows that these children may have a trend to have a worse vitamin A status than their counterparts. The diminished vitamin A status may have preceded active VL, as observed in a recent prospective study conducted in Bangladesh.57 These results reinforce the hypothesis that malnutrition precedes infection.

Several explanations may be offered for why vitamin A levels are reduced during VL. The results obtained in this study raise a number of questions related to the role of vitamin A in infection. One possibility is that a pre-existing immune abnormality predisposes a person to diminished vitamin A status and VL. A second possibility is that children with VL may have reduced vitamin A intake than their healthy relatives. Both of these factors may be active. Genetic regulators of serum retinol levels are still unknown. A case study showed a low level of retinol caused by a mutation in the retinol binding protein gene.48 Low plasma retinol binding protein concentrations were found in persons with a mutation in transthyretin, which complexes with retinol binding protein in the circulation at position 84 of the molecule.49 A potential mutation in STRA-6, the retinol binding protein receptor, which causes Matthew-Wood syndrome, could potentially be of importance in regulating vitamin A function.50,51 Thus, we cannot exclude the possibility of a gene-induced impairment of serum retinol levels in the children with VL. Further studies in the area are needed to corroborate this hypothesis.

In the present study, we compared children with VL with their relatives or with a population in similar socioeconomic conditions to control for the diet offered to the children. Red meat consumption was associated with protection from de-

### TABLE 4

Clinical characteristics of the study groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total, mean ± SD, n = 145</th>
<th>Active VL, mean ± SD, n = 20</th>
<th>History of VL, mean ± SD, n = 32</th>
<th>Asymptomatic infection, mean ± SD, n = 39</th>
<th>No Leishmania chagasi infection, mean ± SD, n = 34</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol (µg/dL)</td>
<td>30.4 ± 11.9</td>
<td>23.3 ± 12.3§</td>
<td>30.9 ± 9.7</td>
<td>32.0 ± 11.6</td>
<td>31.7 ± 12.3</td>
<td>0.035</td>
</tr>
<tr>
<td>MRDR result</td>
<td>0.023 ± 0.019</td>
<td>0.036 ± 0.030§</td>
<td>0.022 ± 0.018</td>
<td>0.021 ± 0.016</td>
<td>0.019 ± 0.019</td>
<td>0.009</td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>1.6 ± 2.7</td>
<td>5.7 ± 5.9</td>
<td>0.9 ± 0.5</td>
<td>0.8 ± 0.7</td>
<td>1.0 ± 0.5</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>Alpha-1-acid glycoprotein (mg/dL)</td>
<td>84.3 ± 35.7</td>
<td>144.9 ± 43.6</td>
<td>78.8 ± 22.3</td>
<td>78.1 ± 24.7</td>
<td>67.3 ± 19.2</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.2 ± 0.9</td>
<td>2.9 ± 0.6</td>
<td>4.4 ± 0.5</td>
<td>4.4 ± 0.9</td>
<td>4.4 ± 0.8</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td>4.1 ± 1.1</td>
<td>5.5 ± 1.3</td>
<td>4.2 ± 1.3</td>
<td>3.8 ± 0.8</td>
<td>3.8 ± 1.0</td>
<td>&lt; 0.0005</td>
</tr>
</tbody>
</table>

† By analysis of variance.

### TABLE 5

Prevalence of low and deficient vitamin A status measured by serum retinol concentrations and uncertain and inadequate vitamin A status measures by the modified relative dose response (MRDR) test in the study groups

<table>
<thead>
<tr>
<th>Vitamin A levels</th>
<th>Total, n = 145</th>
<th>Active VL, n = 20</th>
<th>History of VL, n = 32</th>
<th>Asymptomatic infection, n = 39</th>
<th>No Leishmania chagasi infection, n = 34</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol 20–30 µg/dL</td>
<td>45 (31.0)</td>
<td>4 (20.0)</td>
<td>10 (31.3)</td>
<td>11 (28.2)</td>
<td>20 (37.0)</td>
<td>0.085</td>
</tr>
<tr>
<td>Retinol 100–200 µg/dL</td>
<td>25 (17.2)</td>
<td>7 (28.0)</td>
<td>4 (12.5)</td>
<td>7 (18.0)</td>
<td>7 (13.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Retinol &lt; 10 µg/dL</td>
<td>3 (2.1)</td>
<td>3 (15.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>&lt; 0.0005</td>
</tr>
<tr>
<td>MRDR result 0.030–0.06</td>
<td>34 (23.4)</td>
<td>8 (40.0)</td>
<td>9 (28.1)</td>
<td>7 (17.9)</td>
<td>10 (18.5)</td>
<td></td>
</tr>
<tr>
<td>MRDR result ≥ 0.06</td>
<td>8 (5.5)</td>
<td>3 (15.0)</td>
<td>2 (6.3)</td>
<td>2 (5.1)</td>
<td>1 (1.9)</td>
<td></td>
</tr>
</tbody>
</table>
FIGURE 1. Correlation between antibodies to *Leishmania* spp. (SLA) and A, body mass index z-scores, B, mid-upper arm circumference-for-height (MUAC-for-height), and C, modified relative dose response test (MRDR) (C).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Clinical outcome of <em>Leishmania</em> spp. infection</th>
<th>Asymptomatic infection‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β ± SE OR (95% CI) P</td>
<td>β ± SE OR (95% CI) P</td>
</tr>
<tr>
<td>Age (years)</td>
<td>−0.03 ± 0.17 0.97 (0.70–1.35) &gt; 0.05</td>
<td>0.64 ± 0.31 1.90 (1.03–3.52) 0.041</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>−0.00 ± 0.00 0.85 (0.73–0.99) 0.047 &lt; 0.001</td>
<td>0.15 ± 0.07 1.16 (1.01–1.33) 0.036</td>
</tr>
<tr>
<td>Breastfeeding time (minutes)</td>
<td>−0.02 ± 0.04 0.98 (0.91–1.05) &gt; 0.05</td>
<td>−0.18 ± 0.21 0.84 (0.55–1.27) &gt; 0.05</td>
</tr>
<tr>
<td>Exclusive breastfeeding time (minutes)</td>
<td>0.08 ± 0.16 1.09 (0.80–1.48) &gt; 0.05</td>
<td>−2.19 ± 1.11 0.11 (0.01–0.98) 0.048</td>
</tr>
<tr>
<td>Height/age (z-scores)</td>
<td>0.61 ± 0.60 1.83 (0.57–5.92) &gt; 0.05</td>
<td>−0.40 ± 0.52 0.63 (0.23–1.45) &gt; 0.05</td>
</tr>
<tr>
<td>MUAC/height (z-scores)</td>
<td>−0.46 ± 0.52 0.63 (0.23–1.45) &gt; 0.05</td>
<td>−0.69 ± 0.99 0.50 (0.13–1.92) &gt; 0.05</td>
</tr>
<tr>
<td>MRDR result</td>
<td>26.02 ± 26.96 &lt; 0.00 0.045 &lt; 0.00</td>
<td>−27.34 ± 37.15 &lt; 0.00 0.045</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>1.20 ± 1.25 3.33 (0.29–38.19) &gt; 0.05</td>
<td>8.2 ± 4.7 1.00 &gt; 0.05</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>−0.33 ± 0.46 0.72 (0.29–1.78) &gt; 0.05</td>
<td>−2.93 ± 1.65 0.05 (0.00–1.35) &gt; 0.05</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>−2.31 ± 1.13 0.10 (0.01–0.90) 0.040</td>
<td>3.1 ± 1.31 22.19 (1.71–287.33) 0.018</td>
</tr>
</tbody>
</table>

