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Biodemographic Perspectives on Early Life Influences on Later Life Health

Early origins of inflammation: A life course perspective on the predictors of C-reactive protein in young adults in the Philippines

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Abstract

Inflammation may be an important mechanism linking early environments with subsequent patterns of aging and mortality, although few studies have considered biomarkers of inflammation from a life course perspective. We address this issue in an ongoing cohort study in the Philippines, with over 20 years of prospective data. Concentrations of high sensitivity C-reactive protein were determined in 1,598 individuals, and evaluated in relation to the following: prenatal undernutrition; growth, frequency of infectious disease, pathogen exposure, and patterns of breastfeeding in the first two years of life; and concurrent measures of health behaviors and anthropometric measures of nutritional status, assessed at the time of blood collection. Logistic regression models indicate that prenatal undernutrition and pathogen exposure in infancy are relatively strong predictors of the likelihood of elevated CRP in young adulthood, independent of concurrent measures of adiposity and health behaviors.

Introduction

Life course approaches to health recognize that trajectories of health unfold over time, with exposures having short as well as long-term consequences for outcomes that depend in part on the life stage during which they are experienced (Blackwell, Hayward and Crimmins 2001; Hayward and Heron 1999; Lynch, Kaplan and Shema 1997). Experimental animal models as well as observational data from population-based studies have established that conditions early in life—prenatal and early postnatal environments in particular—shape risk for cardiovascular and metabolic disease later in life through developmental modifications to critical physiological systems (Barker 1994; Gluckman, Hanson and Beedle 2007). Relatively little attention has been given to the long-term impact on immune function and inflammation, although recent work has suggested that inflammation may be an important mechanism linking stressors experienced in early environments with subsequent patterns of aging and mortality (Finch and Crimmins 2004).

Inflammation is a fundamental, highly conserved mammalian response to infection and injury. Recent evidence linking biomarkers of inflammation to the pathophysiology of cardiovascular disease, as well as other non-communicable diseases, has generated much interest in inflammation in general, and high sensitivity C-reactive protein (CRP) in particular (Danesh et al. 2000a; Danesh et al. 2000c; Ridker et al. 2000). As the prototypical acute phase protein, CRP is an important component of innate immunity and represents a first line of defense against a wide range of pathogens (Ballou and Kushner 1992). Pathogen exposure and body fat are important regulators of CRP production, but the vast majority of research investigating the causes and consequences of variation in high sensitivity CRP has been conducted in affluent populations characterized by over-nutrition and low levels of infectious disease.

Considerable insights into the dynamics of inflammation may be gained, therefore, by investigating CRP in a different ecological context characterized by burdens of infectious disease as well as over-nutrition. Such is the case in the Philippines, where rapid socio-economic and lifestyle changes have led to increased consumption of energy-dense processed foods and reduced levels of physical activity (Adair 2004). Like other populations undergoing the "nutrition transition," rates of chronic degenerative diseases have increased dramatically in a single generation, and many individuals who are overweight as adults were exposed to undernutrition and relatively high levels of infection in infancy and early childhood.

The Cebu Longitudinal Health and Nutrition Survey (CLHNS) is an ongoing population-based study that enrolled a cohort of women when they were pregnant in 1983, and that continues to follow them and their offspring to collect sociodemographic, lifestyle, community, and health information. The prospective design of this study, as well as the detailed information collected at multiple time points, provide a unique opportunity for a life course perspective on physiological function and health. Prior research from this population has shown that birth weight, growth in infancy and early childhood, and infectious disease exposure are significantly related to cardiovascular disease risk factors and immune function later in life, independent of current measures of context and lifestyle (Adair, Kuzawa and Borja 2001a; Kuzawa and Adair 2003; McDade et al. 2001). We build on these analyses by testing the hypothesis that early environments are significant predictors of CRP in young adulthood, when the original offspring children are over 20 years old. In particular, prior cross-sectional analyses (McDade et al., in prep) revealed different patterns of association between adiposity and CRP in the Philippines and the U.S., raising the possibility that distinct ecological exposures early in life may have lasting impact on the regulation of inflammation.

