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Predictors of C-reactive protein in Tsimane' 2-15 year-olds in lowland Bolivia

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ABSTRACT

Infectious disease is a major global determinant of child morbidity and mortality, and energetic investment in immune defenses—even in the absence of overt disease—is an important life history variable, with implications for human growth and development. This study uses a biomarker of immune activation—C-reactive protein—to investigate an important aspect of child health among the Tsimane', a relatively isolated Amerindian population in lowland Bolivia. Our objectives are twofold: 1) Describe the distribution of CRP by age and gender in a cross-sectional sample of 536 2 to 15 year-olds; and 2) Explore multiple measures of pathogen exposure, economic resources, and acculturation as predictors of increased CRP. The median blood spot CRP concentration was 0.73 mg/L, with 12.9% of the sample having concentrations greater than 5 mg/L, indicating a relatively high degree of immune activation in this population. Age was the strongest predictor of CRP, with the highest concentrations found among younger individuals. Increased CRP was also associated with higher pathogen exposure, lower household economic resources, and increased maternal education and literacy. The measurement of CRP offers a direct, objective indicator of immune activation, and provides insight into a potentially important pathway through which environmental quality may shape child growth and health.

The World Health Organization estimates that infectious diseases are responsible for one in two premature deaths in low-income countries, claiming over 13 million lives globally per year (WHO, 1998). This burden is born disproportionately by children and infants, for whom infectious diseases and undernutrition exert independent as well as synergistic effects on growth and survival (Lutter et al., 1989; Pelletier et al., 1995; Scrimshaw, 2003). Recent work suggests that even in the absence of overt disease, chronic activation of immune processes is energetically expensive, and may play an important role in growth faltering (Campbell et al., 2003; Solomons et al., 1993). This study uses a biomarker of immune activation—C-reactive protein (CRP)—to investigate child health among the Tsimane', a relatively isolated Amerindian population in lowland Bolivia.

Previous research has emphasized the importance of clean water, hygiene, and proper waste disposal in reducing pathogen transmission, as well as the protective benefits of vaccination, prolonged breastfeeding, adequate child nutrition, and maternal education (Huttly et al., 1997; Mahalanabis et al., 1996; Stephenson, 1999). The majority of research has focused on the proximate determinants of infection, but researchers have also noted that these determinants are embedded in, and shaped by, larger social, economic, and political factors (Frongillo et al., 1997). Such factors have been of central interest to many biological and medical anthropologists, who recognize that ecological, socio-cultural, and political-economic transitions associated with globalization pose adaptive challenges at the local level, with implications for individual well-being (Baker et al., 1986; Dressler, 1991; Goodman and Leatherman, 1998; Little and Leslie, 1999; Schell et al., 1993).

Field-based research on infectious morbidity often relies on caregiver reports of overt symptoms in children, typically recalled over a 7 or 14 day period. This approach is convenient and inexpensive, but is subject to under-reporting, particularly for longer periods of recall (Martorell et al., 1975; Murray and Chen, 1992). Also, linguistic and cultural factors help define idealized states of health, and may contribute to variation in the experience and reporting of symptoms (Hahn, 1995; Kleinman, 1986). Physical examinations by health-care professionals can provide a more objective assessment of child morbidity, but are time-intensive and may not be feasible in some field settings. In addition, both this and the caregiver report methods cannot detect subclinical infectious processes that may not manifest as observable symptoms, but that may nonetheless involve the activation of energetically costly anti-pathogen defenses (Rousham et al., 1998).

The measurement of acute phase proteins such as C-reactive protein (CRP) provides an alternative method for assessing infection. CRP is a central component of the acute phase response, a non-specific, systemic response to infection or injury that provides the body's first line of defense against pathogens. Trace amounts of CRP are normally detectable in circulation, but concentrations increase by a factor of 100 to 1,000 during the 24 to 72 hours following an injury or infectious challenge. Interleukin-6 is primarily responsible for upregulating hepatocyte production of CRP (Ballou and Kushner, 1992; Baumann and Gauldie, 1994; Fleck, 1989). The half-life of circulating CRP is approximately 18 hours, and concentrations remain elevated during the course of infection for about one week following resolution (Gillespie et al., 1991; Mortensen, 1994).

