RELATIONSHIPS AMONG KIDNEY FUNCTION, SYSTEMIC INFLAMMATION, AND AGE IN THE INDIGENOUS SHUAR OF ECUADOR: THE SHUAR HEALTH AND LIFE HISTORY PROJECT

by

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Relationships Among Kidney Function, Systemic Inflammation, and Age in the Indigenous Shuar of Ecuador: The Shuar Health and Life History Project

This article aims to investigate and evaluate the relationships among between kidney function as measured by Cystatin C (CysC) levels, inflammation as measured by levels of C-reactive protein (CRP), and age in the indigenous Shuar of Ecuador. The assay used in this study to measure CysC levels expands the ease with which researchers can analyze and assess kidney function in non-western populations, which has large implications for public health interventions and initiatives around the world. Previously collected dried blood spots (DBS) from 128 indigenous Shuar participants were analyzed for CysC and CRP using enzyme-linked immunosorbent assays (ELISA). Lower levels of CysC mean the kidney is efficient in filtering waste and signify good kidney health, and elevated levels of CRP mean high rates of inflammation. The ELISA for CysC was based on a recently validated protocol that makes kidney function analysis through DBS possible for large community-based studies in non-clinical settings. A series of regression analyses were used to test for biological relationships among CysC, CRP, and age and between two ecological factors, (latrine type and water source). CRP and CysC levels were significantly and positively correlated (p=0.009) although the correlation was attenuated when controlling for age (p=0.078). Water source had a significant effect on CysC levels across age groups (p=0.002) and higher CysC levels were associated with a well/outdoor pipe water source. Further investigation of the association between water source and kidney health would be interesting and could have large implications for targeted public health interventions in many developing nations. This study is the first to utilize a Cystatin C DBS ELISA assay for population-based research.

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INTRODUCTION

The relationship between kidney function and inflammation remains understudied, especially in non-western populations. Western patients with predialytic renal failure have been shown to have increased levels of C-reactive protein (CRP), a common biomarker of inflammation, and CRP levels have been shown to be inversely related to renal function (Panichi et al., 1999). Chronic systemic inflammation as induced by CRP is also related to a number of downstream health problems that have large implications for public health, such as cardiovascular disease (CVD) (Kershaw et al., 2010; McDade et al., 2012; Stenvinkel., 2010; Zee et al., 2002). Research on kidney function normally focuses on severe end-stage renal disease patients, although far more people around the world experience complications as a result of the earlier stages of chronic kidney disease (CKD) such as anemia, muscle dysfunction and cardiovascular disease (Hoy et al., 2010; Dungey et al., 2013). Kidney function can now be measured via an endogenous biomarker, cystatin C (CysC), in non-clinical settings using minimally invasive dried blood spot (DBS) samples (Vogl, 2013). There is a need for further research on the relationship between inflammation and kidney function, as well as ecological factors that may affect levels of each, to address global medical research inquiries. The present study evaluates and investigates cystatin C and CRP levels in the context of an indigenous, non-western population, the Shuar of Ecuador.

One of the two biomarkers assayed and analyzed in this study, CRP (or high-sensitivity CRP), is an acute phase reactant and a commonly measured biomarker of both acute and chronic inflammation. Chronic elevated CRP levels are associated with numerous negative health outcomes including CVD, increased risk for stroke and

peripheral vascular disease (Kershaw et al., 2010; Zee et al., 2002). CRP is such a strong indicator of cardiovascular risk that plasma CRP levels can prospectively identify seemingly healthy adults that are at high risk for a cardiac event (Libby et al., 2002; Ridker et al., 1998; Willerson et al., 2004). Elevated levels of CRP have also been associated with intestinal inflammation in inflammatory bowel diseases (Langhorst et al., 2008). CRP is often used as a biomarker of morbidity and mortality risk in studies of population health, facilitated by assays that use samples collected by minimally invasive means onto filter paper cards (McDade et al., 2004; Brindle et al., 2010).

Community-based studies and clinical studies in western populations sample participants from hygienic, low infectious disease environments that are uncharacteristic of many parts of the world. In contrast, non-western populations such as the Shuar live in rural environments and depend on the subsistence economy with relatively high burdens of infectious and parasitic diseases (McDade et al., 2012; Cepon et al., 2014; Gildner et al., 2016). In economically developing countries, it has been proposed that early life exposures to parasites and infectious challenges moves the body towards a T helper 1 (T_H1) immune phenotype (Yazdanbakhsh et al., 2002; Guarner et al., 2006; Rautava et al., 2004). Deemed the Hygiene Hypothesis, this interpretation states in part that the infectious agents trigger an anti-inflammatory network that “trains” the immune system to establish a regulatory network that can be used to efficiently combat future immunological threats and improve overall immune function. Inflammation is regulated efficiently and stays low except during acute challenges when it rises to a high level before dropping back down after the challenge ends. Industrialized western populations would not develop this regulatory network as strongly due to their relatively hygienic

non-infectious environments. This framework would be true within specific countries as well, with sample population location, number and type of pets and numbers of people per household all contributing to the level of exposure to immune-stimulating pathogens (Von Mutius, 2007; Garn et al., 2007).

Chronic kidney disease (CKD) and reduced renal functioning also pose public health problems in both western and non-western populations. CKD is the gradual loss of kidney function characterized by the kidney's decreased efficiency in filtering wastes and excess fluid from the blood (Levey et al., 2012) and is associated with adverse health outcomes such as kidney failure, CVD, and premature death (Levey et al., 2005). Recent studies also suggest that because of their complex functions, the loss of kidney function can aggravate other pathophysiological processes elsewhere in the body and disrupt homeostasis (Eckardt et al., 2013). It is also increasingly evident that the kidney plays an important role in highly prevalent and complex disorders such as type 2 diabetes and hypertension (Eckardt et al., 2013). Better understanding kidney function can help improve health outcomes worldwide if stakeholders use this information in clinical practice and public health interventions.

Previous studies have documented links between reduced renal function and systemic inflammation in western populations. Plasma concentrations of proinflammatory cytokines are increased proportionally with reduced kidney functioning in patients with CKD, which may suggest that the reduced renal clearance of these cytokines contributes to their accumulation and pathophysiolog in the kidney (Guarnieri et al., 2004). In a western population-based sample, higher levels of CRP were independently associated with higher serum CysC levels, which correlate with

decreased kidney filtration efficiency (Knight et al., 2004). Similar results were observed in another sample population from Europe; patients with CKD frequently presented with chronic elevations in levels of biomarkers of inflammation, and even patients that were in the early stages of CKD (predialysis) had elevated levels of inflammation (Dungey et al., 2013). Studies like these demonstrate links between inflammation and reduced renal function.

A good estimation of kidney function is provided by the glomerular filtration rate (GFR), defined as "the volume of plasma that can be completely cleared of a particular substance by the kidneys in a unit of time" (Laterza et al., 2002). The "gold-standard" methods of determining GFR with exogenous substances are inconvenient, invasive and expensive, so many clinicians choose to utilize endogenous serum creatinine to estimate GFR. However, recent publications have suggested that another endogenous marker, Cystatin C (CysC) is a more useful and accurate marker because it does not return to the bloodstream and is not secreted by renal tubules so levels will closely reflect the kidney's true filtration efficiency with lower levels in plasma/serum indicating higher filtration efficiency (Laterza et al., 2002).

