American Journal of Human Biology

Analysis of variability of high sensitivity C-reactive protein in lowland Ecuador reveals no evidence of chronic low-grade inflammation

Journal:	*American Journal of Human Biology*
Manuscript ID:	AJHB-12-0056.R1
Wiley - Manuscript type:	Original Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	McDade, Thomas; Northwestern University, Anthropology Tallman, Paula; Northwestern University, Anthropology Madimenos, Felicia; University of Oregon, Department of Anthropology Liebert, Melissa; University of Oregon, Department of Anthropology Cepon, Tara; University of Oregon, Department of Anthropology; Sugiyama, Lawrence; University of Oregon, Department of Anthropology Snodgrass, James; University of Oregon, Anthropology;
Keywords:	inflammation, infectious disease, cardiovascular disease, developmental origins of adult disease, ecological immunology

Title: Analysis of variability of high sensitivity C-reactive protein in lowland Ecuador reveals no evidence of chronic low-grade inflammation

Thomas W. McDade[1,2]
Paula S. Tallman[1]
Felicia C. Madimenos[3,4]
Melissa A. Liebert[3,4]
Tara J. Cepon[3,4]
Lawrence S. Sugiyama[3,4,5]
J. Josh Snodgrass[3,4]

[1] Department of Anthropology, Northwestern University
[2] Cells to Society: The Center on Social Disparities and Health at the Institute for Policy Research, Northwestern University
[3] Department of Anthropology, University of Oregon
[4] Institute of Cognitive and Decision Sciences, University of Oregon
[5] Center for Evolutionary Psychology, University of California, Santa Barbara

Abbreviated title: CRP variability in lowland Ecuador

Corresponding author:
Thomas McDade, Ph.D.
Northwestern University
Department of Anthropology
1810 Hinman Avenue
Evanston, IL 60208
847-467-4304 (phone)
847-467-1778 (fax)
t-mcdade@northwestern.edu

Funding: This work was supported by funding from the National Science Foundation (BCS-1027687), the National Institutes of Health (#5DP1OD000516), the L.S.B. Leakey Foundation, and the Wenner-Gren Foundation (7970).

Abstract

Objectives: C-reactive protein is an important component of innate immune defenses, and high sensitivity CRP (CRP) has emerged as an important biomarker of chronic inflammation and cardiovascular disease risk. Prior analyses of CRP variability have reported stable between-individual differences in CRP over time, but a limitation of current knowledge is that it is based on research conducted in post-epidemiologic transition populations.

Methods: This study evaluated CRP variability among adults in the southeastern region of the Ecuadorian Amazon where rates of infectious diseases remain high. Blood samples were collected from 52 adults at four weekly sampling intervals, and quantified using a high sensitivity immunoassay.

Results: Median CRP concentration was 0.52 mg/L. 34.6% of participants had CRP >3 mg/L at one time point, but no individuals had CRP >3 mg/L across two or more sampling intervals, and within-individual correlations revealed low levels of stable, between-individual differences in CRP. The application of current guidelines for the assessment of chronic inflammation failed to detect a single case of "high risk" CRP.

Conclusions: This study is the first to investigate CRP variability in a non-industrialized, high infectious disease environment. It documents a pattern of variation over time that is distinct from prior research, with no evidence for chronic low-grade inflammation. These results may have substantial implications for research on inflammation and diseases of aging globally, as well as for scientific understandings of the regulation of inflammation.

Keywords: inflammation, infectious disease, cardiovascular disease, developmental origins of adult disease, ecological immunology

Introduction

The recent application of highly sensitive laboratory assays for C-reactive protein (CRP) (Macy et al. 1997; Rifai et al. 1999; Roberts et al. 2000) has revealed that chronic, low-grade inflammation is an important predictor of incident cardiovascular disease (CVD) (Ridker 1998), type 2 diabetes (Pradhan et al. 2001), the metabolic syndrome (Ridker et al. 2003), late-life disability (Kuo et al. 2006) and mortality (Jenny et al. 2007). Proponents of the chronic inflammation hypothesis argue that inflammatory processes contribute directly to the pathogenesis of atherosclerosis at multiple levels, while others suggest that inflammation biomarkers like CRP correlate with disease risk, but are not part of the causal pathway (Libby et al. 2002; Lloyd-Jones et al. 2006; Pearson et al. 2003; Tracy 1998).

This line of research depends on a model of inflammation in which individuals reliably differ in their level of baseline inflammatory activity. In other words, biomarkers of inflammation like CRP have to demonstrate a relatively high level of between-individual variation, and low level of within-individual variation across time, in order to serve as useful predictors of disease risk. In practical terms, this situation would allow one to use a single CRP measurement to locate an individual with respect to his or her chronic burden of inflammatory activity.

Prior research on the variability of CRP has validated this approach. For example, in a widely cited analysis, Macy et al. (1997) report high levels of within-individual correlation in CRP concentrations over time. The authors conclude: "Concerning variability from an epidemiological standpoint, our data suggest that over a 6-month period CRP values appear relatively tightly regulated, with some individuals having consistently higher values than others" (p. 56). Similarly, recent investigation of CRP variability in healthy adults over a one year

period demonstrated that the measurement stability of CRP was comparable to that of total cholesterol, a widely accepted indicator of CVD risk (Ockene et al. 2001).