CRP is an important component of innate, non-specific immune defenses involved in activating phagocytes and complement, and opsonizing bacteria, fungi, and parasites (Ballou and Kushner, 1992). As a non-specific defense, CRP increases in response to a range of pathogenic agents, making it a potentially useful marker of infectious burden and degree of immunostimulation. Concentrations above 5 to 10 mg/L have been associated with infection and inflammatory processes in many field-based studies with infants, children, and adolescents (Campbell et al., 2003; Doherty et al., 1993; Filteau et al., 1995; McDade et al., 2000; Rousham et al., 1998; Shell-Duncan and McDade, 2004). Recently, Panter-Brick and colleagues (Panter-Brick et al., 2000) have reported that elevated acute phase proteins (as measured by α_1 -antichymotrypsin) are associated with growth faltering among 10-14 year-old boys in Nepal. Immune processes associated with fighting infection consume energetic resources, and life history tradeoffs between investment in anti-pathogen defenses and growth can be expected (McDade, 2003; McDade and Worthman, 1999). Acute phase proteins such as CRP represent a measure of immune activation that can provide insight into these tradeoffs.

The objectives of this study are twofold: 1) Describe the distribution of CRP by age and gender in a population of 2 to 15 year-olds in lowland Bolivia; and 2) Investigate the association between cultural and economic transitions and increased CRP. We evaluate a series of factors, including household-level measures of pathogen exposure, economic resources, acculturation, and village distance from the regional commercial center. We use CRP as an objective measure of immune activation that may reveal an important pathway through which environmental quality shapes child growth and development.

METHODS

Study design

Research was conducted among the Tsimane', an Amerindian population of approximately 8,000 in the Department of Beni in the Bolivian Amazon (Castillo, 1988; Godoy, 2001; Gullison et al., 1996; Riestler, 1993). Slash-and-burn farming is the primary means of subsistence, supplemented with hunting and gathering, wage labor in logging camps or cattle ranches, or selling crops and forest goods. Markets are poorly developed in remote areas, but people closer to town take advantage of opportunities for wage labor and cash cropping. Like other rural populations that have become incorporated into the larger Bolivian economy, lowland populations increasingly confront problems related to land degradation, impoverishment, and discrimination (Jones, 1995; Morales, 1992; Painter, 1995). The Tsimane' are by no means isolated, but they are currently at the margins of these political-economic processes. At the time of our survey, electricity and running water were not available to any household, and all but the two closest communities in our study were not accessible by road year round.

Data were collected as part of an ongoing study investigating the effects of cultural and economic transitions on Tsimane' well-being. The Maniquí river serves as the main artery of commerce and transportation for many Tsimane' settlements, and twelve communities were selected along the river that vary in distance from the town of San Borja, the regional commercial center (population ~16,000). Baseline sociodemographic, economic, cultural, and health data were collected in the dry season (May-August 2002), along with finger prick blood samples and anthropometric measures

of children between the ages of 2 and 15 years, inclusive. Fifteen was chosen because after this age individuals begin clearing their own plots and become more economically self-sufficient. The study protocol was approved by the Northwestern University Institutional Review Board for research involving human subjects. The Tsimane' Grand Council also approved the study, and parental consent as well as child/adolescent assent was obtained prior to enrollment.

Data collection

An attempt was made to recruit every resident of the 12 villages over the age of 2 years into the study. Census information for this population is not available, so we cannot formally evaluate the proportion included in our sample. Anthropometric measures and blood spot samples were collected in a single day, and virtually everyone who was present that day was included in the sample. However, village residents who happened to be absent were not included. Primary caregivers provided information on a range of household- and individual-level variables, including patterns of work and consumption, education, economic resources, household demographics, attitudes toward acculturation, and perceived health. The age of most children is known with confidence, although ages were estimated for 27 percent of the sample based on comparisons with children of known age in the family or village.

Standard procedures (Lohman et al., 1988) were implemented to collect anthropometric measures of nutritional status, including standing height (without footwear) and weight (in light tropical clothing). Sex-specific standardized scores for height-for-age (HAZ), weight-for-age (WAZ), and weight-for-height (WHZ) were

calculated in EpiInfo (Version 3.2, CDC) using the CDC/WHO 1978 reference curves recommended for international use (WHO Working Group, 1986).

At least one drop of free flowing capillary blood was collected on filter paper for analysis of CRP. Each participant's finger was cleaned with alcohol, and a sterile, disposable microlancet was used to deliver a controlled, uniform puncture. Whole blood was placed directly on standardized filter paper commonly used for neonatal screening (#903, Schleicher and Schull, Keene, NH). This relatively non-invasive blood collection protocol minimizes pain and inconvenience to the participants, and facilitates the collection of large numbers of blood samples despite the constraints of field conditions. After collection, samples were covered, allowed to dry overnight, and then transported to the town of San Borja where they were refrigerated prior to express shipment to the U.S.. Upon arrival at Northwestern University, samples were stored frozen at -30°C until analysis. Samples were exposed to tropical temperatures for less than three days, within the limits necessary to maintain sample integrity for CRP analysis (McDade et al., 2004).