Cystatin C is a low molecular weight cysteine protease inhibitor protein and is produced in all nucleated cells (Dharnidharka et al., 2002). CysC is exclusively eliminated from the circulation by glomerular filtration and is therefore strongly inversely correlated with glomerular filtration rate (GFR). Similar to serum creatinine, high serum values of CysC indicate low GFR and poorer kidney function, while lower values indicate higher GFRs and more efficient kidney function (Dharnidharka et al., 2002). Due to interference of elements from whole blood, attempts to use serum

creatinine to estimate GFR from dried blood spot (DBS) samples have been unsuccessful to date (Vogl, 2013). There is also evidence that GFR is more closely correlated to serum levels of CysC than to serum levels of creatinine (Stevens et al., 2008; Dharnidharka et al., 2002). Furthermore, CysC levels are not significantly affected by muscle mass, diet, or sex as are creatinine levels and CysC remains largely stable after the first 18 months of life as opposed to creatinine levels, which fluctuate throughout childhood and are more labile in response to acute changes in infection status (Andersen et al., 2009). Serum CysC levels, however, can be influenced by some external factors, such as smoking (Knight et al., 2004).

A CysC DBS enzyme-linked immunosorbent (ELISA) assay offers a convenient method for measuring CysC in large-scale population studies and a protocol has recently been published based on research in the department of Laboratory Medicine at the University of Washington (Vogl, 2013). The use of biomarkers in human health research circumvents the potential pitfalls of using self-reported measures for assessing health because they are objective and do not rely on the participant's ability or willingness to recall sensitive health information (Mei et al., 2001; McDade et al., 2007). A biomarker refers to a substance from the body that can be objectively measured and is evaluated as an indicator of either normal biological processes or some biological phenomenon such as response to pathogens (Strimbu et al., 2010). Levels of certain biomarkers like CRP also offer insight into immune/inflammatory responses that can be predictive of future diseases such as CVD (McDade et al., 2007, 2012).

Although the traditional clinical standard for many biomarkers of physiology and health is plasma or serum from venipuncture blood, the collection of such blood in

the field settings is challenging. Samples must be kept cold and then centrifuged and frozen within a prescribed amount of time, which is rarely feasible in a field-based setting. Venipuncture blood is also complicated to store and transport, making it a difficult sampling method for large population-based studies. Fortunately, recent methodological advances have expanded the options for community-based data collection and subsequent health research (McDade et al., 2007). In particular, the use of DBS samples has dramatically improved the ease of obtaining biological field samples in nonclinical settings. Even though most biomarker assays were developed for use with serum or plasma from venipuncture blood, most (although not all) analytes measured in serum/plasma can also be reliably measured from discs of DBS samples that have been reconstituted in an elution buffer (McDade et al., 2007).

Using DBS for biomarker analysis instead of venipuncture blood provides an attractive host of benefits to both the participant and the researcher. DBS sampling involves the collection of whole blood spots from a finger prick on filter paper that can then be packed with desiccant and frozen for long-term storage (McDade et al., 2007). Finger pricks are minimally invasive and a single finger prick can provide the necessary capillary whole blood needed for adequate collection. DBS samples also don't need to be immediately centrifuged or frozen after collection, which gives researchers flexibility in storage and transport.

The present study is the first to use a CysC DBS ELISA for population-level research. The objective of this study was to examine the biological relationship among CysC, which is rarely analyzed in non-western medical research, and CRP, a common biomarker of inflammation, in the indigenous Shuar of Ecuador. Influences of

participant age and sex on CysC and CRP concentrations were analyzed as well as the influences of two ecological variables, latrine type and water source, on CysC.

HYPOTHESES

Hypothesis 1: Levels of inflammation, and therefore levels of CRP, will be higher in Shuar participants with higher CysC concentrations (poorer kidney function).

Higher levels of inflammatory biomarkers like CRP appear to accompany reduced renal function in individuals with chronic kidney disease (CKD) in western models (Garg et al., 2001; Panichi et al., 1999; Dungey et al., 2013; Knight et al., 2014), although the specific relationship between the level of inflammation determined by CRP concentration and estimated GFR has not been found to correlate as may be expected in these populations (Oberg et al., 2004; Ortega et al., 2002). However, it is possible that the western population model does not accurately reflect biological patterns in non-western populations such as the Shuar. Therefore, a positive correlation between CysC and CRP levels is likely for the present population.

Hypothesis 2: Lower household style of life (H-SOL) indices for latrine type and water source (Table I) will correspond to lower inflammation and more robust immune function (lower CRP concentrations) and better kidney health (lower CysC concentrations) in the sampled Shuar participants.

The Hygiene Hypothesis was used as a framework to derive the present hypotheses about how two variables of household infrastructure, latrine type and primary water source, would affect CysC concentration. According to the Hygiene

Hypothesis, less market-integrated styles of life such as using a pit latrine instead of an indoor toilet, and drinking stream water rather than tap water, would facilitate more contact with immune stimulating pathogens and eventually down-regulate inflammation and alter immune function (Yazdanbakhsh et al., 2002). Based on the association between reduced inflammation and better kidney health reported in western populations (Knight et al., 2014; Dungey et al., 2013) it was hypothesized that in the Shuar, lower household style of life (H-SOL) indices for latrine type and water source (Table I) would correspond to more robust immune function (lower CRP concentrations) and therefore better kidney health (lower CysC concentrations).

MATERIALS AND METHODS

Study Population:

The Ecuadorian Shuar are an indigenous population of about 50,000-110,000 that reside in lowland southeastern Ecuador. Shuar live in communities throughout the Upano River Valley (UV) and in the remote region east of the Cutucú Mountains (CC) (Gildner et al., 2016). The Shuar are traditionally forager-horticulturalists and continue to rely on subsistence horticulture hunting and fishing to supply daily dietary needs (McDade et al., 2012). However, UV Shuar are rapidly experiencing economic development and increased production for and consumption from a market-based economy – a process termed market integration (MI) (Cepon-Robins et al., 2014).

The Shuar Health and Life History Proejct (SHLHP; www.bonesandbehavior.org/shuar) is a collaborative research effort that focuses on the indigenous Shuar of Ecuador and involves scientists from several US universities, Ecuadorian health

providers and Shuar colleagues. One of the goals of the SHLHP is to investigate the relationship between biomarkers of immune function and inflammation, such as C-reactive protein, and overall health. The results of these investigations have important implications for expanding knowledge about the health of isolated non-western populations as well as furthering understanding of the regulation of inflammation in the human body. Researchers from the SHLHP facilitate the delivery of assayed health information to participants, the Shuar Federation, and local medical providers to assist in targeting prevention and treatment efforts. Overall research results and interpretations from field sessions are also presented to Ministry of Health colleagues, the Shuar Federation Health directorate, and participant community meetings to contribute to and inform public health policy.

Study Participants:

Researchers collected the field data for this study in the Morona-Santiago region of Ecuador as a part of the Shuar Health and Life History Project over seven field sessions conducted between 2010 and 2014. The present sample included *n*=128 participants (75 females, 53 males) randomly selected from a larger Shuar dataset. The selected participants were scattered across five different communities, two located in the more market-integrated Upano River Valley and three in the remote cross-Cutucú region (Figure I). Participants ranged in age from 12-86 years. To examine differences in CysC and CRP levels across the lifespan, individuals were divided into age sub-groups that split the study population relatively equally: adolescents (12-17 years old, *n*=45), young adults (18-35 years old, *n*=35), and adults (36+ years old, *n*=48).

Ages were determined by birthdates on government-issued identification cards and extensively cross-checked by informants and existing SHLHP genealogical data. (Blackwell et al., 2010; Liebert et al., 2013).

Informed verbal consent was obtained from adult participants; for participants under the age of 15, the local age of consent, both parental verbal consent and child assent were obtained. The study and consent procedures were approved by the Institutional Review Board of the University of Oregon, and the Federación Interprovincial de Centros Shaur (FICSH) and local leaders authorized research in participating communities.

