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C-reactive Protein Across the Ovarian Cycle in Urban Poor and Urban Better-off Bolivian Women

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Abstract

By Mariam K. Karamali

C-reactive protein (CRP) is part of the non-specific inflammatory response that provides a glimpse of immune system functioning. Recent research has identified it as a useful biomarker for assessing health status and risk of many chronic diseases. Previous studies have found that CRP fluctuations throughout the ovarian cycle are due to changes in concentrations of various reproductive hormones. The role of life history strategies to explain the relationship between reproductive hormones and CRP remains speculative. Life history theory suggests that environmental stressors play a key role in helping our body to decide how much energy is allocated to vital life functions, such as reproduction and maintenance. It is hypothesized that due to different environmental stressors, Bolivian women will have higher levels of CRP than women in Western populations and that there is a tradeoff between reproduction and maintenance such that as estradiol levels increase, CRP levels will decrease. A survey was administered to women in La Paz, Bolivia and blood spots were collected on 5-6 days of two consecutive ovarian cycles in 30 Urban poor and 31 Urban better-off Bolivian women. The blood samples were assayed for estradiol, progesterone, and CRP. Results showed that urban poor women had higher BMI (P=0.00) and lower levels of estradiol (p=0.02) compared to urban better-off women. BMI is positively associated with CRP (P=0.009) and negatively associated with estradiol (P=0.004). CRP levels are also mediated by BMI. CRP levels in Bolivia were

lower than reported values from women in Western populations and CRP did not vary significantly between cycle days or cycle phases, contrary to the hypotheses. CRP differences due to socioeconomic status may be observed with a greater sample size and/or may be due to ecological differences including differences in: histories, inactivity, stress, or diet quality between the two populations. In this sample, CRP is independent of hormonal fluctuations in the ovarian cycle and is shaped by individual lifestyle differences, which may allow it to be a useful biomarker.

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I. Introduction

Significance

In the last decade, documentation of linkages of C-reactive protein (CRP) to subsequent chronic diseases such as cardiovascular disease and diabetes has led to recognition of the role of inflammation in chronic disease (Wander et al., 2008). For this reason, CRP is used as a leading biomarker of cumulative systemic burden and chronic disease risk. Previous studies have shown a link between reproductive functioning and maintenance efforts, including fluctuations of CRP across the ovarian cycle (Wander et al., 2008). Environmental pressures mediate reproductive effort and can cause interpopulational variation of hormonal fluctuation across the ovarian cycle. These hormonal fluctuations may have an affect on CRP levels in women. The significance of understanding the interaction between human reproduction and immune function is twofold. When assessing health status through the measurement of CRP, researchers might need to account for not only, the effect of the ovarian cycle, but also the effect of ecological pressures resulting in population differences in reproductive functioning. More importantly, studying the relationship of reproductive functioning and maintenance in non-industrialized Bolivian women will allow for a better understanding of energy allocations in our human ancestors relative to modern human populations.

Life History Theory

The chief concern for biological anthropologists is the understanding of processes that generate variation in biological human forms. For many years, the principal outline for this research has been constructed around the theory of evolution through natural selection (Washburn, 1951). Biologists and bioanthropologists alike, agree that evolution involves changes in allele frequency and those genes that allow offspring to live up to reproductive age and produce viable offspring, are selected for. Recently, however, bioanthropologists are suggesting a mechanistic model stating that that genes and developmental processes can evolve as organisms interact with their environments (Pigliucci & Kaplan 2000, Jablonka & Lamb 2005).

One mechanistic model of evolution stems from life history theory, a method used to understand developmental strategies for different species (Charnov, 1993; Stearns, 1992). This model states that resources are limited and the energy gained through these resources is distributed to three life functions: growth, reproduction and maintenance. Cultural and ecological pressures can influence an individual's energy allocations. After being exposed to different ecological and cultural pressures, humans respond adaptively within a certain range of plasticity (McDade et al., 2003). For example, Jones (1986) identified tradeoffs between interbirth interval and number of surviving offspring in the !Kung women of Africa. He measured success rates at raising children to adulthood for different interbirth intervals. Jones found that the interbirth interval that led to the highest number of surviving offspring was the most common interbirth interval in the population.

