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C-reactive protein by pregnancy and lactational status among Filipino young adult women

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Abstract

Objectives—Pregnancy and lactation involve adaptations in immune regulation, but little is known about cross-cultural variation in inflammatory changes during pregnancy or lactation. Here we report concentrations of C-reactive protein (CRP) in a large cross-sectional sample of healthy Filipino women who vary in parity, gestational and lactational status, and who come from a population previously described as having low CRP.

Methods—Fasting plasma CRP was measured among female participants (ages 20.8-22.4 years) in the Cebu Longitudinal Health and Nutrition Survey (n=822).

Results—Median CRP was 0.2 mg/L in nulliparous women and peaked at 2.0 mg/L in women in their 3rd trimester of pregnancy. Parous but post-partum women had higher CRP compared to nulliparous women, which was largely explained by body composition differences as reflected in waist circumference and skinfold measures. Among post-partum women with infants, CRP was similar in women who were currently breastfeeding compared to those who were not.

Conclusions—At Cebu, women late in gestation have 10-fold higher C-reactive protein compared to nulliparous women, with no evidence that lactation is inflammatory. These population-based findings are similar with findings from prior clinic-based studies and are consistent with the maternal immunological adaptations initiated during pregnancy. The tendency of human females to spend more time than females of other great apes in gestation rather than lactation suggests that the human life history strategy involved increased time spent by reproductively aged females in a pro-inflammatory state.

Keywords

inflammation; reproduction; immunity; cardiovascular disease risk; Philippines

Viviparous placentation represents a potent challenge to maternal immunity. During pregnancy the maternal immune system undergoes significant changes to prevent rejection of the fetus, as marked by a shift from a T-Helper 1 (Th1) cell dominated profile toward T-Helper 2 (Th2) activity (Challis et al., 2009). The predominance of Th2 cytokines during pregnancy is thought to protect the fetus, with experimental animal model work showing

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that Th1 cytokines can have adverse effects on the placenta and fetus (Hahn-Zoric 2002). It has been proposed that suppression of specific maternal immunity to facilitate the maintenance of implantation is compensated by an increase in innate immunity (Sacks et al., 1999).

Consistent with these maternal immunological adaptations, studies of pregnant women consistently report higher levels of pro-inflammatory cytokines and related immune factors, such as C-reactive protein, although they vary in the pattern of changes documented (Watts et al., 1991). An early study reported a progressive increase in CRP across the weeks of gestation (Romem and Artal 1985). Others have reported that CRP is already high in early pregnancy (Sacks et al., 2004), remains elevated at a similar level into later pregnancy (Belo et al., 2005; Picklesimer et al., 2008), or that CRP peaks in mid-pregnancy (Saarelainen et al., 2009). Although at least one study reported ethnic differences in pregnancy CRP (Picklesimer et al., 2008), little is known about the extent of human variation in the magnitude or timecourse of inflammatory changes during pregnancy. We are also aware of no prior human study to report CRP during lactation, when metabolic demands associated with breast milk production remain high but in the absence of an invasive feto-placental unit.

To clarify changes in inflammation by reproductive status, here we report levels of C-reactive protein (CRP) measured in a healthy sample of young adult Filipino women participating in the Cebu Longitudinal Health and Nutrition Survey. Participants varied in reproductive status at the time of CRP measurement, allowing characterization of current inflammation among women who were nulliparous, currently pregnant, or parous but not currently pregnant. Among currently pregnant women, we reported differences in CRP across trimesters, and among parous but currently not pregnant women, by time since last pregnancy and current breastfeeding status.