Laboratory analysis of CRP

Samples were analyzed in the Laboratory for Human Biology Research at Northwestern University using an enzyme-linked immunosorbent assay (ELISA) protocol previously developed for use with blood spots (McDade et al., 2004). Calibrators for blood spots were prepared by diluting delipidated human serum enriched with CRP (standardized against the WHO International Reference Preparation; #X0923, Dako, Carpinteria, CA) with washed erythrocytes, followed by application onto filter paper.

Blood spot calibrators, controls, and samples were included in all assays, and were treated identically throughout the protocol.

Briefly, one 3.2 mm disc of whole blood was eluted overnight in 500 uL buffer, and 100 uL eluate were added in duplicate to microtiter wells precoated with anti-CRP capture antibody (#A0073, Dako). Wells were washed, then incubated with anti-CRP detection antibody conjugated with horseradish peroxidase (#P227, Dako). Wells were washed, and 100 uL chromogen substrate were added to catalyze color formation proportional to the concentration of bound detection antibody. Absorbance was read at 490 nm (BioTek Elx808), and sample concentrations were calculated from the best fit 4-parameter logistic standard curve (KCJunior, BioTek). Samples reading above the highest calibrator were re-analyzed using a higher dilution factor (i.e., discs were eluted in 1000uL wash/elution buffer and the result was multiplied by 2).

Prior validation of assay performance indicates that the blood spot CRP method has good sensitivity, precision, and reliability, and a high correlation between matched plasma and blood spot samples (Pearson $R=0.96$, $N=94$) (McDade et al., 2004). To monitor day-to-day variation across assays during the analysis of samples for this study, two control values were included with each run. Between-assay coefficients of variation (SD/mean) for the low and high controls were 7.9% and 9.0%, respectively.

Data analysis

Complete anthropometric, sociodemographic, and CRP data were available for 536 individuals. All statistical analyses were conducted with Stata for Windows, version 8.0 (StataCorp, College Station, TX). A series of maximum likelihood logistic regression

models were used to predict the likelihood of having CRP concentration greater than 5.0 mg/L. The highly skewed distribution of CRP—even following logarithmic transformation—precluded modeling CRP as a continuous outcome. This cut-off value is based on previous applications of the blood spot CRP method (McDade et al., 2000), as well as previous research in which plasma concentrations of CRP greater than 5-10 mg/L have been associated with infection and inflammatory activity (Ballou and Kushner, 1992; Filteau et al., 1995; Gillespie et al., 1991). Previous analysis of matched plasma-blood spot samples (McDade et al., 2004) indicates that a blood spot CRP concentration of 5.0 mg/L is equivalent to ~6 mg/L plasma CRP.

We expected CRP to be related to 4 sets of variables: 1) variables associated with the individual (e.g., age, sex); 2) variables associated with the level of pathogen exposure (e.g., school attendance, source of drinking water); 3) household-level variables associated with the caregiving environment that may structure pathogen exposure and nutritional status (e.g., cleanliness, economic resources, acculturation); and 4) variables associated with the broader environment of the village (distance to nearest town).

Following this logic, statistical analyses proceeded in three stages. First, age and gender were investigated as predictors of CRP concentration. Age was modeled in years, as a continuous variable. All subsequent models included age and gender as covariates. Second, three sets of household-level variables—representing degree of pathogen exposure, economic resources and market integration, and acculturation—were evaluated as predictors of CRP. We evaluated pathogen exposure variables first—after age and gender—because we considered them to be the most proximate causal factors associated with CRP. We then considered the independent effects of economic factors, followed by

acculturation. We added each variable separately, and evaluated its association with CRP, as well as its impact on the odds ratios of variables already in the model. We also considered two-way interactions between these variables and gender and age. We set the criterion for retaining interaction terms at $p < 0.15$. Since the correlates of CRP have never been investigated in this population (or any similar population), we regarded these analyses as exploratory, and retained variables associated with CRP at $p < 0.10$. No corrections were made for multiple analyses.

Lastly, village distance from San Borja was considered as a group-level indicator of acculturation/market integration. Villages were divided into three groups based on the time required to travel by canoe and/or by foot to San Borja (≤ 1.5 hours, 3-4 hours, 5-8 hours).

Prior to our regression analyses, we used principal components factor analysis to investigate the associations among our measures of pathogen exposure, economic resources, and acculturation (McDade and Adair, 2001). Factor analysis identifies patterns of association among variables by calculating the optimally weighted linear combination of these variables that maximizes the amount of explained variance. The resulting factors provide insight into the nature of lifestyle transitions among the Tsimane'. We used this approach to guide the construction of summary variables, and to avoid collinearity in our regression models.