The !Kung population as a whole, has different food resources available and different environmental conditions compared to other populations allowing them to be characterized by a different interbirth interval. There is a tradeoff in the allocation of resources between female reproduction and survival of offspring and in order to maximize survival of offspring, most women had the 48-month birth interval. The interbirth interval varies across populations indicating population differences in life-history strategies (LHSs), reproductive and developmental traits, across environments.

Natural selection favors those phenotypes, or patterns of energy allocations, that are associated with defense responses and more importantly those phenotypes that result with the highest reproductive success in a specific population. Because allocations to specific functions are mutually exclusive, tradeoffs must be considered. Therefore, according to life history theory, when species allocate more energy to improving reproductive success (age, size at initiating reproduction, number and quality of offspring, etc), there is a resulting decrease in energy allocations toward growth, maintenance, or both (Buttgereit et al., 2000; Read & Allen, 2000; Lochmiller & Deerenberg, 2000).

Most research conducted on life history theory has focused on energy allocations toward reproduction and modulating reproductive effort through the study of reproductive ecology. Through natural selection, humans adapt over time to environmental pressures to enhance reproductive functioning while still sustaining enough resources for maintenance and growth. Many scientists have studied cultural and

environmental pressures in a given population and how these pressures individually affect maintenance, growth, and reproductive functioning. These studies show that the immune system is energetically expensive and that tradeoffs between reproductive functioning and growth exist. However, very few studies have analyzed the tradeoffs in energy allocation between reproduction and maintenance (McDade et al., 2003).

Before analyzing details of this tradeoff of resources between maintenance and reproduction, it is important to understand reproduction and maintenance as separate functions and how they can alter through environmental pressures.

A. Reproduction

Significance of Reproduction

Energy allocation towards reproduction is vital in females to enhance reproductive fitness. It is important to allocate energy to reproduction to increase the likelihood of producing viable offspring and passing on genes that have a selective advantage. Energy dedicated to reproductive functioning is important to sustain the ovarian cycle. The ovarian cycle is a physiological process that has been conserved throughout mammalian evolution and thus inferring its selective advantage in conserving energy resources and oxygen consumption (Price et al., 1981; Strassman, 1996). The primary function of the ovarian cycle is to prepare the uterus for implantation and if implantation does not occur, menses results.

Reproductive Ecology

Given parental resources, natural selection favors the combination of offspring number and size that maximizes the number of offspring that reach reproductive maturity (Lack, 1954). Risks associated with investment in a new offspring include: risking one's survival, possibility of having future offspring, and current offspring survival (Vitzthum, 2008). Because of these risks, species must allocate enough resources towards reproduction in order to minimize these risks and maximize their reproductive success. Reproductive effort is the allocation of resources to producing an offspring that lives to maturity and modulating reproductive effort is adaptive and crucial in maximizing lifetime reproductive success (Wasser & Barash, 1983; Ellison, 1990; Haig, 1990; Peackock, 1990; Vitzthum, 1990).

Reproductive ecology is the study of environmental factors that affect reproductive effort and functioning. Reproductive effort can be adjusted through behavioral or physiological changes (Vitzthum, 2008). A behavioral change such as a shorter period of lactation allows for a new conception sooner and thus alters the reproductive effort (Vitzthum, 1994).