METHODS

Data come from the Cebu Longitudinal Health and Nutritional Survey (CLHNS), which enrolled 3327 pregnant women who gave birth to 3080 singleton, live-born offspring from 1983-1984 in Cebu City, Philippines (Adair et al., 2011). In 2005, 893 of the original cohort of 1447 female offspring were located and interviewed. The present analyses focus on the 822 women who had all necessary 2005 questionnaire and anthropometric data and for whom C-reactive protein was available. Women were asked about their current reproductive status during interviews, during which time anthropometric measures (height, weight, the body mass index), and information on socioeconomic status and pathogen exposure (household income, hygiene index) were obtained. Pathogen exposure was measured using a hygiene score constructed from data on the interviewer's rating of cleanliness in the area of food preparation, and excrement in the immediate area around the house, toilet type, and method/location of garbage disposal. This variable scales from 0-9, with 9 representing the cleanest and most hygienic households. In addition, we used reproductive histories collected in 2007 and 2009 (birth dates of offspring and duration of gestation) to retrospectively identify which women had been early in pregnancy during blood draw in 2005 but were not yet aware that they were pregnant. During blood sampling, women were also asked if they were currently breastfeeding. Because our prior analyses in these women found that CRP was unrelated to smoking (McDade et al 2009), we did not adjust for smoking. This research was conducted under conditions of written informed consent with approval of the Institutional Review Boards of the University of North Carolina (Chapel Hill), Northwestern University (Evanston, Illinois), and the Office of Population Studies Foundation (Cebu, Philippines).

C-reactive protein measurement

Venipuncture blood samples were collected using EDTA-coated vacutainer tubes in the participants' homes in the morning after the overnight fast (~12 hours). Blood samples were kept in coolers on ice packs for no more than 2 h and were then centrifuged to separate plasma prior to freezing at –70°C. Samples were express-shipped in a single batch to Northwestern University on dry ice and stored frozen at –80°C until analysis at the clinical chemistry facility at Northwestern Memorial Hospital in Evanston. CRP concentrations were determined using a high sensitivity immunoturbidimetric method (Synchron LX20, lower detection limit: 0.1 mg/L).

Statistical analysis

All analyses were conducted using Stata 10.1 (College Station, TX). We first calculated descriptive statistics (means and SD for all variables other than CRP, which was reported as median +/- interquartile range to account for skewed distribution). Next, we plotted the distribution of CRP and changes in median (+/- interquartile range) CRP by reproductive status. Finally, we evaluated whether differences in CRP (log-transformed) by reproductive status were independent of potential confounding influences, using tobit regression models to account for left censoring of the distribution due to the large number of observations with values below the lower detection limit of the CRP assay.

RESULTS

At the time of CRP measurement, women in the study were an average of 21 years of age (Table 1). More than half were nulliparous, with the other half currently or formerly pregnant. There were significant differences in height, weight and BMI across women varying in reproductive status. In addition, women who were already pregnant or had reproduced previously came from lower income households with lower hygiene index scores.

Figure 1 shows median (± interquartile range) CRP plotted for nulliparous women (n=479), by trimester among currently pregnant women (n for trimesters 1, 2, 3 = 40, 40, 21), and among parous but not currently pregnant women, by year elapsed since last giving birth. Post-partum women were further stratified on the basis of whether they were currently breastfeeding (n for post-partum year: not breastfeeding = 41, breastfeeding= 70; n for post-partum year 2: not breastfeeding = 106, breastfeeding = 25). There was evidence for increased inflammatory status in later stages of gestation, with women in their 3rd trimester having median CRP 10-fold higher than nulliparous women. Women who had given birth within the past year and more than 1 year ago had median CRP of 0.6 and 0.3, respectively. Women whose youngest offspring was in infancy (<1 year post-partum) had modestly lower CRP values if they were currently breastfeeding (median CRP of 0.55 vs. 0,7 mg/L), although this difference was far from statistically significant (p=0.701).

Because women varied in several characteristics that could confound relationships with CRP, we used tobit regression to evaluate relationships between reproductive status and CRP before and after adjusting for measures of adiposity and the income and hygiene index of households. Findings were similar to the simple descriptive analysis above: compared to nulliparous women, CRP values were significantly higher in women in their 2nd and 3rd trimesters of pregnancy (Table 2). Although median CRP peaked in the 3rd trimester (Figure 2), tobit estimations suggested similar CRP among women in their 2nd and 3rd trimesters of pregnancy reflecting effects on tobit coefficients of 2 women in their 2nd trimesters who had relatively high CRP values (16.1 and 20.9 mg/L). CRP levels were intermediate in parous but not currently breastfeeding women, with lowest CRP levels among women who had not

been pregnant for more than 1 year. Adjusting for adiposity, income and hygiene index attenuated the differences in CRP between reproductively active women and nulliparous women, suggesting that elevated adiposity of pregnant women and mothers contributed to their elevated CRP compared to nulliparous women.