To facilitate interpretation of the associations between CRP concentration and significant explanatory variables—particularly in the presence of interactions—we calculated the predicted probabilities of increased CRP based on regression coefficients from our maximum likelihood models. The variables of interest were set to the desired

level, individual values were retained for other covariates, and the probability of a positive response was calculated based on the coefficients from the full model. In effect, this procedure allows us to calculate the probability of $CRP > 5$ mg/L based on specific sets of variables while controlling for potentially confounding factors by setting them at their mean value.

The following variables were considered as measures of pathogen exposure: absence of a latrine, presence of a kitchen area separated from the main house, presence of a table for preparing/eating meals, distance from nearest neighbor (in minutes), school attendance, household density (number of persons/size of house in m^2), number of children in the household, distance to water source (in minutes), and whether the river was the primary source of water.

We considered a range of measures of household resources and degree of integration into local market activities. These measures included wealth in the form of animals, “traditional” markers of wealth (e.g., canoe, bow, arrows), “modern” markers of wealth (e.g., shotgun, radio, bicycle), number of agricultural plots, wage income earned over the past 2 months (typically associated with logging or ranching), and cash value of goods sold or bartered in the past two weeks.

Acculturation variables included the following measures for mother (or primary female head of household) and father (or primary male head of household) of each child: duration of residence in current village, frequency of travel to San Borja, proficiency in spoken Spanish, years of schooling, writing ability in Spanish, basic math skills, literacy in Tsimane’, and attitudes toward the “traditional” Tsimane’ lifestyle.

The “cluster” option in Stata was specified for all models, with village designated as the clustering variable. This option relaxes the assumption that individual observations are independent, and requires only that observations be independent across clusters (Williams, 2000). This procedure adjusts for the fact that individuals were enrolled at the village level, and provides robust (and more conservative) estimates of variance around regression parameters.

RESULTS

Distribution of elevated CRP by age and sex

Overall, 12.9% of the sample had concentrations of blood spot CRP > 5 mg/L, indicating at least a moderate degree of inflammatory activity (Table 1). Anthropometric measures of nutritional status were similar to other indigenous lowland Amerindian populations, with relatively high levels of stunting, but little evidence of wasting (Foster et al., in press). We found no significant sex differences in nutritional status. More detailed analyses of child growth and nutritional status among the Tsimane’ are provided elsewhere (Foster et al., in press).

The distribution of CRP was highly skewed, with a median value of 0.73 mg/L (range 0.10 to 54.0). The association between CRP concentration and age is presented for females and males in Figures 1 and 2, respectively. For both sexes, concentrations of CRP are highest before 5 years, and lower at older ages. Age (in years) is significantly related to the likelihood of CRP > 5 mg/L (OR=0.88, 95% CI=0.82, 0.93, $p<0.001$): 23.3% of 2-3 year-olds have increased CRP, compared with 15.2% of 4-5 year-olds, 12.1% of 8-9 year-olds, and 2.7% of 14-15 year-olds (Table 1). Males and females were

equally likely to have increased concentrations of CRP, and there were no significant interactions between age and sex.

Measures of pathogen exposure

We hypothesized that increased exposure to pathogens would be associated with increased concentrations of CRP. We do not have direct measures of pathogenicity, and instead use proxy measures of hygiene and likelihood of encountering infectious agents. The results of our factor analysis did not justify the construction of a summary exposure scale, with eigenvalues for the first three factors of 1.77, 1.51, and 1.19, respectively. We therefore added each pathogen exposure variable stepwise to a logistic model including age and gender, and retained those variables associated with CRP at $p < 0.10$.

Preliminary analyses of the association between school attendance and CRP excluded 2-4 year-olds, since five years was the youngest age at which students were enrolled. Among 5-15 year-olds, 75.5% were enrolled in school at the time of the survey. We found evidence for a significant interaction between school attendance (coded as 0, 1) and age in predicting the likelihood of high CRP ($N=391$; $OR=1.60$, 95% $CI=1.23, 2.08$, $p < 0.001$).

To explore this interaction further, we ran separate models including age and gender for 5-15 year-olds attending school and for those not attending school. For those not attending school, the likelihood of $CRP > 5$ mg/L was significantly lower at older ages ($N=96$; $OR=0.53$, 95% $CI=0.39, 0.73$, $p < 0.001$). In contrast, age was a much weaker predictor of increased CRP concentration among those currently attending school ($N=295$; $OR=0.90$, 95% $CI=0.79, 1.02$, $p=0.099$). Figure 3 presents the predicted

probabilities of increased CRP based on these models. For all participants, the likelihood of increased CRP is higher at younger ages. But for children in school the probability is higher at all ages, and for those not in school much lower probabilities are evident by 7-8 years.