A limitation of current knowledge is that it is based primarily on research conducted in post-epidemiologic transition populations with low levels of exposure to infectious diseases. The variability of high sensitivity CRP in environments with higher levels of infectious exposures is not known. There are at least three reasons why this is an important question. First, populations in lower income nations are facing rapidly rising rates of obesity and associated chronic diseases that are supplementing—rather than supplanting—infectious diseases as contributors to morbidity and mortality (Barrett et al. 1998; Basnyat and Rajapaksa 2004; Boutayeb 2006). Globally, three fourths of all deaths due to coronary heart disease occur in low- and middle-income countries (Gaziano et al. 2010). To the extent that inflammation is involved in the pathophysiology of cardiovascular diseases, it is important to understand CRP variability in order to guide future research and prevention efforts around the world.

Second, ecological settings characterized by higher levels of infectious disease pose challenges to the measurement of chronic inflammation. CRP is an acute phase reactant, and concentrations increase rapidly following infection as part of a coordinated mobilization of non-specific cellular and biochemical defenses that are critical for pathogen clearance and healing (Kumar et al. 2004). Acute spikes in CRP production may therefore obscure detection of an underlying "signal" of chronic inflammation. Since acute inflammatory processes are quickly down-regulated following resolution of infection, multiple measures across time are necessary to identify CRP observations that are not influenced by infection. For example, a recent study reports that the prevalence of "high risk" CRP (>3 mg/L) is significantly lower in the U.S. than in a remote Amazonian population with high infectious disease mortality (Gurven et al. 2008).

But since the study was cross-sectional, the significance of elevated CRP is not clear, and may trace to acute infectious responses, chronic activation of inflammatory pathways, or both. An analysis of CRP variability in the context of endemic infectious diseases is necessary to determine the prevalence of chronic inflammation, and to evaluate whether a single CRP measure can reliably indicate chronic inflammation in these settings.

Lastly, comparative research on CRP variability may yield insights into the dynamics of inflammation that are not evident in the hygienic, low infectious disease environments that are typical in the US. Recent research in the Philippines, for example, has documented exceptionally low concentrations of CRP that trace back to higher levels of microbial exposure in infancy (McDade 2009; McDade et al. 2010). The immune system is characterized by considerable developmental plasticity and ecological sensitivity (Blackwell et al. 2010; McDade 2003; McDade 2005; Yazdanbakhsh et al. 2002), and one might therefore hypothesize different patterns of inflammatory activity in individuals who grow up in environments characterized by low versus high levels of infectious disease. The documentation of such differences could have substantial implications for scientific understandings of the regulation of inflammation, and for future research on the associations among inflammation and diseases of aging.

The objective of this paper is to evaluate the pattern of variability in CRP over time in a pre-epidemiological transition setting with a relatively high burden of infectious disease. The study was conducted among the Shuar, a large indigenous population concentrated in the southeastern region of the Ecuadorian Amazon (Descola 1996). The Shuar live in small villages with scattered clusters of households, their economy based on horticulture, hunting, and fishing. Despite accelerating economic and infrastructural development, Shuar continue to depend on subsistence horticulture for daily dietary needs, while also engaging in a mix of small scale agro-

pastoralist production for market sale. Regionally, infectious and parasitic diseases account for more than 15 percent of all deaths, compared to less than 3 percent in the US and Canada (WHO 2011). Mortality risk for children under 5 years is more than three times higher in Ecuador than in the US, with 1 in 4 child deaths attributable to infectious diarrhea (Kosek 2003; WHO 2005). Across all ages, acute respiratory infection, gastrointestinal illness, and vector-borne disease are the primary sources of morbidity in the Ecuadorian Amazon, with higher rates of infectious disease among indigenous groups compared to non-indigenous Ecuadorians (Kuang-Yao Pan 2010). Recent research among the Shuar indicates a high degree of growth stunting, likely due to synergistic influences of infectious disease and marginal nutrition (Blackwell et al. 2009).

Materials and methods

Participants and data collection

Participants were drawn from three Shuar communities located near the town of Sucua in the province of Morona-Santiago, Ecuador. Blood samples and morbidity data were collected at four weekly sampling intervals from 52 adults between the ages of 18 and 49 years, excluding women who were pregnant. This age range was selected in order to limit selection due to mortality. Informed consent was obtained from all participants, and the study protocol was approved by the Northwestern University Institutional Review Board for research involving human subjects.

Anthropometric and demographic data were collected at baseline. Body weight, height, and waist circumference were measured using standard anthropometric techniques (Lohman et al. 1988). The body mass index (BMI) was calculated as the ratio of weight (kg)/height (m^2).

Information was also recorded on participant age and formal education, as well as household composition, structure, assets, and subsistence strategy.

A morbidity questionnaire was administered at each weekly sampling interval to assess the presence of infectious symptoms (Filteau et al. 1995). Participants indicated whether they were currently sick or had been sick in the last week. Participants were asked what symptoms they experienced, when the symptoms began, and whether they had spent any days in bed due to the illness. Responses were used to define a dichotomous variable (0, 1) indicating the presence of infectious symptoms during the preceding week. A value of 1 was assigned if the participant reported diarrhea, fever, urinary tract infection, or cold, and/or any two of the following: cough, runny nose, sore throat, stomach ache, body ache, nausea.

Finger stick capillary whole blood samples were collected on filter paper (dried blood spots, DBS) for the analysis of hsCRP. 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 (Whatman #903, GE Healthcare, Pascataway, NJ). This relatively non-invasive blood collection protocol minimizes pain and inconvenience to the participants, and facilitates the collection of repeat blood samples despite the constraints of remote field conditions like rural Ecuador (McDade et al. 2007).

After collection, DBS cards were allowed to dry at ambient temperatures for approximately four hours, protected by a small mesh cage. After drying, samples were stored in gas impermeable bags with desiccant, in a portable freezer at -20˚C for the duration of field data collection. Samples were exposed to ambient temperatures for less than 12 hours prior to freezing, well within the stability limits of CRP in DBS samples (McDade et al. 2004). Upon

completion of the field study, samples were express shipped to the U.S. where they were stored at -30˚C prior to analysis.