*OR = odds ratio, CI = confidence interval, MUAC = mid-upper arm circumference, MRDR = modified relative dose response.
† Active and visceral leishmaniasis history were included. These groups are compared with the asymptomatic infection and no *L. chagasi* infection groups.
‡ Children with asymptomatic infections are compared with children with active VL and a history of VL.
veloping VL. In addition, a study conducted in mice fed with a hypocaloric, low-protein, zinc and iron diet showed more visceralization of *L. donovani* in these mice than in the control group. The investigators proposed that *Leishmania* spp. visceralization was probably caused by a lymph node barrier dysfunction, increased prostaglandin E₂ production, decreased IL-10 production, and inducible nitric oxide synthesis production. These studies indicate that food intake is possibly a regulator of the immune response to *Leishmania* spp. infection.

Acute infection and inflammation diminish serum retinol levels, probably because of increased excretion of retinol-binding protein and retinol. In contrast, the MRDR test result is not altered by acute abnormalities because a decrease in retinol-binding protein levels would equally affect both 3,4-DHR and retinol. Furthermore, children with a history of disease have decreased vitamin A levels. Thus, we can not attribute the decrease in vitamin A status exclusively to losses caused by acute infection, but to some other factor, including immunogenetics. Surprisingly, there was a high prevalence of vitamin A deficiency in the healthy group, in spite of the vitamin A supplementation and overall improvement in diet.

Higher albumin concentrations were associated with protection from disease and the increased likelihood of self-resolving infection, although the 95% confidence intervals in both cases were very wide. Another risk factor that was associated with higher risk of VL is age, and the results of our study are consistent with the epidemiologic findings of lower vitamin A status in children who develop the disease and higher age as a factor that increases the likelihood of having an asymptomatic infection.

The results in this study emphasize the importance of current and previous nutritional status in the outcome of *Leishmania* spp. infection. Immunogenetic factors are implicated in the outcome of *Leishmania* spp. infection. Simple nutritional measures that might protect against development of disease, such as micronutrients provision from diet, are extremely important because they are modifiable factors that can be addressed in populations exposed to pathogens such as *Leishmania* spp. Our data support the notion that by looking at these measures and other nutritional factors such as breastfeeding time and birth weight, which reflects intrauterine and maternal nutrition, may also be important in the outcome of *Leishmania* spp. infection and should be addressed in high-risk areas. Finally, vitamin A supplementation and breastfeeding are measures that can be implemented.

Received March 25, 2008. Accepted for publication May 30, 2008.

Acknowledgments: We thank Manoel Fernandes (Fundação Nacional de Saúde) for assistance during field work studies, Dr. Goreti Macedo (Biochemistry Engineering Department, Federal University of Rio Grande do Norte) for assistance and advice regarding high-performance liquid chromatography, and Dr. Daniela Martins, Glória Monteiro, and Olívia Souza for laboratory and field support.

Financial support: This study was supported by grant A1030639-15S1 from the National Institutes of Health. Selma M.B. Jeronimo is a researcher of the Conselho Nacional de Desenvolvimento Científico e Tecnológico. Bruna L. Lima Maciel was supported by a fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior. Authors’ addresses: Bruna L. Lima Maciel, José W. Queiroz, Juliana Galvão, Níbia N. Pontes, Roberto Dimenstein, and Selma M.B. Jeronimo, Department of Biochemistry, Federal University of Rio Grande do Norte, CP 1624, Natal, Rio Grande do Norte, 59072-970, Brazil, Tel/Fax: 55-84-3215-3428, E-mail: smbj@cb.ufrn.br. Hênio G. Lacerda, Department of Infectious Diseases, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil. Lúcia F. C. de Souza, Department of Nutrition, Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte, Brazil. Stephen E. McGowan, Department of Internal Medicine, Carver College of Medicine, University of Iowa, Iowa City, IA 52242.

REFERENCES