Approximately 35% of the participants used the Maniquí River as their primary source of water, a practice associated with a two-fold increase in the likelihood of increased CRP (OR=2.00, 95% CI=1.02, 3.95, p=0.044). This association was independent of school attendance, and we found no evidence for an interaction between the use of river water and age. Other measures of pathogen exposure were not related to CRP concentration, and did not alter the associations between CRP and school attendance or water source.

Measures of economic resources and activities

We hypothesized that increased access to economic resources would be associated with lower risk of CRP elevation. We considered multiple types of resources, including wealth, income over the preceding two months due to barter and/or wage labor, and number of agricultural plots. We expected that associations between CRP and economic resources would in part be mediated by pathogen exposure, and therefore added the economic variables to logistic regression models including age, gender, and the pathogen exposure variables identified as significant above.

Factor analysis did not demonstrate strong associations among the economic variables, although the first factor revealed relatively close associations among our three measures of wealth (modern, traditional, and animal; eigenvalue=2.10). We therefore

used a summary wealth measure, with the distribution divided into quintiles to counter the high degree of skew. Household wealth was significantly associated with the likelihood of increased CRP concentration, independent of gender, age, school attendance, and the river as primary water source (OR=0.83, 95% CI=0.71, 0.97, p=0.029). Similar odds ratios were found for each type of wealth when considered separately, suggesting that wealth in general had a protective effect.

Cash income from wage labor in the preceding 2 months was not associated with CRP, nor was the value of goods sold over the preceding two weeks, or the number of agricultural plots tended by the household. However, the value of goods bartered in the preceding two weeks by the male head of household (modeled as quintiles) was a significant predictor of increased CRP (OR=0.78, 95% CI=0.62, 0.99, p=0.042). The protective effect of barter was independent of household wealth, and the addition of barter did not substantially alter the odds ratios of previously considered variables.

Measures of acculturation

Based on previous research in this population reporting a negative association between Spanish language proficiency and child nutritional status (Foster et al., in press), we hypothesized that parental measures of acculturation would be positively associated with CRP concentration. Two main factors emerged from factor analysis, each demonstrating close associations among years of schooling, writing ability in Spanish, basic math skills, and literacy in Tsimane' for both the father (Factor 1, eigenvalue=4.25) and mother (Factor 2, eigenvalue=2.45). We therefore created separate literacy variables that summed values on these traits, and evaluated them as predictors of CRP in logistic

models including age, gender, and the covariates representing pathogen exposure and household resources identified as significant above. Other acculturation variables were not closely correlated with parental literacy, and were therefore considered separately.

Higher scores for maternal literacy (mean=1.01, range=0 to 6) were associated with increased risk of CRP >5 mg/L (OR=1.08, 95% CI=1.01, 1.16, p=0.022). We found no evidence for significant associations with paternal literacy, or with other measures of acculturation including frequency of travel to San Borja, oral Spanish language proficiency, duration of residence in current village, and attitudes toward Tsimane' culture.

Distance from the commercial center

Distance from San Borja represents a village level variable that may serve as a proxy for the degree of cultural and/or economic transition as experienced at the level of the household or individual. We evaluated distance as a predictor of CRP above and beyond our more proximate household- and individual-level variables by adding the distance variable to a full model including all significant covariates identified above. No significant associations were found. Compared with participants living close to San Borja, those in middle-distance communities were 1.66 times more likely to have increased CRP (95% CI=0.74, 3.75, p=0.22), while those in more remote communities were 1.36 times more likely (95% CI=0.65, 2.83, p=0.41). Adding distance to the full model did not substantially alter the odds ratios of other predictors of CRP.

Best and worst case scenarios

The final model for predicting CRP > 5 mg/L is presented in Table 2. With all covariates considered simultaneously, household wealth and the value of bartered goods were of marginal significance ($p < 0.10$), while age, school attendance in interaction with age, maternal literacy, and the use of river water remained strong predictors of increased CRP.

In order to investigate the combined impact of these exposures independent of age, we constructed best-case and worst-case scenarios from the variables in our final model. The best-case scenario was defined by not attending school, collecting water from sources other than the river, a high level of household wealth (fourth quintile of the distribution), a high value of bartered goods (fourth quintile of the distribution), and low maternal literacy (score=0, 57.8% of sample). Under these favorable conditions, the probability of CRP > 5 mg/L was predicted to be 0.03. The worst case scenario was defined by attending school, using the river as a primary water source, low household wealth (second quintile), low value of bartered goods (second quintile) and high maternal literacy (score=3, approximately 1 SD above the mean). Under these circumstances, the probability of increased CRP was predicted to be 0.26, more than an eight-fold increase.