CRP analysis

Samples were analyzed for CRP in the Laboratory for Human Biology Research at Northwestern University using a modified high sensitivity enzyme immunoassay protocol previously developed for use with DBS (McDade et al. 2004). Prior validation of assay performance indicates that the DBS CRP method produces results that are comparable to gold standard serum-based clinical methods (McDade et al. 2004). To minimize between-assay variation, all samples were analyzed using a single lot of capture antibody, detection antibody, and calibration material. In addition, all four samples in a series were included on the same assay plate in order to enhance within-individual comparisons. All samples were run in duplicate, and the average within-assay coefficient of variation (CV; SD/mean) across all samples was 1.9%. Between-assay CVs for low, mid, and high control samples included with all runs were 5.8%, 8.2%, and 6.9%, respectively.

Analysis of DBS samples provides concentrations of whole blood CRP, which will differ from serum CRP due to the presence of lysed erythrocytes and associated matrix effects. However, since DBS and serum results are so highly correlated a conversion formula can be applied to DBS CRP results to calculate serum equivalent values (McDade et al. 2004). We generated a study-specific conversion formula by analyzing n=51 matched DBS and serum samples, collected for a prior assay validation study. DBS samples were analyzed using the same procedures, lot number of reagents, and technician as applied to the Shuar DBS samples. Serum samples were analyzed for high sensitivity CRP in a high throughput clinical laboratory, on the Beckman Coulter Synchron DXC platform. The correlation between DBS and serum

values was high (Pearson R = 0.98) and the resulting Deming regression conversion formula was as follows: serum (mg/L) = 1.84 x DBS (mg/L).

Statistical analysis

Statistical analyses were conducted with Stata for Windows, version 11.1 (StataCorp, College Station, TX). All analyses used log transformed (base 10) serum-equivalent CRP concentrations unless noted otherwise. Random effects analysis of variance (loneway procedure) was applied to estimate intra-class correlations and between- and within-individual variance components.

Results

The average age of participants was 32.8 years, with 61.5 percent of the sample comprised of women (Table 1). Median CRP concentration across all observations was 0.52 mg/L, with comparable concentrations in females (0.55 mg/L) and males (0.48 mg/L). Mean BMI in the sample was 25.8 kg/m^2 (SD 2.7), and BMI was positively correlated with CRP at baseline (Pearson R=0.29, p<0.05). Age was not significantly associated with CRP (R=0.03, P=0.85). Reports of cigarette smoking and alcohol consumption were infrequent (4 or fewer participants at each interval), and were not associated with CRP.

Infectious symptoms were reported for 27.4% of the observations. There were no significant differences in rates of reported symptoms across the four weekly intervals (Pearson χ^2=4.52, P=0.21). Only 19 individuals (36.5%) reported no infectious symptoms during the course of the study, 18 reported one infectious episode (34.6%), and 15 individuals (28.9%) reported symptoms at two or more intervals (only one individual reported symptoms across all

four weeks). There was a significant association between infectious symptoms and reports of staying in bed due to illness during the prior week (Pearson χ^2=8.38, P=0.004).

Concentrations of CRP were significantly associated with infectious symptoms: median CRP was 0.39 mg/L for individuals reporting no infectious symptoms during the prior week, compared to 1.01 mg/L for observations with infectious symptoms (Wilcoxon rank sum z = -3.22, P=0.001) (Figure 1).

We evaluated stability of CRP within individuals by correlating CRP values across each sampling interval (Table 2). Correlations were generally strong and positive, with Pearson r values ranging from 0.451 to 0.767. This analysis was repeated excluding CRP values associated with symptoms of infectious disease: CRP values were set to missing for observations where a participant reported symptoms of infectious disease during the week preceding blood collection. Correlations increased substantially, ranging from 0.712 to 0.862 across the four time points.

Multiple measures across time allow us to estimate the proportion of CRP variation that can be attributed to between-individual variance (σ_b^2) and within-individual variance (σ_w^2). Between-individual variance is the amount of variation in average response across individuals over the course of the study. If total variation is represented by ($\sigma_b^2 + \sigma_w^2$), then the quantity (σ_b^2)/($\sigma_b^2 + \sigma_w^2$) is the intraclass correlation coefficient (ICC), which estimates the proportion of variance attributable to between-individual factors. If the ICC is high, then individual differences in average CRP would be interpreted as relatively stable over time. For log-CRP values, ICC=0.634, indicating that 63.4% of total variance can be ascribed to between-individual factors (Table 3). The ICC was substantially higher (0.721) when observations associated with reports of infectious symptoms were removed.

Figure 2 presents the pattern of variability in CRP within and across individuals over the 4 weekly sampling intervals. Considerable variation is apparent, but the pattern is not consistent across the CRP distribution. As the mean CRP concentration for each individual increases, within-individual variation also increases due to the fact that individuals who produced high CRP at one interval also produced low CRP at other intervals. Of particular note is the complete absence of within-individual clusters of CRP observations >3 mg/L: No participants had CRP >3 mg/L across two or more sampling intervals.

This pattern of variation suggests that the ICC may not provide an adequate representation of between- versus within-individual sources of variation across the full range of CRP values. To evaluate this possibility, we divided the sample into two groups based on the distribution of CRP values, and calculated the ICC separately for each group, using log-CRP values. For individuals with mean CRP ≤ 1 mg/L across all four sampling intervals (N=27), ICC=0.602. For those with mean CRP > 1 mg/L (N=25) ICC=0.000, indicating no contribution of between-individual factors to explaining the variation in hsCRP concentration.