Biomarkers:

Finger prick capillary whole blood samples were collected on filter paper for the analysis of CysC and CRP. Blood samples were collected following standard procedures for dried blood spots (McDade et al., 2007). Briefly, a finger prick using a sterile, disposable lancet was used to obtain three to five 50 μl drops of whole capillary blood spotted onto standardized filter paper. Blood spot samples were air-dried for 4 hours protected by a small mesh cage and then sealed in airtight bags with desiccant and frozen in the village clinic freezer at -20℃ for 1–3 weeks (the duration of field collection). They were allowed to come to room temperature for transport by plane to the University of Oregon (~12 hours), after which they were stored at -30℃ until analysis. CRP and CysC in DBS samples are generally very stable at ambient temperatures and can be shipped via mail with minimal shipping label requirements (McDade et al., 2004; Vogl, 2013).

DBS samples were analyzed for levels of CRP using a modified ELISA protocol previously developed for use with DBS (McDade et al., 2004) in the Snodgrass Human Biology Laboratory at the University of Oregon. 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). The modified assay protocol from McDade et al (2004) has been used in other SHLHP studies and has been validated against both the published protocol and against blood plasma samples (Blackwell et al., 2010; McDade et al., 2012). Color change of samples and the same eight standards were read at a wavelength of 450 nm on a spectrophotometer and CRP concentrations were calculated and recorded in mg/L.

The same DBS samples as used for CRP were also analyzed for levels of CysC using an ELISA protocol that was recently modified for use with DBS and validated (Vogl, 2013). Because the protocol was published as a thesis and not in the peer-reviewed and indexed literature, it has not been used by many other labs. To the author's knowledge the present study is the first to apply the CysC DBS protocol to population-based research. Color change was read at a wavelength of 450 nm on a spectrophotometer and CysC concentrations were calculated and recorded in mg/L.

Sociodemographic and Lifestyle Data:

To assess the relative level of MI, questionnaires based on a modified version of the Material Style of Life (SOL) Index (Bindon et al., 1997; Leonard et al., 2002) were administered to all participants. One of the scales resulting from this questionnaire is referred to as Household Style of Life (H-SOL), which assesses household

infrastructure. This H-SOL scale was based on the Shuar-specific SOL measurements from Liebert et al. (2013) and provides information on factors including household permanence, access to modern infrastructure such as water and electricity and pathogen risk. High H-SOL scores correspond to a greater level of participation in a market-integrated lifestyle (Liebert, 2016). The present study incorporates two of these indices into analyses, type of latrine and water source, because of their links to pathogen exposure and overall immune function (Cepon-Robins et al., 2014). Levels of the two variables are presented in Table I taken from Liebert (2016).

Statistical Analysis:

All analyses were conducted using SPSS version 21.0. As with many biomarkers, CysC and CRP concentrations had skewed distributions, which were normalized with natural log-transformations so standard parametric statistics could be used. The log-transformed values are referred to as lnCysC and lnCRP and all model analyses were done on log-transformed values. Univariate Analysis of Variance (ANOVA) tests were used to understand the interactions between age, sex and lnCRP as well as between age, sex and lnCysC. Correlations and partial correlations examined the effect of CRP, age and age category on lnCysC concentrations. A series of regression analyses were used to test the relationships between levels of CysC and different variables (CRP, age, sex, participant water source, etc.). Age and sex were controlled for in the regression models. To control for multicolinearity, certain variables such as age, CRP concentrations and CysC concentrations were centered on the mean value

("ln_CRPcenter", etc.) Estimated marginal means were utilized in the analyses to adjust for covariance.

RESULTS

Descriptive Statistics:

Table II presents selected descriptive statistics including both raw and log-transformed (lnCysC and lnCRP) concentrations of CysC and CRP for all participants subdivided by age category and sex. All reported CysC and CRP concentrations are raw DBS values, and are not serum or plasma-equivalent concentrations. The sample comprised 58.6% women (*n*=75) and 41.6% men (*n*=53) and the average age of both sexes was roughly 30 years old. Median CysC concentration across the entire sample was 0.37 mg/L while median CRP concentration across the entire sample was 0.38 mg/L.

Table III presents the frequency of the latrine type variable for all participants, derived from the Household Style of Life (H-SOL) scale assessing household infrastructure. Almost half (46.1%) of all participants recorded not having any type of formal latrine (*n*=59). Roughly 33% of participants recorded a pit toilet as their main latrine (*n*=42) and 21% recorded using either an indoor or outdoor toilet as their primary latrine (*n*=27).

Table IV presents the frequency of the water source variable for all participants, derived from the same H-SOL scale assessing household infrastructure. About 20% of all surveyed participants' primary water source was a river or stream (*n*=26) and about 80% of participants regularly used a well or outdoor pipe for obtaining water (*n*=102).

No participants in the present study reported the third water source variable, indoor pipe, as their primary water source.

2x2 ANOVAs:

2x2 ANOVAs were used to understand the interactions between age, sex and lnCRP as well as between age, sex and lnCysC. For lnCRP values, sex alone did not have a significant main effect (p=0.069) but there was a significant difference in CRP levels across age categories (p<0.001). The interaction between age categories and sex did not have a significant effect on CRP levels. Post hoc analyses using a Bonferroni correction indicated that adolescents (12-17 years old) had significantly lower CRP levels than young adults (18-35 years old) (p=0.022) and older adults (36+ years old) (p<0.001).

Similar results were found when lnCysC was used as the outcome variable instead of lnCRP. Sex did not have a significant main effect (p=0.381) but there was a significant difference in CysC levels across age categories (p=0.040). As with CRP values, there was no significant effect for the interaction term between age category and sex. Post hoc analyses using a Bonferroni correction indicated that young adults had significantly lower CysC levels than adults (p=0.035). Graph I shows this association broken down by age category and sex.

Correlations:

Table V presents the pooled correlation results between CysC levels and CRP levels with all age categories combined (not controlling for age). Levels CysC and CRP

were significantly and positively correlated, r(128) = .23, p=0.009, which supports the initial hypothesis that CysC and CRP would be positively correlated. However, further investigation showed that the correlation was attenuated when controlling for age (p=0.078)(Table VI). Subsequent correlations controlling for age categories revealed that CysC and CRP levels were not significantly correlated for either in the adolescent age category (Table VII, p=0.183) or the young adult category (Table VIII, p=0.948). However, CysC and CRP were significantly and positively correlated among older adult Shuar participants, r(48) = 0.45, p=0.001 (Table IX). This suggests that, when age was not controlled for, adult participants drove the significant positive correlation between CysC and CRP.

Regressions:

A series of stepwise regression models that used lnCysC as the dependent variable served to further examine the preliminary results. The first regression model tested the effect of CRP on CysC when controlling for sex and age and found that CRP does have a significant main effect on CysC levels (p=0.039). The second regression model added an interaction term variable between centered age and centered CRP values, which gave a significant F change of p=0.013 (from p=0.039 without the interaction variable) (Table X). This significant association underscored that one cannot discuss the effect of CRP on CysC without also discussing age because age significantly moderates the relationship between the two biomarkers. A graphical representation of this regression model with trendlines added by age category is shown in Graph II. Because the analyses interpreted a regression model with log-transformed predictors

and outcomes, a coefficient value of 0.045 for the centered lnCRP variable was calculated. Using this information, it could be said that for any 10% increase in the centered lnCRP concentration, the expected ratio of the two geometric means for lnCysC would be 1.10^(0.045)=1.0043. Stated another way, any 10% increase in centered lnCRP values, would result in a ~0.43% increase in lnCysC while sex and age are held constant.

Next the selected household style of life indices (latrine type and water source) and their effects on CysC levels were examined. Latrine type was not significantly associated with CysC levels (None vs. Pit Toilet, p=0.425), (None vs. Indoor/Outdoor Toilet, p=0.118). Water source had a significant effect on CysC levels across age groups independent of the effects of age and sex (p=0.002). The interaction term variable between age centered and water source was not significant (p=0.321). In other words, even once controlling for age and sex, water source was a significant predictor of CysC because age did not moderate that relationship. Higher CysC levels were associated with the well/outdoor pipe water source as opposed to the river/stream water source, which supported the Hygiene Hypothesis-based theory that higher H-SOL indices would correlate with higher levels of CysC.