DISCUSSION

This study is among the first to report CRP in a large healthy sample of women varying in reproductive status. In these young adult women from Cebu, Philippines, CRP was markedly higher among women who were in more advanced stages of pregnancy. This was reflected in the fact that 80% of nulliparous women had CRP<1.0 mg/L, whereas this figure was less than 30% among women in their 2nd and 3rd trimesters. Similarly, median CRP concentrations were roughly 10-fold higher among women in their 2nd or 3rd trimesters compared to women who were nulliparous at the time of measurement. Among parous women whose youngest offspring had been born within a year, CRP remained high compared to nulliparous women, which was partially explained by greater adiposity among parous women. In contrast, there was no evidence for elevated CRP among the subset of these women who were currently lactating.

We have previously shown that CRP levels in this population are low compared to age-, sex- and BMI-matched NHANES data from the US (McDade et al., 2009), and this is reflected here in the sizeable number of women with CRP levels below the 0.1 mg/L detection limit of the assay. We have speculated that the relatively low CRP at Cebu could relate to differences in early infectious disease ecology and immune system priming (McDade et al., 2010). Despite these overall low CRP levels, our finding of elevated CRP among pregnant women agrees with prior observations in clinically-based samples (Belo et al., 2005; Picklesimer et al., 2008; Romem and Artal 1985). However, the pattern of inflammation reported by pregnancy status has varied by study and population. Evidence for peak inflammatory status in later stages of pregnancy, akin to what we find at Cebu, was reported in the first study of CRP in pregnancy (Romem and Artal 1985). Subsequent studies have been few, and highlight variation, with some finding that CRP reaches high levels early in pregnancy and remains relatively stable (Belo et al., 2005), and with at least one study finding peak CRP in the second trimester (Picklesimer et al., 2008). The cause of this variation across populations remains uncertain, but underscores the need for additional cross-cultural work on the course of inflammatory change during pregnancy.

Although CRP was generally elevated late in gestation, variation in inflammatory state among these women was notable, which is likely to have implications for offspring physiologic programming, timing of parturition, fetal growth and health outcomes (Challis et al., 2009; Kuzawa and Quinn 2009). We recently reported that inflammatory markers measured *outside* of pregnancy have modest relationships with birth outcomes in this sample (Kuzawa et al., 2012), suggesting that differences in basal inflammatory regulation could impact offspring birth outcomes and potentially have long-term impacts on health. Although the present analyses were cross-sectional and thus incapable of addressing impacts on offspring, in the future we hope to longitudinally investigate these questions at Cebu.

Evidence for pro-inflammatory changes during gestation has intriguing implications for models of the evolution of human reproductive and life history strategy. Humans in subsistence level societies often wean their offspring several years earlier than other great apes (Kaplan et al., 2000). This allows human females to resume ovulation and initiate the next pregnancy earlier, and contributes to higher completed fertility (Bogin 1988; Kaplan et al., 2000). Thus, human females may often spend more of their adult reproductive lives in pregnancy compared to other great apes, which typically spend 5-7 years breastfeeding each

offspring. The evidence for pro-inflammatory gestational changes presented by us here, and by others elsewhere (Belo et al., 2005; Watts et al., 1991), and the lack of evidence for pro-inflammatory changes during lactation, suggests that the human life history strategy was likely accompanied by females spending more of their reproductive lives in a Th-1 biased pro-inflammatory state. Outside of pregnancy, chronic low grade inflammation is energetically costly (McDade et al., 2008), and can lead to decline in cognition and other functions (Libby et al., 2002; Sorci and Faivre 2009). It is presently not clear whether the immune changes that occur during normal healthy pregnancy, and the attendant shift to a pro-inflammatory state, carry similar health or energetic costs.

Limitations of this analysis include our reliance upon cross-sectional data, which limits our ability to differentiate changes in CRP during reproduction from other sources of bias. For instance, women who reproduced at a younger age tended to come from lower income households with higher pathogen exposure as indicated by the hygiene index. While this could contribute to differences in CRP across women varying in reproductive status, it is notable that the early reproducers also had lower BMIs and adiposity, which is expected to lead to lower CRP based upon prior work in this and other populations (McDade et al., 2009). Thus, confounding by body composition differences across these groups would be expected to lead to lower CRP among the parous but not currently pregnant women, which is the opposite of the pattern documented here. Other forms of bias cannot be ruled out.