DISCUSSION

Infectious disease is a major contributor to child morbidity, mortality, and growth faltering, particularly in low income nations. We used CRP to investigate the correlates of immune activation in a population of 2 to 15 year-olds undergoing cultural and economic transition. As expected, CRP concentrations were highest in early childhood,

and increased CRP was associated with higher degrees of pathogen exposure and lower levels of household economic resources. Only one measure of acculturation—a summary measure of maternal education and literacy—was positively associated with increased CRP.

Overall, the median concentration of CRP in this sample was 0.73 mg/L, and 12.9% had concentrations of CRP > 5 mg/L. Although there has been recent interest in measuring acute phase proteins in population-based surveys, few datasets are available for comparison. Prior research in Samoa used a similar blood spot assay protocol to measure CRP in 760 2 to 20 year-olds. In this relatively well-nourished South Pacific population, 6.2% of the sample had concentrations of CRP > 5 mg/L, with higher CRP evident at younger ages (McDade et al., 2000). A negative association between CRP and age has also been reported for children under 5 years in rural Gambia (Filteau et al. 1995). Dramatically lower concentrations of CRP have been reported in two recent studies conducted with western populations. In a sample of 10-11 year-olds from England and Wales, the geometric mean plasma CRP concentration was 0.15 mg/L, with an interquartile range of 0.06 to 0.47 mg/L (Cook et al., 2000). In the third National Health and Nutrition Examination Survey (NHANES) conducted with a representative sample of the U.S. population, only 7.1% of 8-16 year-old boys and 6.1% of 8-16 year-old girls had detectable concentrations of serum CRP (≥ 0.22 mg/L). If these analyses had been conducted with blood spots instead of plasma/serum, the concentrations of CRP would be even lower due to the diluting effects of red blood cells (McDade et al., 2004). All together, these studies suggest a relatively high degree of energetic investment in immune activity among Tsimane' children.

The significance of increased CRP concentrations among the Tsimane' is not clear, although research in other populations is suggestive. For example, a vitamin A supplementation trial in rural Ghana recorded symptoms of infectious disease in children under five years for a week preceding blood collection. For individuals in the placebo group, higher concentrations of CRP were found among those reporting symptoms of infection, including fever, cough, vomiting, and/or diarrhea (Filteau et al. 1995). Among 10-14 year-old boys in Nepal, high concentrations of acute phase proteins have been associated with stunted growth (Panter-Brick et al. 2000). These findings indicate that high concentrations of CRP among the Tsimane' may indicate a high burden of infectious disease, with implications for child growth and overall health. Additional longitudinal analyses will be required to evaluate these implications.

Age is a major predictor of CRP, with nearly one in four 2-3 year-olds having CRP>5 mg/L, compared with only one in forty by 14-15 years. Two processes likely account for this age-related decline. First, young children spend more time in contact with the ground, and likely encounter more pathogens as a result. Domestic animals roam freely across most Tsimane' homes, and infants and young children are commonly seen sitting or playing in the dirt.

Second, non-specific, innate immune processes—of which CRP is a central component—may be more active early in life since specific immune defenses coordinated by T and B lymphocytes are relatively naïve. In many cases pathogens will be encountered for the first time, whereas older children will have developed a more diverse repertoire of long-lived memory lymphocytes that coordinate stronger, targeted responses to pathogens upon subsequent encounters (Goldsby et al., 2000; Wilson, 1990).

Antigen-specific immune responses are slower and less efficient early in life, contributing to higher vulnerability to infection. Previously established trajectories of child growth in many impoverished settings—characterized by rates of growth relative to reference values that are poorest early in life, and then stabilize as children “track” in the lower percentiles—likely reflect, at least in part, this age-dependent vulnerability to infection (Eveleth and Tanner, 1990). These parallel patterns of growth impairment and infectious disease risk underscore the importance of using objective indicators of immune activity such as CRP to investigate infection as a well-known, but relatively understudied, pathway through which environmental quality may affect child growth.