The pattern of variation in Figure 2 also draws attention to low CRP—rather than high or average CRP—as a potential outcome of interest. All individuals had at least one CRP value <3 mg/L, and all but one individual had at least one CRP value <1.5 mg/L. If we consider only the lowest CRP value produced by each individual, the median CRP concentration for the sample was 0.24 mg/L.

For clinical and epidemiological purposes the following CRP cut-off values have been recommended to assess an individual's relative risk of cardiovascular disease: <1 mg/L (low), 1.0 to 3.0 (average), >3.0 mg/L (high). In the U.S., approximately 1/3 of adults fall into each of these categories (Pearson et al. 2003). Current guidelines recommend that individuals be

sampled twice, preferably two weeks apart, and CRP results averaged. Values >10 mg/L and results associated with symptoms of an acute infectious/inflammatory condition should not be used. Applying these criteria to weeks 1 and 3 in our study, no individuals are in the "high risk" category, and only one individual approaches the 3 mg/L cut-off (mean CRP = 2.83 mg/L). More than two-thirds (70.4%) of the sample is classified "low risk."

Figure 3 presents the pattern of CRP variation within individuals across time, but only for the 18 individuals (34.6%) with at least one CRP result >3 mg/L. A pattern of acute elevation in CRP is evident, followed by reduction in CRP well below 3 mg/L. While several individuals have elevated "high risk" CRP at each time point, inspection of multiple measures over time reveals that different individuals are represented in this category at each sampling interval.

Discussion

This study is the first to consider high sensitivity CRP variability in a non-industrialized, high infectious disease environment. Our analysis demonstrates a pattern of variation over time that is distinct from prior studies in the US, and suggests that chronic low grade inflammation is not prevalent in this environment. These results may have substantial implications for research on inflammation and diseases of aging globally, as well as for scientific understandings of the regulation of inflammation.

Three sets of findings converge on the conclusion that chronic inflammation is absent among the Shuar. First, within-individual correlations in CRP concentrations are lower than previously reported in the US, as is the proportion of variance that can be attributed to between-individual factors. In prior studies of CRP variability, the average Pearson r between adjacent sampling intervals was 0.84 (Macy et al. 1997), and the ICC was estimated at 0.783 (Ockene et

al. 2001). For the Shuar, correlations across sampling intervals and ICC values were substantially lower, even when observations associated with symptoms of infectious disease were removed from the analysis. And given that we sampled every week, rather than every three weeks or three months as in prior research (Macy et al. 1997; Ockene et al. 2001) it is likely that correlations and ICC values would be even lower among the Shuar with a longer sampling interval.

Second, it is clear from figure 2 that there are no individuals with clusters of CRP values > 3 mg/L. Rather, individuals with high CRP at one time point also produce low CRP at other time points. This pattern contrasts with prior analyses in the US, where clusters of high CRP values are evident for a subset of individuals, and where these individuals do not produce CRP values <3 mg/L (Macy et al. 1997; Ockene et al. 2001; Pearson et al. 2003). Consistent with this distinct pattern of variation, the ICC approaches zero for Shuar individuals with mean CRP > 1 mg/L, underscoring the absence of stable between-individual differences in chronic inflammation in the part of the CRP distribution where they should be most evident.

Third, the application of consensus guidelines for the measurement of chronic inflammation in clinical and public health practice (Pearson et al. 2003) failed to detect a single case of "high risk" CRP among the Shuar, and more than two-thirds of the sample was classified as "low risk." In contrast, only 1/3 of the US adult population is "low risk", and approximately one-third of adults have "high risk" levels of CRP > 3 mg/L (Pearson et al. 2003; Woloshin and Schwartz 2005).

These findings underscore the critical importance of multiple CRP measures in determining the prevalence of chronic inflammation, and in testing the hypothesis that inflammation predicts CVD risk. For example, at time point 2 (Figure 3), seven individuals had

CRP >3 mg/L. A study using a single CRP measure would be justified in omitting four of these observations due to their association with reported symptoms of infectious disease, and assigning the remaining three individuals to the "high risk" category. However, two weeks later, all three individuals had CRP <3 mg/L, indicating misclassification at the beginning of the study. Challenges in distinguishing acute from chronic activation of inflammatory pathways may explain why a recent study in rural lowland Bolivia failed to detect significant associations between a single CRP measurement and atherosclerosis despite high levels of inflammation (Gurven et al. 2009).

In the US, median CRP has been estimated at 1.6 and 2.2 mg/L for adult men and women, respectively (Ford et al. 2004; Ford et al. 2003). Median CRP is lower among the Shuar at 0.52 mg/L. Similarly, we have reported previously that the median CRP for older women in the Philippines is 0.90 mg/L (McDade et al. 2008). Lower CRP despite higher levels of endemic infectious disease represents something of a paradox. But in light of the distinct pattern of CRP variability reported here, we propose that our findings constitute tentative support for the hypothesis that the dynamics of inflammation may differ significantly across populations, and that these differences may trace back to ecological factors during critical stages of immune development.

Infectious microbes have been part of the human ecology for millennia, and it is only recently that more hygienic environments in affluent industrialized settings have substantially reduced the level and diversity of exposure (Armelagos et al. 2005; Rook 2009). Microbial exposures—particularly saprophytic mycobacteria, lactobacilli, and many helminthes common in rotting vegetable matter, soil, and untreated water—represent normative ecological inputs that guide the development of the immune system, and in the absence of such inputs, poorly

regulated or self-directed inflammatory activity may be more likely to emerge (McDade 2003; Yazdanbakhsh et al. 2002). Prior research has shown that higher levels of microbial exposure in infancy predict lower levels of chronic inflammation in adulthood, as well as reduced risk for atopy, asthma, and autoimmunity—all conditions with an inflammatory component (McDade et al. 2010; Radon et al. 2004; Rook 2010; Rook and Stanford 1998; Yazdanbakhsh et al. 2002).