DISCUSSION

In this study, we examined the relationships among kidney function, inflammation, and two ecological variables, latrine and water source in a Shuar sample. Age mediated the significant positive correlation between CysC and CRP although the positive correlation itself was consistent with our hypothesis about their correlation due

to the inverse relationship between kidney function and inflammation. Higher CysC levels were associated with the well/outdoor pipe water source as opposed to the river/stream water source, which supported the Hygiene Hypothesis-based theory that more "sophisticated" H-SOL indices would correlate with higher levels of CysC.

The Age Effect on CysC Levels:

The analysis showed an interesting pattern of CysC concentrations across different age groups. Further investigation of the age effect showed that there is a significant quadratic trend for the effect of age on lnCysC concentration (p=0.010). Table XI shows the regression model summary incorporating centered age-squared values from the participants to support the quadratic trend. Graph III also shows this trend with a quadratic trendline. The significant quadratic trend may suggest that the same phenomenon was related to the elevated CysC concentrations in both the adolescent and adult groups. A more likely explanation, however, is that different factors like the consequences of an immature immune system and the age-related decline in kidney function, were responsible for the elevation of CysC concentrations within the two different age groups.

Elevated CysC levels in the oldest age category (36+ years) may have a fairly straightforward explanation - kidney function is widely known to decline with age in western populations (Rodwell et al., 2004). Research has suggested that as age increases there's a cumulative effect of exposure to salt, low exercise etc. resulting in a noticeable decline in kidney function and many cellular processes (Lin et al., 2010; Sanders, 2004). The weakening of multiple biological and immunological pathways cumulatively

causes a significant decrease in cell function (Rodwell et al., 2004). According to a recent study that evaluated serum CysC levels in western participants from the United States and the Netherlands over the age of forty, a non-linear pattern of age-correlated decline was even evident even in individuals without clinical risk factors for kidney disease (Odden et al., 2010). Although the majority of studies evaluating measures of kidney function have been conducted in western populations (due in part to the feasibility of venipuncture blood sampling), the strong non-linear association of age with CysC levels observed by Odden and colleagues appears to hold true in the Shuar as well.

The reasons for elevated levels of CysC in the adolescent participants measured in this study are not as intuitive. Although kidney health and incidences of kidney disease in adolescent populations are not well documented, the higher levels of CysC in Shuar adolescents relative to Shuar young adults do not seem to be similar to adolescents from western populations (Fadrowski et al., 2010). Using the Hygiene Hypothesis as a biological framework, it was hypothesized that growing up in an infectious environment with exposure to many bacterial, viral and parasitic infections stimulates the development of a robust adaptive immune system and encourages immunological memory that would fully mature by young adulthood. Young adults (18-35 years old) would suffer fewer infections with less inflammation and have overall better immune health than adolescents (12-17 years old) with developing immune systems or than older adults (36+) experiencing natural age-related decline in cellular function and/or a “fading” of protective immunological memory (Simon et al., 2015). Shuar adolescents with maturing immune systems would theoretically be exposed to

more inflammation-inducing pathogens that would elevate CRP levels and in turn elevate CysC levels relative to Shuar young adults.

Although high levels of CysC do not necessarily mean that an individual has CKD, high levels seem to be indicative of early renal impairment and CKD (Omar et al., 2015). A recent study examining early stages of chronic kidney disease among indigenous and non-indigenous populations in Australia found that rates of kidney disease were consistently higher in indigenous Australian populations than in non-indigenous Australian populations (Stumpters et al., 2013). This seems to be true of many indigenous populations as compared to their western counterparts (Zhang et al., 2008; Valeggia et al., 2015). The study also found that the factors contributing to kidney disease among indigenous people were generally attributed to combinations of risk factors such as highly infectious environments, low birth weight, infant malnutrition etc. These risk factors also seem plausible for the Shuar and could be contributing to or exacerbating the elevation of levels of CysC in Shuar adolescents and older adults relative to younger adults.

The Effect of Water Source on CysC Levels:

The well/outdoor pipe water source instead of the river/stream source was significantly associated with higher levels of CysC across all age categories, which supported the second hypothesis and is logical considering that the underlying cause of this association is ecological and not affected by biological consequences of aging. The fact that age was not moderating the relationship between water source and CysC levels

was especially interesting since age moderated all of the other biological relationships tested in this study.

Bacteria such as *Escherichia coli* in stagnant well water may be a factor in the elevated levels of CysC and could contribute to poorer kidney function among the Shuar by causing diseases such as pyelonephritis. Pyelonephritis is a type of urinary tract infection (UTI) that affects one or both kidneys and is commonly caused by *E. coli* that could reside in stagnant well water (Roberts, 1991). The presence of *E. coli* in well water is a common problem. In Ecuador specifically, a non-profit organization that surveyed the water in 23 wells in the village of Muisne found that every well was contaminated with *E. coli* or other coliform bacteria (Horizon International Solutions, 2011). Contamination of well water in Shuar communities could contribute to a higher prevalence of UTIs and a higher incidence of diseases like pyelonephritis, which in turn would elevate levels of CysC. *E. coli* is the most frequent cause of pyelonephritis and infection with the bacterium is associated with an acute inflammatory response that often results in damage to the renal tubules (Roberts, 1991). Experiments in mice have also confirmed that the Sat cytotoxin secreted by *E. coli* damages kidneys via dissolution of the glomerular membrane (Guyer et al., 2002). Guyer and colleagues found that Sat damaged the kidney epithelium during upper urinary tract infections, thus contributing to the pathogenesis of UTIs.

Correlations with Serum CysC and CRP Concentrations:

To examine differences in CysC and CRP concentrations between the Shuar and western populations, CysC and CRP mean concentrations as measured from DBS were

converted to serum equivalent values using conversion equations derived from comparisons of matched DBS and serum samples (Vogl, 2013; Geeta Eick, personal communication). For the Shuar participants sampled, serum equivalent CysC values ranged from undetectable to 0.46 mg/L, compared to the range of 0.53-1.02 mg/L reported for western adults (Finney et al., 2000). CRP serum equivalent values ranged from 0.03-3.72 mg/L with an average concentration across age categories of 0.5 mg/L. The average concentration of serum CRP in healthy western adults falls between 0.98 and 1.4 mg/L (Macy et al., 1997; Erlandsen et al., 2000). The relatively low CRP concentrations observed in the Shuar compared to western populations are consistent with other literature from the SHLHP (McDade et al., 2012). The relatively low levels of CysC and CRP are important to note when extrapolating the findings of this paper to other populations (Liebert et al., 2013).

Limitations:

This study has some important limitations. First, the sample size was relatively small. Although efforts were made to maintain relatively equal distributions of participants across categories, some frequencies such as the number of participants between the two water sources, were uneven. Second, DBS collection was limited to volunteers, which may suggest a bias towards Shuar participants who were especially interested in their health, thereby limiting the generalizations of the findings. Third, the interpretation of these results is largely speculative because it is difficult to gain a comprehensive view of an individual's health from two biomarkers alone. Finally, CRP is a labile biomarker of inflammation and, by measuring its level in only one DPS

sample per person, our data may not provide the best framework for evaluating chronic inflammation in the Shuar participants (McDade et al., 2012).

Conclusions:

Elevated levels of CysC in Shuar adolescents could point to a highly infectious disease environment and early exposure to pathogens before full immune system maturation that result in increased inflammation and decreased kidney function relative to young adult Shuar. However, it is important to note that serum equivalent values of CysC across all Shuar participants were below the interval range observed in many western populations (Finney et al., 2000) suggesting that overall, the Shuar have good kidney health with higher filtration efficiency than is typically observed in western adults.