The specific objectives of this study are twofold: 1) To investigate prenatal and postnatal undernutrition, and postnatal infectious disease exposure as predictors of CRP in young adulthood; and (2) To test simultaneously the associations between concurrent and early life factors as predictors of CRP in young adulthood to evaluate the relative importance of exposures early in life. To the best of our knowledge, this study is the first to test the hypothesis that birth weight is associated with CRP in adulthood using data from a prospective birth cohort, and the first test of the hypothesis that infectious disease exposure early in life has a lasting impact on CRP.

Methods

Participants and data collection

The Cebu Longitudinal Health and Nutrition Survey (CLHNS) began in 1983 with the recruitment of 3,327 pregnant women representative of the childbearing population in Cebu City (Adair, Kuzawa and Borja 2001b). The women and their children have been followed through multiple rounds of data collection since 1983, including the most recent survey conducted in 2005. The 2005 survey included 1,885 index children, 1,627 of whom provided complete anthropometric, CRP, and interview data for these analyses. An additional 29 women pregnant at the time of the survey were not included in the analyses due to the effect of pregnancy on inflammation, yielding a final sample size of 1,598.

We evaluated how our sample differed from the original cohort as assessed when the study started in 1983. Compared to those lost to follow up, participants remaining in the study had higher mean birthweights (mean (SE) difference = 53.0 (15.7) grams, $p<0.001$) and were born to mothers with less formal education (0.33 (0.13) years, $p<0.05$). They were also more

likely to come from families that owned their homes in 1983 (74.1 vs. 58.0%, $p<0.001$), and were more likely to live in rural communities (25.7 vs. 21.7%, $p<0.01$). Participants did not differ with respect to household income or assets at baseline.

Participants provided information on household demographics, economic activities and resources, environmental quality, and health behaviors in face-to-face interviews conducted in their homes. Interviewers also provided assessments of household and neighborhood attributes. Standard procedures (Lohman, Roche and Martorell 1988) were implemented to collect anthropometric measures of standing height (without footwear), weight (in light clothing), and waist circumference. Subscapular, triceps, and supra-iliac skinfold thicknesses were measured in triplicate to the nearest 0.5 mm with precision calipers, and averaged.

CRP analysis

Blood samples were collected into EDTA-coated vacutainer tubes in the participants' homes in the morning after an overnight fast. Blood samples were kept in coolers on ice packs for no more than 2 hours and were then centrifuged to separate plasma prior to freezing at -70˚C. Samples were express shipped to Northwestern University on dry ice and stored frozen at -80˚C until analysis. CRP concentrations were determined using a high sensitivity immunoturbidimetric method (Synchron LX20, lower detection limit: 0.1 mg/L).

Data analysis

Maximum likelihood logistic regression (Stata Corporation, College Station, TX) was used to model the likelihood of having CRP concentration in the top tertile of the distribution ($\geq$0.7 mg/L). Since we are investigating CRP as an indicator of chronic, low grade inflammation

that may contribute to the development of cardiovascular disease, individuals with CRP > 10 mg/L were removed from the regression analyses, as recommended by a recent joint scientific statement from the American Heart Association and Centers for Disease Control and Prevention (Pearson et al. 2003). This same statement also recommends using 3 mg/L as a cut-off value for identifying individuals at elevated risk for cardiovascular disease. Given the exceptionally low concentrations of CRP in our sample, we have elected to model the likelihood of CRP in the top tertile of the distribution, rather than > 3 mg/L. The tertile approach has been used in several population-based studies of CRP (Danesh et al. 2000b). For comparative purposes, we also ran our models with CRP > 3 mg/L as the outcome.

Analyses proceeded in four stages. First, we considered prenatal nutrition as a predictor of CRP in adulthood, using birth weight (measured shortly after delivery) as an indicator. We also considered preterm delivery, and included gender in all of our models. Second, in a separate model, we considered aspects of the postnatal environment, including measures of socioeconomic status (maternal education, household assets, household income, home ownership), duration of breastfeeding, pathogen exposure, and frequency of infectious morbidity. We then combined prenatal as well as early postnatal factors to evaluate their relative independence, and to derive a model of the early life predictors of CRP in young adulthood.