These mechanisms may explain, in part, why the Shuar do not chronically produce CRP: Inflammatory mediators increase acutely in response to infection, but robust mechanisms are in place to effectively down-regulate inflammation to very low levels of activity. Anti-inflammatory cytokines like interleukin-10 may play important roles in this process, and we have recently reported high concentrations of IL-10 among young adults in the Philippines compared to the US (McDade et al. 2011). From this perspective, the lowest CRP value an individual produces over time may be the best predictor of CVD risk: The lowest value is least likely to reflect acute phase activity, and a lower level of basal CRP production may indicate enhanced ability to keep inflammation under control.

The variability of CRP in high infectious disease environments complicates efforts to detect with certainty the "signal" of chronic inflammation despite the "noise" of acute phase activity. Measurement of additional inflammatory mediators (e.g., IL-6, IL-10), and the implementation of more dynamic models of inflammation (e.g., response to vaccination), may provide important insights. These types of measures, as well as studies of CRP variability in other populations, will be necessary to evaluate further the hypothesis that ecological factors during development are important determinants of the how inflammation is regulated in adulthood. The implications for links between inflammation and CVD will also require further investigation. The model proposed here suggests that inflammation will predict CVD only in

ecological settings where chronic inflammation is prevalent. However, if lifetime exposure to inflammation increases disease risk, then it is possible that acute—but frequent—exposures to inflammation may contribute to CVD even in pre-epidemiologic transition settings like lowland Ecuador or Bolivia (Gurven et al. 2008).

To the extent that the Shuar represent an infectious disease ecology that was more common in the past than today, the levels of chronic inflammation documented recently in post-epidemiologic transition populations like the US are unusual by historical standards. If the pattern of CRP variability reported here reflects the development of a distinct inflammatory phenotype in high infectious disease environments, it is reasonable to hypothesize that global trends toward increased overweight/obesity and reduced microbial exposure may both contribute to rising rates of CVD around the world. These are important issues that we hope will be addressed by future research on the regulation of inflammation in diverse ecological settings.

References

Armelagos GJ, Brown PJ, and Turner B. 2005. Evolutionary, historical and political economic perspectives on health and disease. Soc Sci Med 61(4):755-765.

Barrett R, Kuzawa CW, McDade T, and Armelagos GJ. 1998. Emerging and re-emerging infectious diseases: The third epidemiologic transition. Annu Rev Anthropol 27:247-271.

Basnyat B, and Rajapaksa LC. 2004. Cardiovascular and infectious diseases in South Asia: The double whammy. Br Med J 328(7443):781.

Blackwell AD, Pryor G, Pozo J, Tiwia W, and Sugiyama LS. 2009. Growth and market integration in Amazonia: A comparison of growth indicators between Shuar, Shiwiar, and nonindigenous school children. Am J Hum Biol 21(2):161-171.

Blackwell AD, Snodgrass JJ, Madimenos FC, and Sugiyama LS. 2010. Life history, immune function, and intestinal helminths: Trade-offs among immunoglobulin E, C-reactive protein, and growth in an Amazonian population. Am J Hum Biol 22(6):836-848.

Boutayeb A. 2006. The double burden of communicable and non-communicable diseases in developing countries. Trans R Soc Trop Med Hyg 100(3):191-199.

Descola P. 1996. The spears of twilight: life and death in the Amazon jungle New Nork: New York Press.

Filteau S, Morris S, Raynes J, Arthur P, Ross D, Kirkwood B, Tomkins A, and Gyapong J. 1995. Vitamin A supplementation, morbidity, and serum acute-phase proteins in young Ghanaian children. Am J Clin Nutr 62(2):434-438.

Ford ES, Giles WH, Mokdad AH, and Myers GL. 2004. Distribution and Correlates of C-Reactive Protein Concentrations among Adult US Women. Clin Chem 50(3):574-581.

Ford ES, Giles WH, Myers GL, and Mannino DM. 2003. Population Distribution of High-Sensitivity C-reactive Protein among US Men: Findings from National Health and Nutrition Examination Survey 1999-2000. Clin Chem 49(4):686-690.

Gaziano TA, Bitton A, Anand S, Abrahams-Gessel S, and Murphy A. 2010. Growing epidemic of coronary heart disease in low- and middle-income countries. Curr Probl Cardiol 35(2):72-115.

Gurven M, Kaplan H, Winking J, Eid Rodriguez D, Vasunilashorn S, Kim JK, Finch C, and Crimmins E. 2009. Inflammation and infection do not promote arterial aging and cardiovascular disease risk factors among lean horticulturalists. PLoS ONE 4(8):e6590.

Gurven M, Kaplan H, Winking J, Finch C, and Crimmins EM. 2008. Aging and inflammation in two epidemiological worlds. J Gerontol A Biol Sci Med Sci 63(2):196-199.

Jenny NS, Yanez ND, Psaty BM, Kuller LH, Hirsch CH, and Tracy RP. 2007. Inflammation biomarkers and near-term death in older men. Am J Epidemiol 165(6):684-695.

Kosek M, Bern, Caryn, Guerrant, Richard. 2003. The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. Bulletin of the World Health Organization 81(3):197-204.

Kuang-Yao Pan W, Erlien, Christine, Bilsborrow, Richard E. 2010. Morbidity and mortality disparities among colonist and indigenous populations in the Ecuadorian Amazon. Soc Sci Med 70(3):401-411.