This study is the first to utilize a Cystatin C DBS ELISA assay for population-based anthropological research. DBS ELISAs such as the one used here expand the ease with which researchers can analyze and assess kidney function in non-western populations, which has large implications for public health interventions and initiatives in these communities. One of the goals of the SHLHP is to assist in targeting prevention and treatment efforts for the Shuar, in part by presenting findings to the Ministry of Health to contribute to public health policy. The majority of the world's population is considered non-western so it is critical to test biological and ecological relationships characterized in western populations and make sure they hold true in non-western populations like the Shuar. Results can offer insight into the health of the Shuar participants sampled as well as patterns in indigenous health around the world.

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List of Tables, Figures and Graphs:

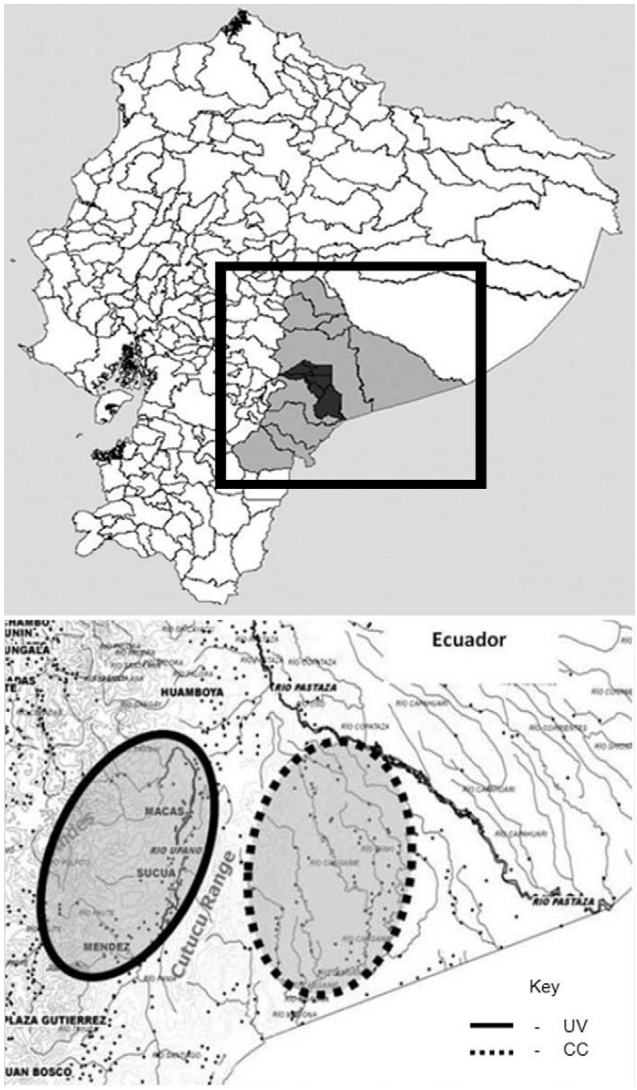


Figure I. Map of Ecuador highlighting Shuar territory in the Morona-Santiago region (top). Participants in this study came from five villages; two located in the more market-integrated Upano River Valley (UV) and three in the remote cross-Cutucú (CC) region (bottom). (Cepon-Robins et al. 2014).

SOL Scales	Items & Scoring (if applicable)
Household-SOL (n = 7)	Walls (*0 = palmwood; 1 = mixed; 2 = milled lumber; 3 = cinder block*) Floors (*0 = dirt; 1 = palmwood; 2 = milled lumber; 3 = concrete; 4 = tile*) Latrine (*0 = none; 1 = pit; 2 = indoor toilet without water; 3 = outdoor toilet with water; 4 = indoor toilet with water*) Water Source (*0 = river/stream; 1 = well/outdoor pipe; 2 = indoor pipe*) Electricity (*0 = none; 1 = lights only; 2 = outlets*) Rooms (*total number*) Houses (*total number*)

Table I: Material goods and household items included in the Shuar style of life (SOL) scales. The present analyses focus on two indices, latrine type and water source. (Liebert, 2016).

Descriptive Statistics	**Full Population (n=128)**	**Age 12-17 (n=45)**	**Age 18-35 (n=35)**	**Age 36+ (n=48)**	**Male (n=53)**	**Female (n=75)**
Average Age (Years)	30.04	13.51	25.74	48.67	29.64	30.32
CysC Concentration (mg/L)	0.37	0.37	0.37	0.4	0.38	0.37
lnCysC Concentration	-1.02	-1.01	-1.12*	-0.95*	-0.99	-1.04
CRP Concentration (mg/L)	0.38	0.19	0.49	0.48	0.27	0.46
lnCRP Concentration	-1.6	-2.17∓	-1.42∓	-1.2∓	-1.85	-1.42
Sex Ratio M:F	53:75	22:23	10:25	21:27	N/A	N/A

Table II: Descriptive statistics for full population (n=128) as well as broken down by age category and sex. Natural log transformed values are presented as lnCysC and lnCRP.

*lnCysC concentrations vary significantly between the 18-35 age group and the 36+ age group (p=0.035).

∓ lnCRP concentrations vary significantly between the 12-17 age group and the 18-35 age group (p=0.022) and between the 12-17 age group and 36+ age group (p<0.001).

BanoCategory/Latrine Type

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	None	59	46.1	46.1	46.1
	Pit Toilet	42	32.8	32.8	78.9
	Indoor or Outdoor Toilet	27	21.1	21.1	100.0
	Total	128	100.0	100.0	

Table III: The latrine type variable, from the H-SOL scale, helps assess participants' relative level of market integration and household infrastructure. Frequency of participants with each type of latrine is shown (None, Pit Toilet or Indoor/Outdoor Toilet).

AguaCode/Water Source

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	River/Stream	26	20.3	20.3	20.3
	Well or Outdoor Pipe	102	79.7	79.7	100.0
	Total	128	100.0	100.0	

Table IV: The water source variable, from the H-SOL scale, helps assess participants' relative level of market integration and household infrastructure. Frequency of participants with each primary water source is shown (River/Stream or Well/Outdoor Pipe).

Correlations

		ln_CRP	ln_CysC	Age
ln_CRP	Pearson Correlation	1	.231**	.370**
	Sig. (2-tailed)		.009	.000
	N	128	128	128
ln_CysC	Pearson Correlation	.231**	1	.241**
	Sig. (2-tailed)	.009		.006
	N	128	128	128
Age	Pearson Correlation	.370**	.241**	1
	Sig. (2-tailed)	.000	.006	
	N	128	128	128

Table V: Bivariate correlation table between lnCRP and lnCysC values, pooled for all participants, not controlling for age.
**Correlation is significant at the 0.01 level (2-tailed).

Correlations

Control Variables			ln_CRP	ln_CysC
Age	ln_CRP	Correlation	1.000	.157
		Significance (2-tailed)	.	.078
		df	0	125
	ln_CysC	Correlation	.157	1.000
		Significance (2-tailed)	.078	.
		df	125	0

Table VI: Partial correlation showing the attenuated relationship between levels of CysC and CRP when controlling for age (p=0.078).

Correlations[a]

		ln_CRP	ln_CysC
ln_CRP	Pearson Correlation	1	.202
	Sig. (2-tailed)		.183
	N	45	45
ln_CysC	Pearson Correlation	.202	1
	Sig. (2-tailed)	.183	
	N	45	45

a. AgeCategory = 12-17

Table VII: Bivariate correlation table for the adolescent age category (12-17) between lnCRP and lnCysC values. Correlation is not significant (p=0.183)

Correlations[a]

		ln_CRP	ln_CysC
ln_CRP	Pearson Correlation	1	.011
	Sig. (2-tailed)		.948
	N	35	35
ln_CysC	Pearson Correlation	.011	1
	Sig. (2-tailed)	.948	
	N	35	35

a. AgeCategory = 18-35

Table VIII: Bivariate correlation table for the young adult age category (18-35) between lnCRP and lnCysC values. Correlation is not significant (p=0.948).