During maturation, an organism's body is able to integrate information from prevailing conditions, which may alter reproductive functioning. This can approximate future conditions. For example, when the environment is temporarily undergoing poor conditions, reproductive effort is delayed. This model of adaptability and life history

theory is known as the flexible response model (Vitzthum 1990, 1997, 2001). The model favors an adapationist approach to women's reproductive functioning in response to: nutrient availability, environment, energetics, and exercise. Whether or not energy is invested in reproduction depends on: the probability of successful reproduction, probability of environmental conditions changing, risk to future reproductive opportunities, and the expected duration until the end of fecundity. If a woman is able to acclimate to suboptimal local conditions and continue to reproduce during those conditions, she will have a selective advantage over a woman who is unable to do so, as long as there is a chance of a successful birth in those suboptimal conditions. In accordance with this explanation is the developmental energetics model which states that women born in resource-poor conditions will mature later, grow more slowly, be more sensitive to energetic stress and have a lower fecundity (Ellison 1990, 1994). Energetics are also important in understanding the variation in ovarian functioning (Ellison, 2003; Ellison et al., 1993, 2007, Jasienska & Thune, 2001; Lipson, 2001). The amount of body fat in a woman may alter her energy expenditure, flux, and balance, which could shape the initiation and maintenance of her ovarian cycling (Jasienska and Thune, 2001). Finally, exercise may also alter ovarian cycling. Research has shown a dose-dependent relationship between exercise and amenorrhea (Prior 1985, 1987). Aside from energetics, environment may also alter reproductive functioning. Rural Bolivian women, age 25-35 years, who lived at higher atlititudes (≥3100 m) were found to have higher progesterone levels than rural Bolivian women who lived in low altitudes (Vitzthum et al., 2000).

Of main focus, however, are physiological changes that affect reproductive functioning. One such mechanism is the varying age at which reproductive maturation may occur. Another mechanism includes hormonal changes in the hypothalamus-pituitary-ovary (HPO) axis. It is known that the hypothalamus secretes gonadotropic releasing hormone (GnRH) causing the pituitary glands to secrete the gonadotropin hormones, which are the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH). FSH is vital for an immature ovum to develop and LH triggers ovulation (Figure 1). Release of FSH and LH from the pituitary gland causes the ovaries to secrete estradiol and progesterone, which then provide a feedback and modulate GnRH release from the hypothalamus.

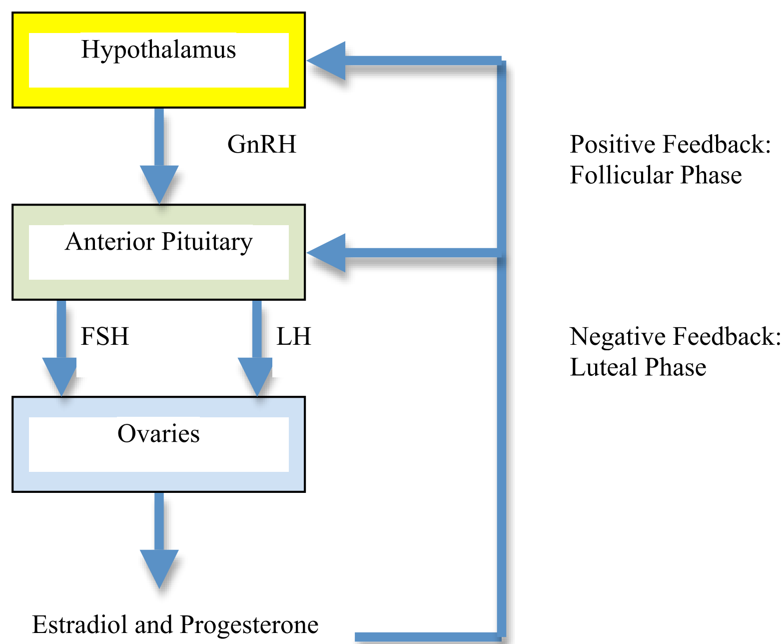

Figure 1. The hypothalamic-pituitary-ovary axis that modulates levels of hormones that affect reproductive functioning.

Given the physiological mechanisms driving reproductive functioning, environmental stressors can alter energy allocated to reproduction. For example, during periods of environmental stress (nutrient deprivation or excess demand for energy), leptin can affect GnRH release from the hypothalamus, thereby affecting concentrations of reproductive hormones.