Kumar R, Clermont G, Vodovotz Y, and Chow CC. 2004. The dynamics of acute inflammation. J Theor Biol 230(2):145-155.

Kuo H-K, Bean JF, Yen C-J, and Leveille SG. 2006. Linking C-reactive protein to late-life disability in the National Health and Nutrition Examination Survey (NHANES) 1999-2002. J Gerontol A Biol Sci Med Sci 61(4):380-387.

Libby P, Ridker PM, and Maseri A. 2002. Inflammation and atherosclerosis. Circulation 105:1135-1143.

Lloyd-Jones DM, Liu K, Tian L, and Greenland P. 2006. Narrative review: Assessment of C-reactive protein in risk prediction for cardiovascular disease. Ann Intern Med 145(1):35-42.

Lohman TG, Roche AF, and Martorell R. 1988. Anthropometric Standardization Reference Manual. Champaign, IL: Human Kinetics Books.

Macy EM, Hayes TE, and Tracy RP. 1997. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. Clin Chem 43(1):52-58.

McDade T. 2003. Life history theory and the immune system: Steps toward a human ecological immunology. Yearb Phys Anthropol 4:100-125.

McDade T, Rutherford, J.N., Adair, L., Kuzawa, C. 2009. Population differences in C-reactive protein concentration and associations with adiposity: Comparing young adults in the Philippines and the U.S. Am J Clin Nutr 89(4):1237-1245.

McDade TW. 2005. Life history, maintenance, and the early origins of immune function. Am J Hum Biol 17(1):81-94.

McDade TW, Burhop J, and Dohnal J. 2004. High-sensitivity enzyme immunoassay for C-reactive protein in dried blood spots. Clin Chem 50(3):652-654.

McDade TW, Rutherford J, Adair L, and Kuzawa CW. 2010. Early origins of inflammation: microbial exposures in infancy predict lower levels of C-reactive protein in adulthood. Proc R Soc Lond [Biol] 277(1684):1129-1137.

McDade TW, Rutherford JN, Adair L, and Kuzawa C. 2008. Adiposity and pathogen exposure predict C-reactive protein in Filipino women. J Nutr 138(12):2442-2447.

McDade TW, Tallman PS, Adair LS, Borja J, and Kuzawa CW. 2011. Comparative insights into the regulation of inflammation: Levels and predictors of interleukin 6 and interleukin 10 in young adults in the Philippines. Am J Phys Anthropol 146(3):373-384.

McDade TW, Williams S, and Snodgrass JJ. 2007. What a drop can do: Dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. Demography 44(4):899-925.

Ockene IS, Matthews CE, Rifai N, Ridker PM, Reed G, and Stanek E. 2001. Variability and classification accuracy of serial high-sensitivity C-reactive protein measurements in healthy adults. Clin Chem 47(3):444-450.

Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, III, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL et al. . 2003. Markers of Inflammation and Cardiovascular Disease: Application to Clinical and Public Health Practice: A Statement for Healthcare Professionals From the Centers for Disease Control and Prevention and the American Heart Association. Circulation 107(3):499-511.

Pradhan AD, Manson JE, Rifai N, Buring JE, and Ridker PM. 2001. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA 286(3):327-334.

Radon K, Ehrenstein V, Praml G, and Nowak D. 2004. Childhood visits to animal buildings and atopic diseases in adulthood: an age-dependent relationship. Am J Ind Med 46:349 - 356.

Ridker PM, Buring JE, Cook NR, and Rifai N. 2003. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: An 8-Year follow-up of 14 719 initially healthy American women. Circulation 107(3):391-397.

Ridker PM, Buring, Julie E., Shih, Jessie, Matias, Mathew, Hennekens, Charles H. 1998. Prospective Study of C-Reactive Protein and the Risk of Future Cardiovascular Events Among Apparently Healthy Women. Circulation 98(8):731-733.

Rifai N, Tracy RP, and Ridker PM. 1999. Clinical efficacy of an automated high-sensitivity C-reactive protein assay. Clin Chem 45(12):2136-2141.

Roberts WL, Sedrick R, Moulton L, Spencer A, and Rifai N. 2000. Evaluation of four automated high-sensitivity C-reactive protein methods: Implications for clinical and epidemiological applications. Clin Chem 46(4):461-468.

Rook GAW. 2009. Review series on helminths, immune modulation and the hygiene hypothesis: The broader implications of the hygiene hypothesis. Immunology 126(1):3-11.

Rook GAW. 2010. 99th Dahlem conference on infection, inflammation and chronic inflammatory disorders: Darwinian medicine and the 'hygiene' or 'old friends' hypothesis. Clin Exp Immunol 160(1):70-79.

Rook GAW, and Stanford JL. 1998. Give us this day our daily germs. Immunol Today 19(3):113-116.

Tracy RP. 1998. Inflammation in cardiovascular disease:Cart, horse, or both? Circulation 97(20):2000-2002.

WHO. 2005. The World Health report: 2005: Make every mother and child count. Geneva: World Health Organization Press

WHO. 2011. Life Tables for WHO Member States. World Health Statistics: World Health Organization Press.

Woloshin S, and Schwartz LM. 2005. Distribution of C-reactive protein values in the United States. New Engl J Med 352(15):1611-1613.

Yazdanbakhsh M, Kremsner PG, and van Ree R. 2002. Allergy, parasites, and the hygiene hypothesis. Science 296(5567):490-494.

Tables

Table 1. Sample descriptive statistics. Mean (SD) values are presented for continuous variables; % values are presented for categorical variables.