Correlations[a]

		ln_CRP	ln_CysC
ln_CRP	Pearson Correlation	1	.447**
	Sig. (2-tailed)		.001
	N	48	48
ln_CysC	Pearson Correlation	.447**	1
	Sig. (2-tailed)	.001	
	N	48	48

a. AgeCategory = 36+

Table IX: Bivariate correlation table for the adult age category (36+) between lnCRP and lnCysC values. **Correlation is significant at the 0.01 level (2-tailed), (p=0.001).

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.265[a]	.070	.055	.251576	.070	4.706	2	125	.011
2	.318[b]	.101	.080	.248290	.031	4.330	1	124	.039
3	.382[c]	.146	.118	.243066	.044	6.387	1	123	.013

a. Predictors: (Constant), Sex, AgeCenter

b. Predictors: (Constant), Sex, AgeCenter, ln_CRPCenter

c. Predictors: (Constant), Sex, AgeCenter, ln_CRPCenter, lnCRPCenter_AgeCenter_Interact

Table X: Model summary for the second regression model tested shows that the addition of the age centered by CRP centered interaction variable gave a significant F change of p=0.013.

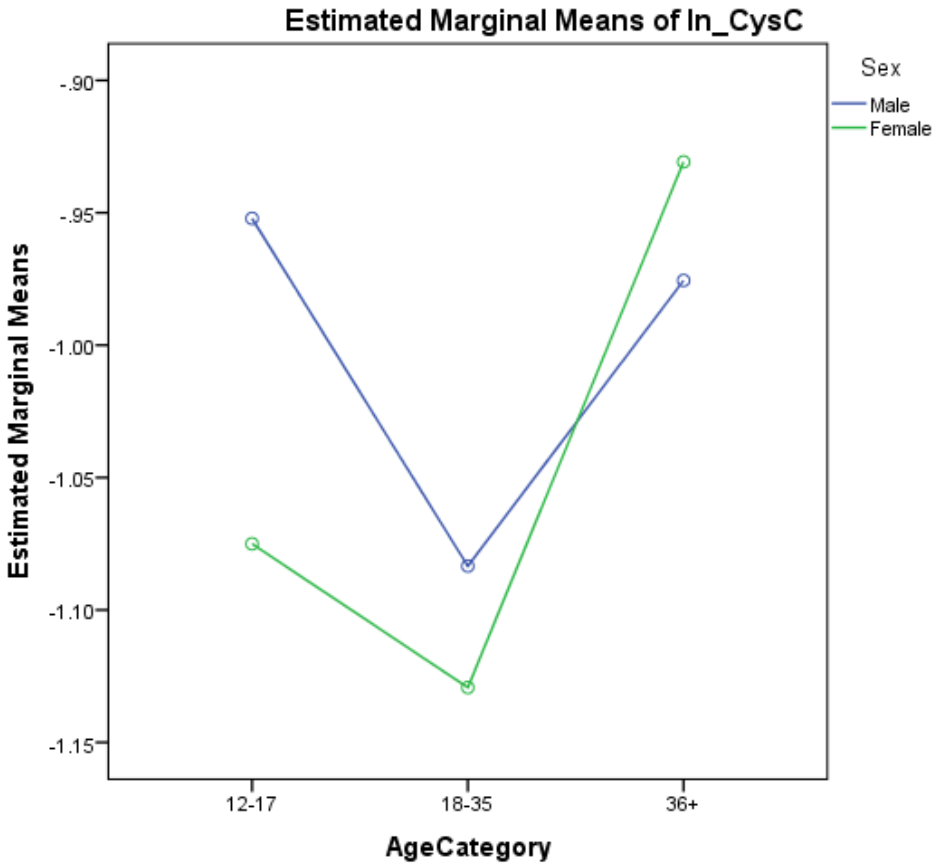
Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.265[a]	.070	.055	.251576	.070	4.706	2	125	.011
2	.345[b]	.119	.098	.245820	.049	6.922	1	124	.010

a. Predictors: (Constant), Sex, AgeCenter

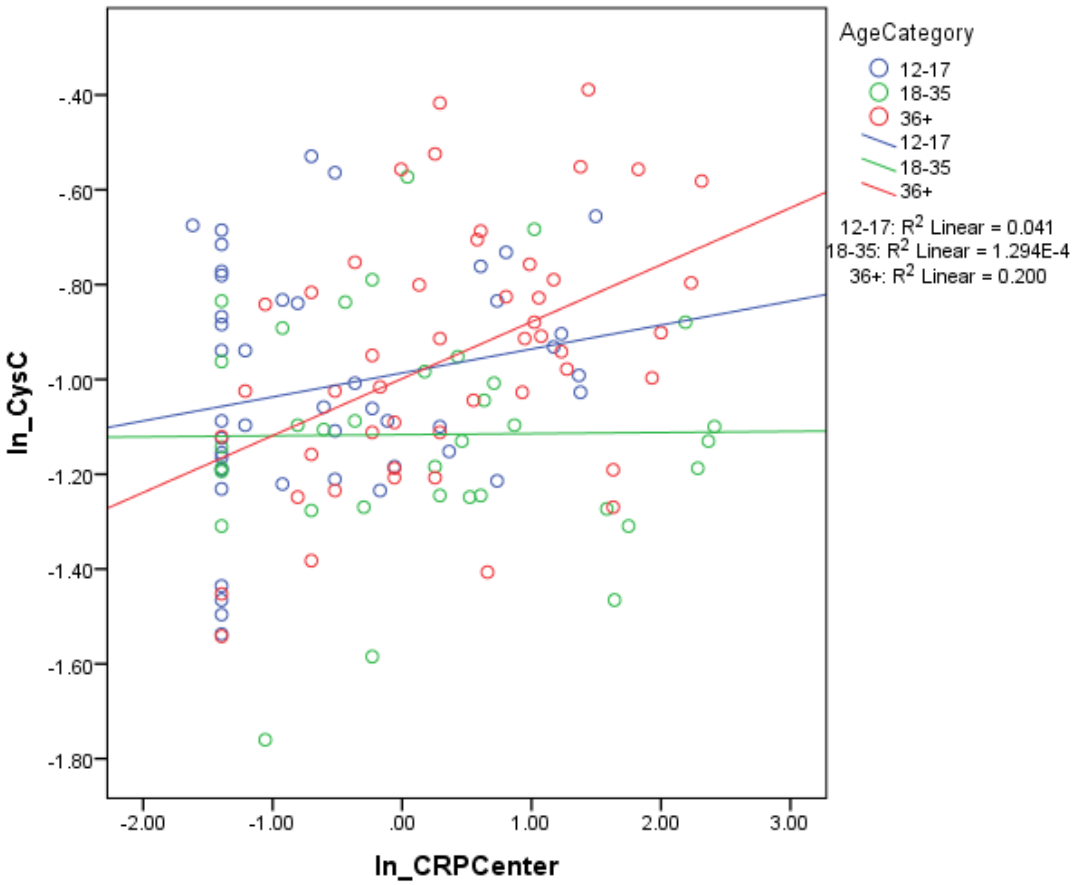
b. Predictors: (Constant), Sex, AgeCenter, AgeCenterSquared

Table XI: Regression model summary showing that adding a centered age squared variable produces a significant F change (p=0.010) and indicates a significant quadratic trend for the effect of age on lnCysC concentration.