In sum, we find evidence for substantial elevation in inflammatory status among pregnant women, with highest levels of CRP in women late in gestation. Parous but not currently pregnant women had CRP levels that were intermediate to those among pregnant and nulliparous women, while there was no evidence for elevated CRP in currently lactating women. This pattern is consistent with the immune adaptations initiated during pregnancy, and suggest that the evolution of the human life history was accompanied by a shift to increased time spent by females in a pro-inflammatory state.

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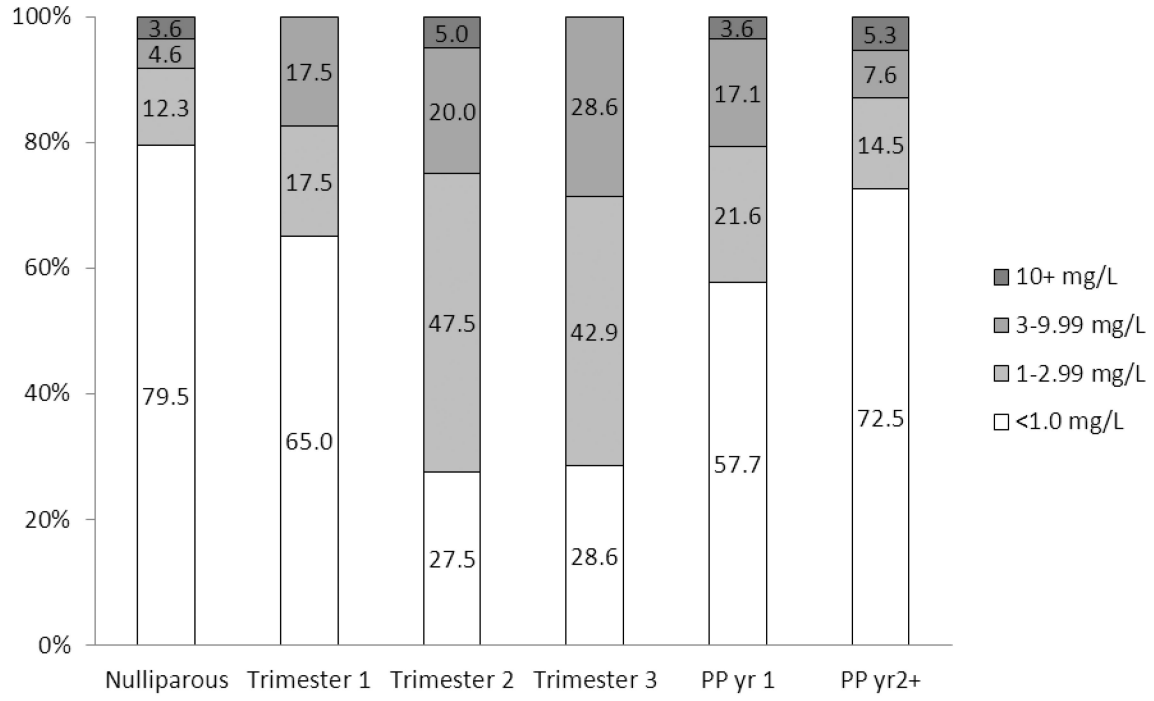
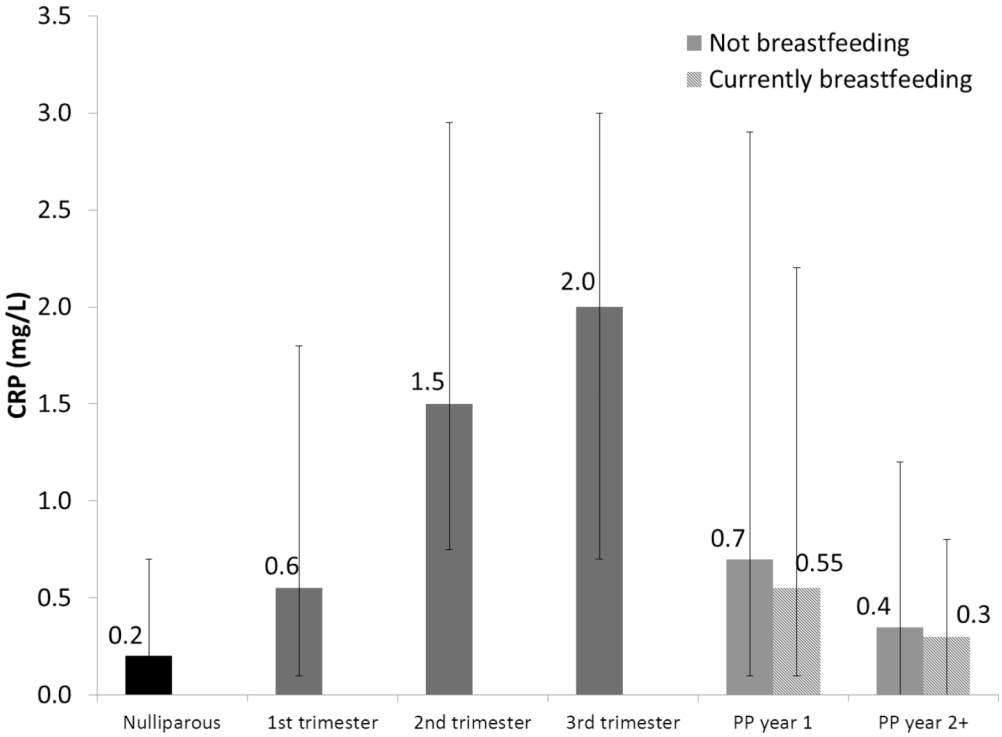