Age (yrs)	32.8 (8.9)
Formal education (yrs)	6.4 (3.1)
Household size (persons)	6.9 (2.7)
Household size (rooms)	2.6 (1.2)
Electricity in the house	67.3%
Refrigerator in the house	34.6%
Water available in the house	23.1%
Body mass index (kg/m^2)	25.8 (2.7)
Waist circumference (cm)	83.4 (7.7)

Table 2. Pearson correlations in CRP concentrations across 4 weekly sampling intervals (N).

	sample 1	sample 2	sample 3
sample 2	0.732[a]* (52)		
	0.765[b] (34)*		
sample 3	0.767* (52)	0.693* (52)	
	0.862 (27)*	*0.712* (25)*	
sample 4	0.679* (52)	0.593* (52)	0.451* (52)
	0.786 (33)*	*0.735* (29)*	*0.714* (27)*

([A]Pearson correlations performed using log-transformed CRP values)

([B]Pearson correlations performed using log-transformed CRP values, excluding observations associated with reports of infectious symptoms)

*p<0.001

Table 3. Variance components and intra-class correlation for CRP in lowland Ecuador and the United States. Values from a prior study of CRP biovariability in the U.S. are included for comparison (Ockene et al. 2001).

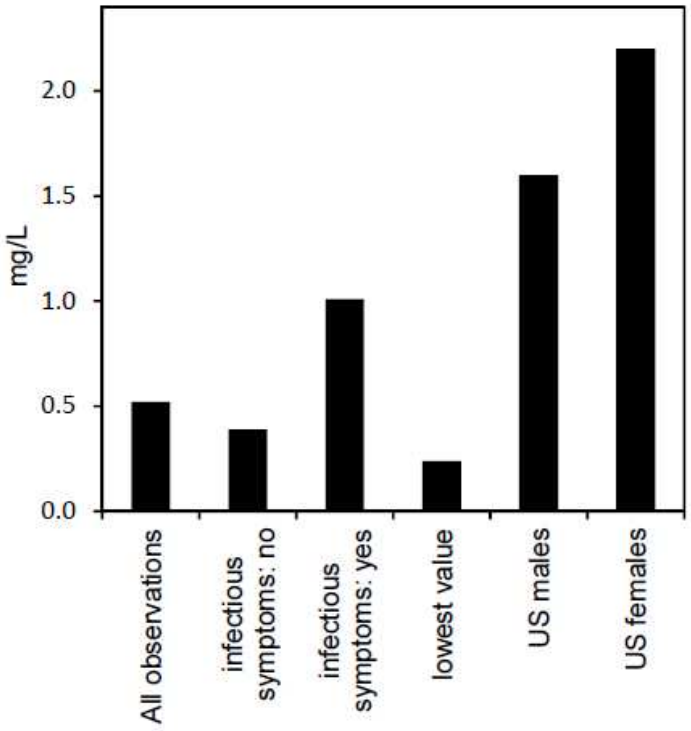
	Variance components		Intra-class correlation
	σ_b	σ_w	
Ecuador			
CRP	0.426	1.941	0.046
log-CRP	0.792	0.602	0.634
log-CRP, no infectious symptoms	0.858	0.534	0.721
log-CRP, mean CRP ≤ 1 mg/L	0.821	0.668	0.602
log-CRP, mean CRP > 1 mg/L	0.00	0.522	0.000
United States			
CRP	1.66	1.19	0.658
log-CRP	0.99	0.52	0.783