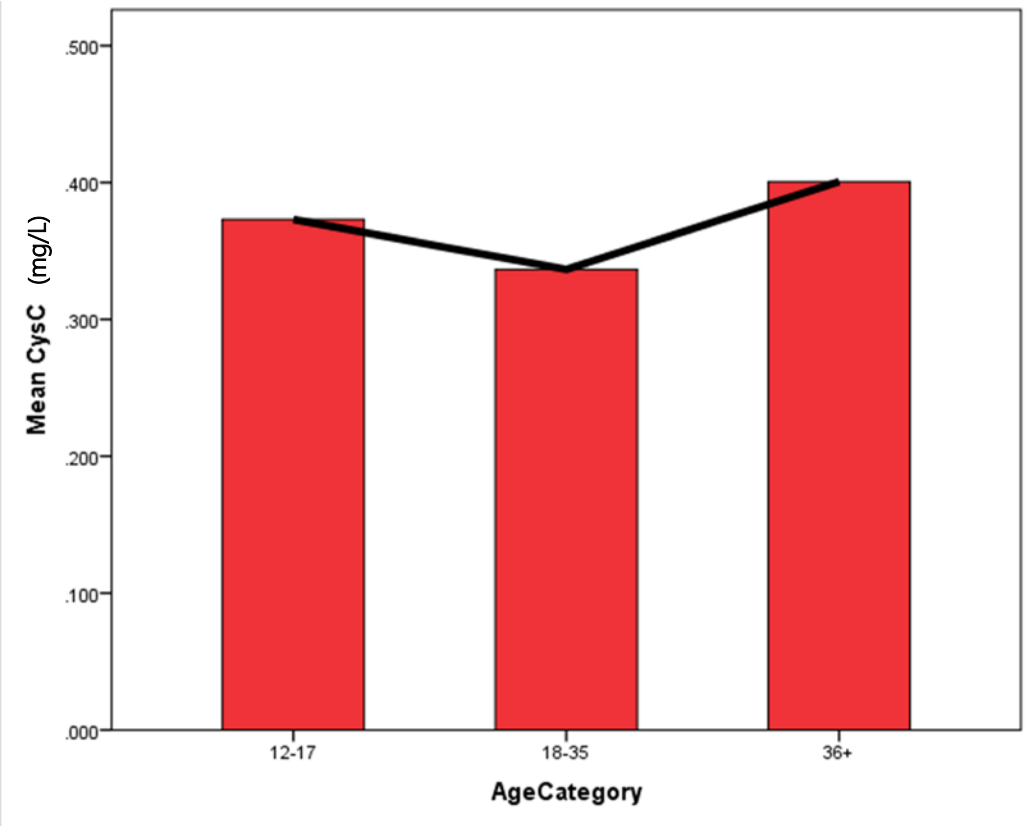


Graph I: Estimated marginal means of lnCysC concentration for three age categories grouped by sex (Males 12-17, n=22), (Females 12-17, n=23), (Males 18-35, n=10),

(Females 18-35, n=25), (Males 36+, *n*=21) and (Females 36+, *n*=28). Values in this graph are presented as natural log transformed data.



Graph II: Regression lines (shown by age category) demonstrate that age moderates the significant positive relationship between levels of CRP and CysC. Relationship is driven by the adults (36+, *n*=48) with an R value of 0.447 (*p*=0.001). CRP values are presented centered on the mean to control for multicolinearity and both CRP and CysC values are natural log transformed.



Graph III: Average CysC concentrations (mg/L) across all three age categories (12-17, *n*=45), (18-35, *n*=35), (36+, *n*=48). Age squared values from the participants were evaluated and a significant quadratic trend (*p*=0.010) was evident. Trendline shows the significant quadratic trend for the effect of age on mean CysC concentration.

Bibliography:

Andersen, T.B., Eskild-Jensen, A., Frøkiaer, J. and Brøchner-Mortensen, J., 2009. Measuring glomerular filtration rate in children; can cystatin C replace established methods? A review. Pediatric nephrology (Berlin, Germany), 24(5), p.929.

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: 836-848.

Brindle, E., Fujita, M., Shofer, J., & O'Connor, K. A. 2010. Serum, plasma, and dried blood spot high sensitivity C-reactive protein enzyme immunoassay for population research. Journal of Immunological Methods, 362(1-2), 112–120.

Cepon TJ, Liebert MA, Gildner TE, Urlacher SS, Colehour AM, Snodgrass JJ, Madimenos FC, and Sugiyama LS. 2014. Soil-transmitted helminth prevalence and infection intensity among geographically and economically distinct Shuar communities in the Ecuadorian Amazon. J Parasitology 100: 598-607.

Dharnidharka, V.R., Kwon, C. and Stevens, G., 2002. Serum cystatin C is superior to serum creatinine as a marker of kidney function: a meta-analysis. American Journal of Kidney Diseases, 40(2), pp.221-226.

Dungey, M., Hull, K.L., Smith, A.C., Burton, J.O. and Bishop, N.C., 2013. Inflammatory factors and exercise in chronic kidney disease. Intl J Endocrinology, 2013.

Eckardt, K.U., Coresh, J., Devuyst, O., Johnson, R.J., Köttgen, A., Levey, A.S. and Levin, A., 2013. Evolving importance of kidney disease: from subspecialty to global health burden. Lancet (London, England), 382(9887), p.158.

Erlandsen, E.J. and Randers, E., 2000. Reference interval for serum C-reactive protein in healthy blood donors using the Dade Behring N Latex CRP mono assay. Scandinavian journal of clinical and laboratory investigation, 60(1), p.37.

Fadrowski, J.J., Navas-Acien, A., Tellez-Plaza, M., Guallar, E., Weaver, V.M. and Furth, S.L., 2010. Blood Lead Level and Kidney Function in US Adolescents. Arch Intern Med, 170(1), pp.75-82.

Finney, H., Newman, D.J. and Price, C.P., 2000. Adult reference ranges for serum cystatin C, creatinine and predicted creatinine clearance. Annals of clinical biochemistry, 37(1).

Garn, H. and Renz, H., 2007. Epidemiological and immunological evidence for the hygiene hypothesis. Immunobiology, 212, pp.441-452.

Garg, A.X., Blake, P.G., Clark, W.F., Clase, C.M., Haynes, R.B. and Moist, L.M., 2001. Association between renal insufficiency and malnutrition in older adults: results from the NHANES III. Kidney international, 60(5), pp.1867-1874.

Gildner, T.E., Cepon-Robins, T.J., Liebert, M.A., Urlacher, S.S., Madimenos, F.C., Snodgrass, J.J. and Sugiyama, L.S., 2016. Regional variation in Ascaris lumbricoides and Trichuris trichiura infections by age cohort and sex: effects of market integration among the indigenous Shuar of Amazonian Ecuador. Journal of Physiological Anthropology, 35(1), p.28.

Guarner, F., Bourdet-Sicard, R., Brandtzaeg, P., Gill, H.S., McGuirk, P., Van Eden, W., Versalovic, J., Weinstock, J.V. and Rook, G.A., 2006. Mechanisms of disease: the hygiene hypothesis revisited. Nature Clinical Practice Gastroenterology & Hepatology, 3(5), pp.275-284.

Guarnieri, G., Biolo, G., Zanetti, M. and Barazzoni, R., 2004, September. Chronic systemic inflammation in uremia: potential therapeutic approaches. In Seminars in nephrology (Vol. 24, No. 5, pp. 441-445). WB Saunders.

Guyer, D.M., Radulovic, S., Jones, F.E. and Mobley, H.L., 2002. Sat, the secreted autotransporter toxin of uropathogenic Escherichia coli, is a vacuolating cytotoxin for bladder and kidney epithelial cells. Infection and immunity, 70(8), p.4539.

Horizon International Solutions. 2011. "Small Ecuadorian Clean Water Project Improves Health in Village." Yale University Department of Biology. Available at: http://www.solutions-site.org/node/321 [Accessed February 10, 2017].

Hoy, W.E., Ingelfinger, J.R., Hallan, S., Hughson, M.D., Mott, S.A. and Bertram, J.F., 2010. The early development of the kidney and implications for future health. Journal of developmental origins of health and disease, 1(04), pp.216-233.