Third, we considered concurrent predictors of CRP, assessed at the time of blood collection when the cohort averaged 20.9 years of age. These predictors included anthropometric measures of adiposity (waist circumference, sum of triceps, subscapular, and supra-iliac skinfold thicknesses, body mass index), indicators of pathogen exposure, current symptoms of infectious disease, individual health behaviors shown previously to be related to inflammation (smoking, alcohol consumption, oral contraceptive use), and measures of SES

comparable to those used to characterize the postnatal environment. Lastly, we conducted simultaneous analysis of early life and concurrent variables to evaluate their independent contributions to predicting the likelihood of elevated CRP in young adulthood.

Information on infectious morbidity was collected during the first two years of life as part of in-home interviews conducted at bimonthly intervals following birth. Mothers were asked whether their infant had shown symptoms of diarrhea, respiratory infection, and/or fever during the week preceding the interview. We constructed separate variables summing the number of bimonthly intervals in which individuals had diarrhea, respiratory infection, or fever, as well an overall infectious morbidity variable summing the number of intervals in which any of these ailments were present. In order to consider the potential impact of timing of exposure, separate morbidity variables were constructed for 2 to 6 months, 8 to 12 months, and 14 to 24 months, inclusive.

In order to assess current infectious morbidity, at the time of blood collection, we asked participants if they were currently experiencing any symptoms of infection. Symptoms included runny nose, cough, fever, diarrhea, sore throat, as well as the more general categories of "flu," "cold," and "sinusitis". Responses were used to construct a single variable indicating the presence or absence of any infectious symptoms at the time of blood collection.

We evaluated the association between pathogen exposure and CRP using multiple proxy measures of the likelihood of exposure to infectious microbes, including household crowding (number of persons/number of rooms), type of toilet (no toilet, pit, flush/water sealed), and source of drinking water (bottled, piped municipal supply, closed well with pump, open sources: uncovered well, spring, river, rain). Domestic animals (e.g., pigs, goats, chickens) were frequently kept in and around participant's homes in 1983, and exposure to animal feces was

assessed by summing the number of bimonthly intervals that the interviewer observed that the infant was crawling, and that animals were present in the home. Separate variables were constructed for exposure to animal feces between 6 and 12 months of age, and between 14 and 24 months.

We also constructed a pathogen exposure variable based on five measures, each scored on a three point scale (0=low exposure, 1=moderate, 2=high) and assessed at baseline and at the time of CRP measurement: cleanliness of the food preparation area, means of garbage disposal, presence of excrement near the house, level of garbage and excrement present in the neighborhood surrounding the household. Cronbach's alphas for the scales representing postnatal and current pathogen exposure were 0.46 and 0.71, respectively. Although reliability of the postnatal scale was low, we retained it due to its comparability with the current pathogen exposure scale. We also evaluated its components separately to consider whether the summary variable was obscuring significant associations.

Results

The median CRP concentration for the entire sample was 0.2 mg/L, with no evidence of difference between males and females. Fifty one participants had concentrations of CRP >10 mg/L. The presence of infectious disease symptoms at the time of blood collection was the only significant predictor of CRP >10 mg/L (OR=3.89, 95% CI=2.17, 7.00, $p<0.001$). No anthropometric, socioeconomic, or other environmental quality variables were significantly related to this level of CRP.

Early life predictors of CRP

We first modeled the likelihood of elevated CRP in relation to variables representing the quality of the prenatal nutritional environment, excluding the 51 participants with CRP >10 mg/L. Birth weight was a significant predictor of adult CRP, with higher birth weights associated with lower likelihood of elevated CRP (Table 2). Consideration of birth weight as a categorical variable, divided into quartiles, suggests that the association with CRP is approximately continuous throughout the distribution, and is not due solely to an association with very low birth weights. Preterm delivery was not associated with CRP.