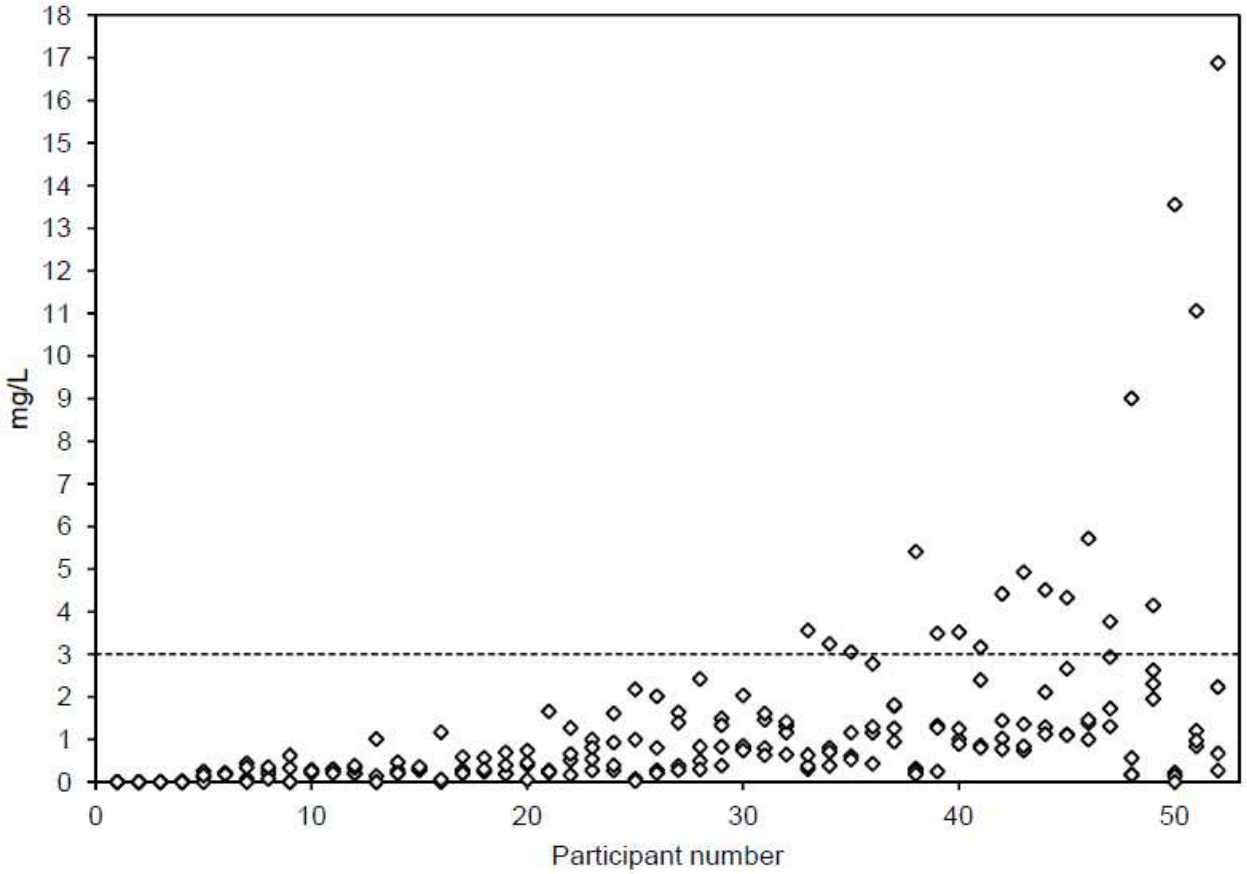
Figure legends

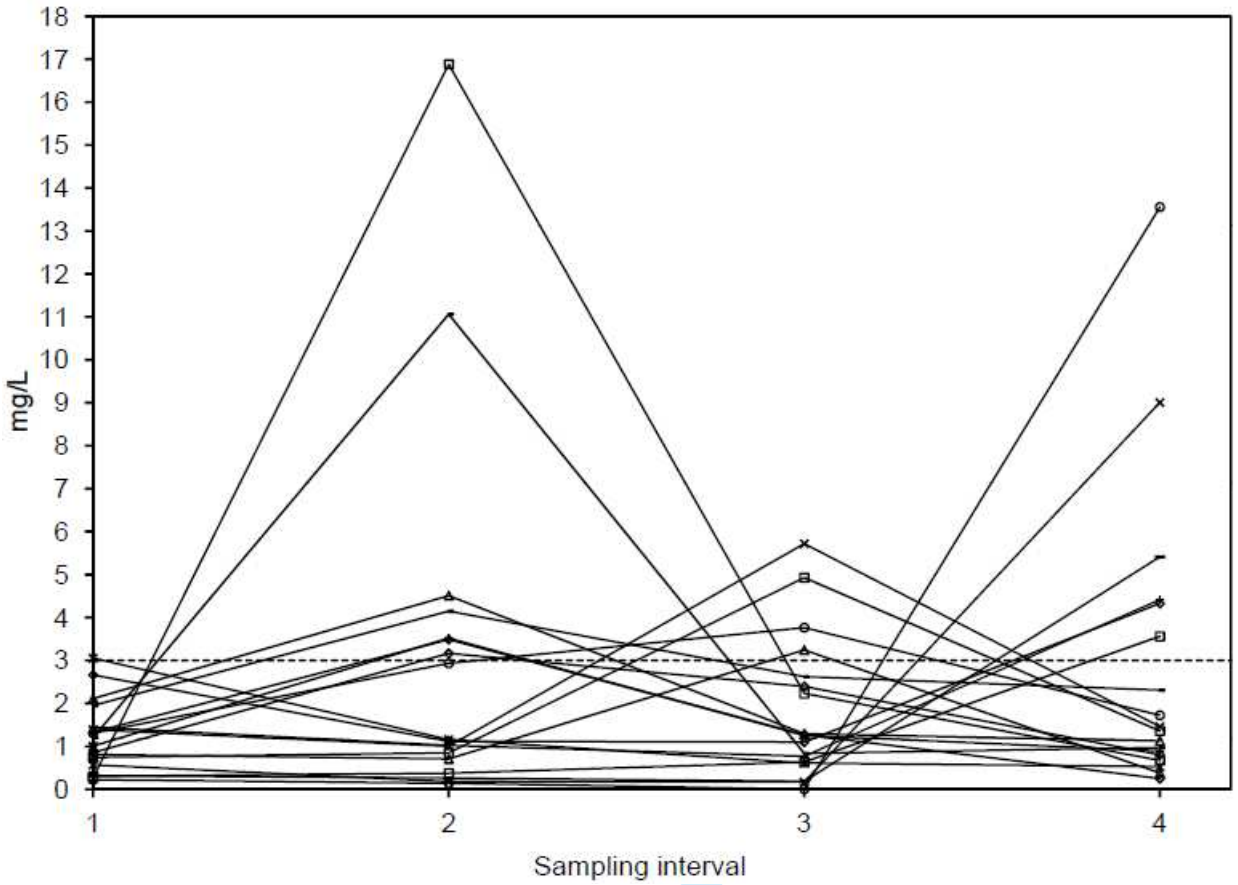
Figure 1. Median CRP concentrations in lowland Ecuador. Values were calculated for all observations (n=208), for the subset of observations when symptoms of infectious disease were present (n=57) and absent (n=151), and using only the lowest CRP result obtained for each participant (n=52). For purposes of comparison, median CRP values for US males and females are reported based on data from the National Health and Nutrition Examination Survey (Ford et al. 2003, 2004).

Figure 2. Variation in CRP concentration for each participant across 4 weekly sampling intervals. Results are presented in rank order, based on the mean CRP concentration for each individual across all sampling intervals.

Figure 3. Variation in CRP across 4 weekly sampling intervals for individuals with CRP >3 mg/L at one or more intervals (n=18).




mg/L
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0
10
20
30
40
50
Participant number


mg/L
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
1
2
3
4
Sampling interval