Kershaw, K.N., Mezuk, B., Abdou, C.M., Rafferty, J.A. and Jackson, J.S., 2010. Socioeconomic position, health behaviors, and C-reactive protein: a moderated-mediation analysis. Health Psychology, 29(3), p.307.

Knight, E.L., Verhave, J.C., Spiegelman, D., Hillege, H.L., De Zeeuw, D., Curhan, G.C. and De Jong, P.E., 2004. Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. Kidney international, 65(4), pp.1416-1421.

Langhorst, J., Elsenbruch, S., Koelzer, J., Rueffer, A., Michalsen, A. and Dobos, G.J., 2008. Noninvasive markers in the assessment of intestinal inflammation in inflammatory bowel diseases: performance of fecal lactoferrin, calprotectin, and PMN-elastase, CRP, and clinical indices. The American journal of gastroenterology, 103(1), p.162.

Laterza, O.F., Price, C.P. and Scott, M.G., 2002. Cystatin C: an improved estimator of glomerular filtration rate?. Clinical chemistry, 48(5), pp.699-707.

Levey, A.S., Eckardt, K.U., Tsukamoto, Y., Levin, A., Coresh, J., Rossert, J., Zeeuw, D.D., Hostetter, T.H., Lameire, N. and Eknoyan, G., 2005. Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney international, 67(6), pp.2089-2100.

Levey, A.S. and Coresh, J., 2012. Chronic kidney disease. The Lancet, 379(9811), pp.165-180.

Libby, P., Ridker, P.M. and Maseri, A., 2002. Inflammation and atherosclerosis. Circulation, 105(9), pp.1135-1143.

Liebert MA, Snodgrass JJ, Blackwell AD, Madimenos FC, Cepon TJ, and Sugiyama LS. 2013. Implications of market integration for cardiovascular and metabolic health among an indigenous Amazonian Ecuadorian population. Ann Hum Biol 20: 228-242.

Liebert MA. 2016. Stress and culture change among indigenous Shuar from Amazonian Ecuador: Integrating evolutionary, developmental, and biocultural perspectives. PhD Dissertation, University of Oregon.

Lin, J., Hu, F.B. and Curhan, G.C., 2010. Associations of diet with albuminuria and kidney function decline. Clinical Journal of the American Society of Nephrology, 5(5), pp.836-843.

Macy, E.M., Hayes, T.E. and Tracy, R.P., 1997. Variability in the measurement of C-reactive protein in healthy subjects: implications for reference intervals and epidemiological applications. Markers, 52, p.58.

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

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

McDade TW, Tallman PS, Madimenos FC, Liebert MA, Cepon TJ, Sugiyama LS, and Snodgrass JJ. 2012. Analysis of variability of high sensitivity C-reactive protein in lowland Ecuador reveals no evidence of chronic low-grade inflammation. Am J Hum Biol 24: 675-681.

McDade, T.W., 2012. Early environments and the ecology of inflammation. Proceedings of the National Academy of Sciences, 109(Supplement 2), pp.17281-17288.

Mei, J.V., J.R. Alexander, B.W. Adam, and W.H. Hannon. 2001. “Use of Filter Paper for the Collection and Analysis of Human Whole Blood Specimens.” Journal of Nutrition 131:1631S–36S.

Oberg, B.P., McMenamin, E., Lucas, F.L.E.E., McMonagle, E., Morrow, J., Ikizler, T.A.L.P. and Himmelfarb, J., 2004. Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. Kidney international, 65(3), pp.1009-1016.

Odden, M.C., Tager, I.B., Gansevoort, R.T., Bakker, S.J., Katz, R., Fried, L.F., Newman, A.B., Harris, T., Sarnak, M.J., Siscovick, D. and Shlipak, M.G., 2010. Age and cystatin C in healthy adults: a collaborative study. Nephrology Dialysis Transplantation, 25(2), pp.463-469.

Omar, M., Abdel-Razek, W., Abo-Raia, G., Assem, M. and El-Azab, G., 2015. Evaluation of serum cystatin C as a marker of early renal impairment in patients with liver cirrhosis. International journal of hepatology, 2015.

Ortega, O., Rodriguez, I., Gallar, P., Carreño, A., Ortiz, M., Espejo, B., Jimenez, J., Gutierrez, M., Oliet, A. and Vigil, A., 2002. Significance of high C‐reactive protein levels in pre‐dialysis patients. Nephrology Dialysis Transplantation, 17(6), pp.1105-1109.

Panichi, V., Migliori, M., De Pietro, S., Taccola, D., Bianchi, A.M., Norpoth, M., Giovannini, L., Palla, R. and Tetta, C., 1999. C-reactive protein as a marker of chronic inflammation in uremic patients. Blood purification, 18(3), pp.183-190.

Rodwell, G.E., Sonu, R., Zahn, J.M., Lund, J., Wilhelmy, J., Wang, L., Xiao, W., Mindrinos, M., Crane, E., Segal, E. and Myers, B.D., 2004. A transcriptional profile of aging in the human kidney. PLoS Biol, 2(12), p.e427.

Sanders, P.W., 2004. Salt intake, endothelial cell signaling, and progression of kidney disease. Hypertension, 43(2), pp.142-146.

Simon, A.K., Hollander, G.A. and McMichael, A., 2015, December. Evolution of the immune system in humans from infancy to old age. In Proc. R. Soc. B (Vol. 282, No. 1821, p. 20143085). The Royal Society.

Stenvinkel, P., 2010. Chronic kidney disease: a public health priority and harbinger of premature cardiovascular disease. Journal of internal medicine, 268(5), pp.456-467.

Stevens, L.A., Coresh, J., Schmid, C.H., Feldman, H.I., Froissart, M., Kusek, J., Rossert, J., Van Lente, F., Bruce, R.D., Zhang, Y.L. and Greene, T., 2008. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: a pooled analysis of 3,418 individuals with CKD. American Journal of Kidney Diseases, 51(3), pp.395-406.

Strimbu, K. and Tavel, J.A., 2010. What are biomarkers?. Current Opinion in HIV and AIDS, 5(6), p.463.

Stumpers, S.A. and Thomson, N.J., 2013. Review of kidney disease and urologic disorders among Indigenous people. Australian Indigenous HealthInfoNet.

Rautava, S., Ruuskanen, O., Ouwehand, A., Salminen, S. and Isolauri, E., 2004. The hygiene hypothesis of atopic disease—an extended version. Journal of pediatric gastroenterology and nutrition, 38(4), pp.378-388.

Ridker, P.M., Buring, J.E., Shih, J., Matias, M. and Hennekens, C.H., 1998. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation, 98(8), pp.731-733.

Roberts, J.A., 1991. Etiology and pathophysiology of pyelonephritis. American Journal of Kidney Diseases, 17(1), pp.1-9.

Valeggia, C.R. and Snodgrass, J.J., 2015. Health of indigenous peoples. Annual Review of Anthropology, 44, pp.117-135.

Vogl, P., 2013. *Measurement of cystatin C in dried blood spot specimens.* (Masters Thesis, University of Washington).

Von Mutius, E., 2007. Allergies, infections and the hygiene hypothesis–the epidemiological evidence. Immunobiology, 212(6), pp.433-439.

Willerson, J.T. and Ridker, P.M., 2004. Inflammation as a cardiovascular risk factor. Circulation, 109(21 suppl 1), pp.II-2.

Yazdanbakhsh, M., Kremsner, P.G. and Van Ree, R., 2002. Allergy, parasites, and the hygiene hypothesis. Science, 296(5567), pp.490-494.

Zee, R.Y. and Ridker, P.M., 2002. Polymorphism in the human C-reactive protein (CRP) gene, plasma concentrations of CRP, and the risk of future arterial thrombosis. Atherosclerosis, 162(1), pp.217-219.

Zhang, Q.L. and Rothenbacher, D., 2008. Prevalence of chronic kidney disease in population-based studies: systematic review. BMC public health, 8(1), p.117.