Leptin is a hormone that is important in regulating energy intake and reflects individual energy balance and fat stores (Houseknecht et al., 1998). When leptin is released in the body, it can bind to a receptor in the arcuate nucleus, located in the brain, to send a message stating that the body has enough energy and is not in a stressful situation. Similarly, GnRH receptors also reside in the arcuate nucleus (Houseknecht et al., 1998). Therefore, when levels of leptin are high, the body is informed that there is enough energy to contribute to reproductive functioning. However, when the body is under stress, leptin may act directly through the arcuate nucleus or through the ovaries, to suppress release of reproductive hormones and to decrease the amount of energy spent on reproduction at a given instance (Clarke & Henry, 1999). Alternatively, leptin may regulate the secretion of other hormones of the pituitary, such as growth hormone as well as prolactin, which can act on the ovaries (Clarke & Henry, 1999). These environmentally influenced physiological changes in concentrations of hormones in the HPO axis can alter the likelihood of ovulation, conception, and implantation (Jasienska & Ellison, 1998). These changes, as well as environmental variation, including developmental conditions, can also shape adult physiology, especially reproductive functioning (Purifoy, 1981; Ellison, 1990; Vitzthum, 1990; Chisolm, 1993).

The Ovarian Cycle

The feedback mechanism of the HPO axis can be manifested in the form of the monthly female ovarian cycle. The ovarian cycle consists of physiological changes in fertile women and some of these changes can be attributed to circadian rhythmicity. It has been found that LH levels vary throughout the day with a circadian rhythmicity, particularly in the early follicular phase of the ovarian cycle. Studies show that the variation of LH everyday is modulated by ovarian cycle. Interaction between ovarian cycle and circadian rhythmicity is involved in the timing of the preovulatory LH surge (McNamara, 2004). Recent studies have shown that FSH might also have a diurnal variation (Mortola et al., 1992).

The inherent fluctuations of these pituitary hormones throughout the day affect the overall variation in levels and pattern of fluctuation of ovarian hormones that characterize the cycle. The ovarian cycle is comprised of menses and 3 phases over the period of 28 days, including: the follicular (preovulatory) phase, ovulatory (periovulatory) phase, and the luteal (postovulatory) phase with ovulation as a dividing event between the follicular and luteal phases (Losos et al., 2002).

The start of menses is considered the first day of the ovarian cycle and it lasts 2-7 days. It is characterized by a 10-80 mL loss of blood due to shedding of the uterine lining (Losos et al., 2002). During menses, levels of LH and FSH are low. Ovarian hormones, such as estradiol and progesterone, are also low. The first phase of the cycle is the

follicular phase, which typically lasts from the end of menses to day 13 of the ovarian cycle. During the follicular phase, the rise in FSH causes ovarian follicles to grow forming an ovum. As these follicles grow, they release estradiol, which initiates the formation of a new endometrium layer. Estradiol peaks in the follicular phase and throughout this phase, it suppresses the production of LH. However, when the egg has matured, LH production is stimulated and reaches its peak. When LH reaches its peak, a mature follicle releases an egg to the fallopian tube, which marks day 14 of the cycle, termed ovulation. Day 15-28 is known as the luteal phase and occurs when the corpus luteum, what is formed in the ovary after the egg is released into the fallopian tube, secretes progesterone. Progesterone is important in preparing the endometrium for implantation. FSH and LH are secreted to help cause the follicle to transform into the corpus luteum. Once the corpus luteum begins to atrophy, levels of progesterone decrease and trigger menstruation. This corpus luteum can be saved if the egg is fertilized resulting in an embryo that produces human chorionic gonadotropin, a hormone that preserves the corpus luteum and is unique to the embryo (Losos et al., 2002).