Measures of infectious morbidity in the first year of life were not significant predictors of CRP, although frequency of diarrhea in the second year was negatively associated with CRP in young adulthood. Similarly, frequency of exposure to animal feces in the house between 6 and 12 months of age was negatively associated with later CRP: For each bimonthly interview interval where fecal contamination was likely the odds of elevated CRP in adulthood were 16 percent higher. Of the postnatal growth measures, only weight gain between birth and six months was positively associated with CRP. And of the measures of socioeconomic status, only home ownership in 1983 predicted CRP in 2005: Infants born into homes not owned by their families were marginally more likely to have elevated CRP as young adults. Measures of the total duration of breastfeeding, or the duration of exclusive breastfeeding, were not significantly associated with CRP in young adulthood. Breastfeeding variables were also not significant in interaction with measures of infectious disease or pathogen exposure in infancy.

Associations between CRP and prenatal and postnatal factors were similar when all early life factors were considered simultaneously, although the association with birth weight was

slightly attenuated. Exposure to animal feces and diarrhea morbidity in infancy remained as relatively strong predictors of lower likelihood of elevated CRP in young adulthood.

Current predictors of CRP

We next considered concurrent predictors of CRP, assessed at the time of blood collection in 2005. Anthropometric measures of adiposity and symptoms of infectious disease at the time of blood collection were the primary predictors of elevated CRP (Table 2). For females, waist circumference was the strongest adiposity measure related to CRP, with a one standard deviation increase in waist circumference (7.6cm) increasing the odds of elevated CRP by nearly twofold. For males, skinfold thickness was the only adiposity measure significantly related to CRP, with a one standard deviation increase in skinfold thickness associated increasing the likelihood of high CRP by a factor of 1.6.

For all participants, the likelihood of elevated CRP more than doubled for individuals reporting infectious disease symptoms at the time of blood collection. Our summary household pathogenicity variable was positively associated with CRP, although this association was of marginal statistical significance. Oral contraceptive use was infrequent in this population, but was associated with elevated CRP. Measures of current individual and household SES were not related to CRP.

Early life and current predictors of CRP

Our final model evaluated whether associations between adult CRP and exposures in utero and infancy were independent of current predictors of CRP, many of which may be related to environments early in life. Associations with CRP changed little when early life and current predictors were considered simultaneously, with the exception of a considerable strengthening of the association between CRP and birth weight, and an attenuation of the association with infant

weight gain (Table 2). The association with home ownership in 1983 dropped out of the full model, likely due to the inclusion of more proximate determinants of variation in CRP.

Individuals born with smaller birth weights, and infants with less exposure to animal feces and diarrhea morbidity, were significantly more likely to have elevated CRP as young adults, providing support for the hypothesis that nutritional and pathogenic exposures early in life have long term effects on inflammation. The association between birth weight and elevated CRP strengthened considerably with the addition of current variables, providing further support for an independent effect of early undernutrition.

Figure 1 presents the predicted probability of elevated CRP based on regression coefficients from the final model in Table 2. Variables of interest were set to low, mid, and high levels and individual values were retained for other covariates, allowing us to compare the relative strength of the independent associations between these variables and CRP. Measures of adiposity were the strongest predictors of elevated CRP. For women with waist circumference one standard deviation above the sample mean, the predicted probability of elevated CRP was 0.41, compared to 0.27 for women at the sample mean. A similar, but slightly weaker pattern of association between skinfold thickness and CRP was evident for men. For individuals with a birth weight of 2.50 kg, the predicted probability of elevated CRP in young adulthood was 0.31. A one kilogram increase in birth weight was associated with a reduced probability of 0.25. Moving from the lowest to the highest levels of diarrhea morbidity and exposure to animal feces reduced the predicted probability of elevated CRP from 0.31 to 0.24, and 0.31 to 0.23, respectively.

Lastly, we re-ran our models using CRP > 3 mg/L as the outcome to provide a basis for comparison with findings using this cut-off value. Only 115 individual had CRP concentrations

greater than 3 mg/L (and less than 10 mg/L). The pattern of associations for waist circumference, skinfold thickness, and animal feces exposure in infancy were similar to those reported in the final model in table 2. Birth weight, diarrhea morbidity, weight gain in infancy, and oral contraceptive use dropped out of the model, and associations with current pathogen exposure (OR=1.77, 95% CI=1.09, 2.87) and current symptoms of infection (OR=4.03, 95% CI=2.62, 6.22) strengthened considerably.