School attendance and consumption of river water are likely to increase CRP concentrations by facilitating pathogen transmission, while a potential mechanism linking maternal literacy to CRP is less intuitive. Although maternal age was not associated with child CRP concentration, we found higher literacy scores in younger mothers (Spearman $\rho = -0.50$, $p < 0.001$), likely reflecting the relatively recent emergence of schools in Tsimane’ communities. A higher literacy score may therefore represent in part inexperience of young mothers that increases the risk of infection among their children. In addition, to the extent that school-based educations de-value local knowledge, literate mothers may have had limited opportunities for learning about the local environment, with potential implications for child health. Consistent with our finding on maternal literacy, previous research with the Tsimane’ has reported a positive association between father’s education and reported child illness (Byron, 2003). In contrast, a large body of literature has suggested that education is positively linked to child health in populations

around the world, although the causality of this association has been questioned (Desai and Alva, 1998). Clearly this issue merits further investigation.

Ecological comparisons—with village distance from the commercial center of San Borja serving as a proxy for exposure to, and possibly engagement in, novel lifestyles—did not reveal any significant associations with CRP concentration. This is perhaps not surprising given that previous analyses among the Tsimane' found no major effect of village distance on multiple measures of nutritional status (Foster et al., in press). However, several household level factors were associated with CRP, highlighting the importance of evaluating the heterogeneity of experience within communities with respect to acculturation (McDade and Adair, 2001).

Strengths of our study include the application of CRP as a direct, objective indicator of inflammation that provides insight into the degree of energetic investment in immune activity. The majority of research on child health relies on maternal morbidity reports or anthropometric measures of growth, but our biomarker approach allows us to investigate directly a critical physiological mechanism related to host defense. In addition, we have a large range of household-level measures that allow us to move beyond the ecological level of analysis and test more directly the association between cultural and economic transitions and child health. Lastly, our analyses include children between the ages of 2 and 15 years, whereas the majority of prior research on infectious disease has focused on children 5 years of age and under.

A limitation of the study is our measurement of CRP at a single time point. A CRP concentration of greater than 5 mg/L can represent a contemporaneous acute infection, recent recovery from acute infection, or ongoing acute phase activity in

response to chronic pathogenic challenge or other pro-inflammatory stimuli. A single measure cannot differentiate among these possibilities, and serial measures will be required for a more accurate assessment of immune activation at the individual level. Alternatively, other acute phase proteins such as α_1 -antichymotrypsin have been proposed as better measures of chronic immunostimulation due to their longer half life (Calvin et al., 1988). Future analyses in this ongoing study will address these issues with additional measures of infection and child health.

Conclusion

We found high concentrations of CRP in Tsimane' children and adolescents, likely reflecting an elevated burden of infectious disease that may contribute to the high degree of growth faltering in this population. Age was the strongest predictor of increased CRP, with the highest concentrations found in young children. Measures of pathogen exposure, household resources, and maternal literacy were also significant predictors of CRP. Measuring CRP in dried blood spots provides a minimally-invasive tool for gaining insight into immune activation and degree of investment in anti-pathogen defenses, processes that may be particularly important to child growth and health in low resource settings.

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Table 1. Prevalence of elevated CRP and mean (standard deviation) z-scores for height-

Age (yrs)	N	% female	% CRP>5 mg/L	HAZ	WAZ	WHZ
2.0-3.9	86	53.5	23.3	-1.90 (1.11)	-0.99 (1.01)	0.23 (0.95)
4.0-5.9	99	45.5	15.2	-1.73 (1.70)	-0.75 (1.28)	0.41 (1.12)
6.0-7.9	94	44.7	10.6	-1.59 (1.35)	-0.74 (1.11)	0.44 (0.91)
8.0-9.9	91	39.6	12.1	-1.75 (1.29)	-0.98 (0.88)	0.49 (0.86)
10.0-11.9	71	45.1	9.9	-1.53 (0.95)	-0.91 (0.77)	-
12.0-13.9	58	48.3	8.6	-1.63 (0.87)	-0.73 (0.76)	-
14.0-15.9	37	64.9	2.7	-1.66 (0.61)	-0.64 (0.71)	-
Total	536	47.2	12.9	-1.69 (1.25)	-0.84 (1.00)	0.40 (0.97)

for-age (HAZ), weight-for-age (WAZ), and weight-for-height (WHZ) by age group.

Table 2. Maximum likelihood multiple logistic regression model predicting the probability of CRP>5 mg/L (Log likelihood=-187.9, p<0.001, N=536).

Variable	Odds ratio	P value	95% confidence interval	
School attendance (0, 1)	0.45	0.203	0.13	1.54
Age (years)	0.70	<0.001	0.62	0.79
School attendance x age	1.29	<0.001	1.12	1.49
River as water source (0, 1)	2.27	0.028	1.09	4.71
Household wealth (quintiles)	0.86	0.082	0.73	1.02
Value of barter (quintiles)	0.80	0.059	0.63	1.01
Maternal literacy (range 0 to 6)	1.09	0.022	1.01	1.17

Figure legend

Figure 1. Median and inter-quartile range CRP concentration with age, girls.