Previous Studies

Many studies have been conducted to analyze changes in the HPO axis that modulate reproductive effort through onset of ovulation, number of ovulatory cycles or levels of estradiol and progesterone. These studies have collected hormonal data through the use of saliva, urine and blood spot samples. Studies analyzing hormone levels across the cycle have utilized different methods to determine ovulation. One such method uses

salivary progesterone, with progesterone levels greater than 110 pmol/L to indicate ovulation (Vitzthum et al., 2002). Another method uses the estradiol to progesterone ratio such that ovulation is identified as the second day of a 5-day dramatic decline in the estradiol to progesterone ratio (Wander et al., 2008). Other studies predict ovulation as day 13-15 in the ovarian cycle.

Results from these studies show interpopulational variation among reproductive hormones. One study observed that Asians in the United States and the United Kingdom have 55% - 90% of those estradiol levels seen in Caucasian women in those countries (Dickinson et al., 1974; Purifoy, 1981). Another study reported low ovarian steroids in the !Kung San (Vanderwalt et al., 1978). A study conducted by Ellison et al (1989) shows that the Lese women of Zaire ovulate only 33% of the time, while women in Boston ovulate 58% of the time. While the women from Boston had a mean salivary progesterone level of 232 pmol/L, the Lese women had a mean salivary progesterone level of 132 pmol/L, therefore suggesting that Western women had increased allocation of resources towards reproduction (Lipson & Ellison, 1992; Ellison et al., 1989).

Tradeoff

The presence of interpopulational variation with respect to ovarian hormones shows reproductive effort is modulated differently from one population to another. These populations, with different living conditions and social ecologies, consequently have different life history strategies. As a result, for a given amount of finite resources, the

amount of energy allocated towards reproduction varies across different populations. According to life history theory, variations in amount of energy allocated to reproduction could be due to a decrease in overall finite resources available or due to tradeoffs in energy allocations between reproduction, growth and maintenance.

Biologists that study population dynamics model life history tradeoffs in a population using the Euler-Lotka equation. This model calculates fitness from life history variables including mortality and fertility, which represent functions of survival and reproduction (Kot, 2001).

Theory would predict that an increased allocation of resources towards reproduction would result in decreased allocation of resources towards growth or maintenance. Few studies have conducted research on tradeoffs between reproduction and maintenance (McDade et al., 2003). Some energy allocated to maintenance is given to sustain and prepare the immune system for host invasion and repair. Studies in this field show that work done by the immune system is energetically expensive and there are tradeoffs between maintenance and growth and maintenance and reproduction (Buttgereit et al., 2000; Read & Allen, 2000; Lochmiller & Deerenberg, 2000). To assess relative amounts of energy allocated to reproduction and maintenance, hormones across the ovarian cycle will be measured and CRP, a biomarker of the immune system, will be measured, respectively.

Life history is a theoretical projection when studying reproductive ecology. Now adding maintenance, it will require us to be more sophisticated than we have been.

B. Maintenance

Immune Responses to Pathogens

Human interaction with the environment allows humans to be under constant insult by different parasites and pathogens. When humans are infected with microbes and undergo attack by persistent pathogens, the immune system responds with antibodies, cytokines and activity by natural killer cells and T cells, specifically cytotoxic T lymphocytes (CTLs). CTLs are vital in antiviral defense because they attack virus-infected cells and limit virus replication in vivo, allowing them to determine viral load (Nowak & Bangham, 1996). Viral load, which can be limited by CTLs, is an important determinant of disease and individual variation in CTLs can affect outcomes of a disease between individuals. This underlying variation in immune response activity can affect baseline health statuses of individuals, how susceptible they are to persistent pathogens, and therefore can affect levels of their biomarker, which are associated with health status.

C - reactive protein in the human body

C-reactive protein (CRP) is part of the non-specific immune response that provides the body's first line of defense against pathogens. CRP is a protein with a

pentameric disc shape that is found in blood (Thompson et al., 1999). It is synthesized in the liver and is produced in response to the release of cytokines including: IL-6, IL-1, and TNF-α (Libby et al., 2002; Ballou & Kushner, 1992). It binds to phosphocholine, which is expressed on cells in the body, and to bacteria that are dying so it can activate the complement system and cause inflammation (Thompson et al., 1999).