Discussion

Consistent with a large body of prior research, primarily conducted in affluent western populations, measures of current adiposity are strong predictors of CRP in the Philippines, with higher levels of body fat associated with higher CRP. However, aspects of the prenatal and early postnatal environment are also significant independent predictors of adult CRP, providing evidence in support of the hypothesis that early environments have long term implications for the regulation of inflammation. We investigated this issue in an ongoing, longitudinal study that began collecting data while participants were in utero, and that includes detailed information on a wide range of individual, household, and community factors over a 22 year period. This is a major strength of our analysis, and increases our confidence that the reported associations are not due to confounding or omitted variable bias.

To the best of our knowledge, this study is the first to document an association between birth weight and CRP in adulthood using data from a prospective birth cohort. The size of this association is considerable: A one kilogram decrease in birth weight is associated with a 25 percent increase in the predicted probability of elevated CRP in this sample. While current measures of body fat are stronger predictors of CRP in adulthood, the fact that birth weight is

positively associated with adult body fat suggests that our results are likely to provide conservative estimates of the true impact of prenatal undernutrition on inflammation in this population.

The association between birth weight and CRP is consistent with recent findings from the MIDSPAN Family Study in Scotland, in which CRP concentrations in adulthood (age 30 to 59 years) were negatively associated with birth weight as recalled by study participants (Sattar et al. 2004). In addition, low birth weight has recently been associated with elevated CRP in five-year old children in Bangladesh, suggesting that effects of birth weight on CRP may emerge in childhood (Raqib et al. 2007). The negative association between birth weight and adult CRP is also consistent with the large number of studies linking lower birth weights with the adult onset of cardiovascular disease and diabetes as well as indicators of risk including blood pressure, total and HDL cholesterol, and insulin resistance (Barker 1994; Gluckman et al. 2007; Rich-Edwards et al. 1999). Inflammation may therefore be an important mechanism accounting for, at least in part, the consistently documented association between lower birth weight and increased risk for cardiovascular and metabolic disease later in life.

Compared to prior research, concentrations of CRP in this sample were exceptionally low. For example, in the United States, median CRP has been reported to be 2 mg/L for adults 17 years and older (King, Egan and Geesey 2003), and 0.9 mg/L for 20 to 29 year-old men (Ford et al. 2003). In a recent analysis of CRP concentrations in the general populations of Glasgow, Scotland, and Augsburg, Germany, geometric mean concentrations for 25 to 34 year-olds ranged from 0.81 to 1.25 mg/L for men and women. Much of the difference in CRP concentrations across populations may be attributed to differences in body fat, with higher levels of overweight and obesity in the U.S. and Europe. However, in prior analyses we have found that even in the

ranges of the body fat distribution that overlap between Cebu and the U.S., age- and gender-matched levels of CRP are elevated in the U.S. (McDade et al., in prep). To the extent that burdens of infectious disease and pathogen exposure were higher in the Philippines than in the U.S. twenty years ago, the lower concentrations of CRP among adults in our study are difficult to reconcile with the hypothesis that pathogenic environments in infancy have lasting pro-inflammatory effects.

Rather, we find evidence for a negative association between adult CRP and pathogen exposure and diarrhea morbidity in infancy. These associations are broadly consistent with the "hygiene hypothesis," in which low levels of pathogen exposure early in life bias immune development and regulatory processes in ways that increase the likelihood of inflammatory conditions such as allergy, asthma, and autoimmune disease later in life (Rook and Stanford 1998; Radon et al. 2007). Recent research has underscored the important roles that plasticity and ecological responsiveness play in the development and function of the immune system, and pathogenic exposures early in life may comprise a critical set of inputs that promote the development of effective anti-inflammatory regulatory networks (McDade 2005; Yazdanbakhsh, Kremsner and van Ree 2002). However, positive associations between childhood infections and adult morbidity have also been reported, suggesting that the timing, type, and intensity of infectious exposures may be critical determinants of their long-term effects on inflammation (Kemp and Björkstén 2003; Blackwell, Hayward and Crimmins 2001). Clearly, more research in this area is needed.