[a]PP yr = years post-partum (since most recent pregnancy)

Fig. 1.
The distribution of CRP by current reproductive status[a]



[a]error bars = interquartile range. PP = post-partum

Fig. 2.
Median CRP among women varying in current reproductive status[a]

TABLE 1

Characteristics of Filipino women[a]

	All (n=822)	Nulliparous (n=479)	Pregnant (n=101)	Post-partum (n=242)	p-value[b]
Age (years)	21.5 (0.3)	21.5 (0.3)	21.4 (0.3)	21.5 (0.3)	0.523
Height (cm)	151.2 (5.4)	151.5 (5.5)	150.8 (5.3)	150.7 (5.3)	0.152
Weight (kg)	46.7 (8.2)	45.7 (8.2)	47.2 (7.7)	48.6 (8.1)	0.0001
BMI (kg/m^2)	20.4 (3.2)	19.9 (3.2)	20.7 (3.1)	21.4 (3.2)	0.0001
Triceps SF (mm)	20.7 (6.2)	21.0 (6.2)	20.0 (5.6)	20.5 (6.4)	0.226
Subscapular SF (mm)	18.9 (6.6)	18.6 (6.7)	18.6 (6.0)	19.4 (6.7)	0.291
Hygiene index (1-9)	6.1 (1.7)	6.4 (1.6)	5.7 (1.7)	5.8 (1.8)	0.0001
Household income (pesos)	639 (1603)	764 (2044)	424 (411)	482 (572)	0.0001
CRP (mg/L)	0.3 (0.0, 1.3)	0.2 (0.0, 0.7)	1.3 (0.5, 2.8)	0.45 (0.0, 1.9)	0.0001

[a] all mean (SD) except for CRP = median (interquartile range), CRP values of 0 denote value below assay detection limit; SF = skinfold thickness.

[b] significance of differences across reproductive status groups from oneway ANOVA, except for CRP based upon joint F test from tobit model.

TABLE 2

TOBIT regression models predicting CRP (mg/L)

	Model 1 β (SE)	p-value	Model 2 β (SE)	p-value
Trimester 1	1.60 (0.53)	0.003	1.58 (0.53)	0.003
Trimester 2	3.47 (0.52)	0.0001	3.28 (0.52)	0.0001
Trimester 3	3.03 (0.71)	0.0001	2.94 (0.71)	0.0001
Post-partum yr 1	1.85 (0.34)	0.0001	1.61 (0.34)	0.0001
Post-partum yr 2+	1.03 (0.32)	0.002	0.94 (0.32)	0.004
Triceps skinfold (mm)			−0.06 (0.03)	0.061
Subscapular skinfold (mm)			0.12 (0.03)	0.0001
Income (1000 pesos)			−0.17 (0.12)	0.165
Hygiene index (1-9)			0.02 (0.07)	0.723