Because of its role in the immune system, CRP is a biomarker that is helpful in identifying an individual's current infectious disease status, cardiovascular risk, and risks for many chronic diseases (Wander et al., 2008). In the absence of an infection, CRP levels are found in the body with a concentration of less than 3 mg/L (Pearson et al., 2003). Its definition as an acute phase protein means that following an inflammatory stimulus, the plasma concentration of CRP rises by at least 25%. During an active infection, the immune system may increase plasma concentrations of CRP (Kushner, 1988). Concentrations of CRP remain elevated for one week during infection before they begin to subside (Gillepsie et al., 1991; Mortensen, 1994).

Significance of CRP

CRP is used as a high sensitivity assay to analyze health status and inflammation and is used in this study because previous findings suggest that it varies with the ovarian cycle. This variation can be important in portraying an accurate and clear depiction of degree of tradeoff between reproduction and maintenance. Anthropologically speaking, higher levels of CRP in populations are indicative of infectious burden and show that

pathogenic agents surround the environment and inhabit human hosts. Immune processes that are associated with fighting infections require a lot of energy expenditure (McDade et al., 2005). Because a lot of the body's energy resources are dedicated to combating pathogens, a tradeoff between antipathogen resources and growth or reproduction can be predicted (McDade et al., 2003; McDade & Worthman, 1999). A population with a high level of CRP could indicate that much of its energy resources are devoted to maintenance and fewer resources are devoted to growth and reproduction. This could be manifested through physical characteristics and birth rates of the population.

Infection and CRP

Epidemiologic evidence suggests that during inflammatory responses, CRP levels increase (Kushner, 1988). Interleukin 6 (IL-6) is a proinflammatory cytokine and is produced in activated leukocytes, adipocytes, and endothelial cells. CRP is a downstream mediator of the acute phase response and is derived via IL-6 dependent hepatic biosynthesis. IL-6 induces gluconeogenesis suggesting that inflammation may be important in the etiology of diabetes (Pradhan et al., 2001). This can result in a high level of CRP in the blood.

Chronically increased basal levels of CRP pose an increased risk of diabetes, cardiovascular disease, and hypertension in patients (Pradhan et al., 2001; Dehghan et al., 2007). CRP can be used to predict cardiovascular risk and mortality in diseased and healthy men and women (Kushner et al., 2006). For example, the Center for Disease

Control has recommended the following when classifying cardiovascular risk using CRP values from serum or plasma: Low risk: CRP<1.0 mg/L; Normal risk: CRP ≥ 1.0 mg/L, ≤ 3.0 mg/L; High risk: CRP >3.0 mg/L (Pearson et al., 2003). CRP is involved in inflammation pathways for many different diseases and serves not only as a marker to identify inflammation, but also serves as an intervening variable on the causal pathway (Ross, 1999; Libby et al., 2002; Dandona et al., 2004). CRP can induce proatherosclerotic activities in vascular endothelial cells, thereby promoting the development of cardiovascular disease (McDade et al., 2006).

When an injury or an infection occurs, CRP levels can increase up to 50,000 fold with its peak at 48 hours (Pepys & Hirschfeld, 2003). During a mild inflammation or viral infection, CRP levels increase to a concentration of 10-40 mg/L. In an active inflammation or bacterial infection, CRP concentration increases to 40-200 mg/L while severe bacterial infections and burns cause the highest concentration in CRP of greater than 200 mg/L (Clyne & Olshaker, 1999). Previous assays for CRP were insufficient in detecting levels of CRP to characterize vascular disease confidently. This led to the development of high sensitivity CRP assays (Ridker, 2003).

Pregnancy and CRP

Aside from infection, pregnancy can greatly increase CRP levels. A study conducted by Watts et al (1991) shows that median CRP values for women in labor are about 13 mg/L. Increased levels of estradiol during pregnancy are associated with

increased CRP production in the liver. Alternatively, high levels of CRP may reflect an immunologic response to the fetus or an ongoing maternal inflammation.