Limitations of our study include the use of a single CRP measure, which makes it more difficult to differentiate acute episodes of inflammation from chronic, low-grade increases in CRP concentration. We also rely on proxy—rather than direct—measures of pathogen exposure

and bimonthly morbidity interviews to capture the quality of the early environment with respect to infectious disease. While neither set of variables provides a complete picture of infection/pathogen burden, the study is unique in its wide range of measures and the prospective nature of data collection. Lastly, the significance of elevated CRP for cardiovascular and metabolic disease risk in this population is not clear, particularly given the relatively low concentrations compared to western populations. Future follow up surveys will be required to determine whether elevated concentrations of CRP, and the antecedent conditions associated with increased inflammation, place Filipinos on the path to chronic degenerative disease.

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Table 1. Descriptive statistics for female and male participants. Mean (SD) values are presented for continuous variables.

	Female (N=701)	Male (N=897)	Total (N=1,598)
Age (yrs)	20.9 (0.3)	20.9 (0.3)	20.9 (0.3)
Birth weight (kg)	2.999 (0.408)	3.026 (0.432)	3.015 (0.422)
Weight gain, 1st year (kg)	4.667 (0.872)	5.219 (0.929)	4.978 (0.945)
Home ownership, 1983 (%)	74.6	73.7	74.1
Waist circumference (cm)	68.1 (7.7)	72.1 (7.4)	70.3 (7.8)
Sum of three skinfold measures (mm)	62.3 (19.8)	37.5 (17.9)	48.4 (22.4)
Number of intervals with high levels of exposure to animal feces, 6-12 mos	1.20 (1.25)	1.27 (1.29)	1.24 (1.28)
Symptoms of infection (%)	14.4	13.6	14.0
Oral contraceptive use (%)	3.8		
CRP (median, 25th, 75th %ile)	0.2 (0.1, 0.9)	0.3 (0.1, 0.9)	0.2 (0.1, 0.9)

Table 2. Maximum likelihood multiple logistic regression models predicting the probability of CRP in the top tertile (>0.5 mg/L), excluding individuals with CRP>10 mg/L.

	Prenatal factors		**Postnatal factors (1st two years)**		**Prenatal and postnatal factors**		**Concurrent factors**		**Full model**	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Female	0.97	0.77, 1.21	1.07	0.84, 1.35	1.05	0.83, 1.34	0.01**	0.00, 0.20	0.01**	0.00, 0.18
Birth weight (kg)	0.77*	0.59, 0.99			0.79+	0.60, 1.03			0.70**	0.53, 0.93
Weight gain, 0-6 mos (kg)			1.27**	1.09, 1.47	1.25**	1.07, 1.45			1.14+	0.97, 1.34
Diarrhea morbidity, 12-24 mos			0.90*	0.81, 0.99	0.90*	0.81, 0.99			0.89*	0.80, 0.98
Frequency of exposure to animal feces in home, 6-12 mos			0.86**	0.78, 0.94	0.86**	0.78, 0.94			0.87**	0.79, 0.95
House ownership			0.82+	0.63, 1.05	0.81+	0.63, 1.04				
Waist circumference (cm)							1.01	0.98, 1.04	1.01	0.97, 1.05
Waist circumference x female							1.08***	1.03, 1.13	1.08***	1.03, 1.13
Skinfold thickness (mm)							1.02**	1.01, 1.04	1.02**	1.01, 1.04
Skinfold thickness x female							0.98*	0.96, 0.99	0.98*	0.96, 0.99
Symptoms of infection							2.54***	1.86, 3.48	2.58***	1.88, 3.55
Oral contraceptive use							2.65*	1.17, 6.00	2.78*	1.20, 6.48
Pathogenicity scale (0-2)							1.24+	0.93, 1.67	1.28+	0.95, 1.73
Log likelihood	-915.16		-902.13		-900.64		-854.19		-842.20	
Model p value	0.09		<0.0001		<0.0001		<0.0001		<0.0001	

+p<0.10, *p<0.05, **p<0.01, ***p<0.001

Figure 1. Predicted probability of CRP in top tertile