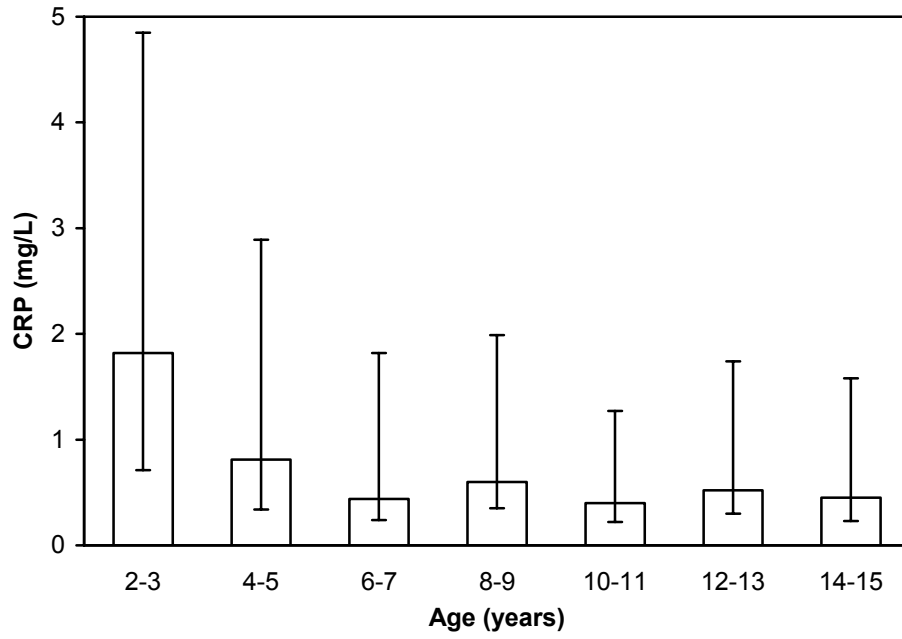


Figure 2. Median inter-quartile range CRP concentration with age, boys.

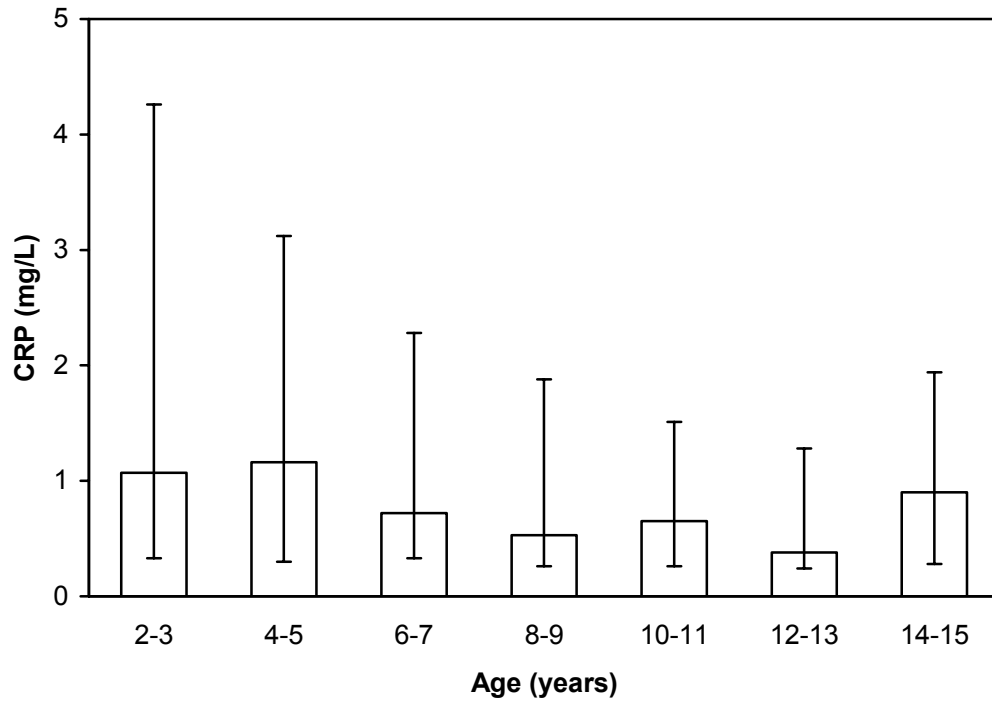


Figure 3. Predicted probability of CRP>5 mg/L according to age and school attendance for 5 to 15 year-olds.