Additional Factors that Alter CRP

There are many different factors that affect CRP levels in the human body and these factors can be divided into two categories – biological traits and lifestyle factors. Biological traits are traits that an individual is unable to control such as: sex, age, and birth weight. Lifestyle factors that individuals can alter and maintain include: obesity, physical fitness, stress, socioeconomic status, diet, and medication use. These factors are associated with increased levels of cytokine IL-6, resulting in higher levels of CRP during these conditions (Yudkin et al., 2000).

Perhaps one of the most important factors when analyzing CRP is sex. Adult females tend to have higher CRP levels than adult males (Wener et al., 2000; Ford et al., 2003). The presence of this sex difference in basal CRP levels prompts the search for physiological differences that might cause this occurrence. Previous research on sex and exogenous hormones affecting CRP levels has prompted the recent investigation in the role of endogenous hormones and levels of CRP with a particular examination of the female ovarian cycle. Increased levels of estradiol are associated with increased levels of IL-6 production by dendritic cells and consequently, increased levels of CRP, which will be discussed shortly (Fish, 2008). In addition to sex, age also affects CRP levels such that increases in CRP levels are associated with increases in age (Chenillot et al, 2000;

Ford et al. 2003). Increases in age are associated with increases in cumulative pathogen exposure resulting in an increase in levels of IL-6, which can cause an increase in levels of CRP (Mysliwska et al., 1998). Birth weight is another factor that affects CRP and is inversely related to CRP. There are two possible mechanisms that explain the inverse relationship between CRP and low birth weight. The first mechanism suggests that low birth weight suppresses the immune system making it more susceptible to infections early on in life, resulting in increased levels of CRP. The second mechanism suggests that trans-placental passage of maternal hormones in response to maternal stressors may lead to persistent inflammatory responses in offspring (Tzoulaki et al., 2008).

Given age and sex of an individual, medications may influence CRP levels. For both males and females, anti-inflammatory drugs, such as corticosteroids and statins, are associated with decreasing CRP levels (Sin et al., 2004). However, the relationship between CRP levels and oral contraceptive use and hormone replacement therapy in women is of particular interest. Most studies have shown that birth control pills tend to elevate CRP levels (Dreon et al., 2003). Plasma CRP levels were 2 times higher among women who used an oral contraceptive (with <50 μg of estradiol) than women who did not use an oral contraceptive (Dreon et al., 2003). In addition, treatment with 17β-oestradiol as HRT or combined oral contraceptive with ethinylestradiol shows a significant increase in CRP levels, suggesting that increased CRP levels are associated with natural and synthetic oestrogen (Kluft et al., 2002).

Multiple studies conducted on the relationship between plasma concentrations of CRP levels and HRT show that increased plasma concentrations of CRP are associated with women using HRT and are not associated with women not using HRT (Ridker et al., 1999; Pradhan et al., 2002). Women administered low doses of estradiol HRT result in lower levels of CRP compared to CRP levels in women who were administered higher doses of estradiol HRT (Decensi et al., 2002; Ropponen et al., 2005). Results from studies examining CRP and progesterone HRT, however, show that women taking progesterone HRT experience decreases in levels of CRP (Cushman et al., 1999; Skouby et al., 2002). Although most researchers agree that a positive association between CRP levels and estrogen HRT exists, most researchers debate the relationship between estrogen HRT and CRP versus the combination of estrogen plus progesterone HRT and CRP. Ridker et al (1999) found no difference in CRP levels between women using estrogen alone and women using estrogen plus progesterone suggesting both estrogen HRT and combined HRT had the same effect. This finding is contrasted by Mikkola & Clarkson (2002), who found that CRP levels are 60% higher among women using estrogen HRT. However, there is no effect on CRP levels in women using estrogen plus progesterone HRT, suggesting that the two therapies have different effects.

The major lifestyle factor that can affect CRP levels is body weight. Adipose tissue produces approximately 25% of the body's circulating IL-6, the cytokine that regulates CRP production (Das, 2001). A study conducted by Visser et al (1999) states that increasing BMI in women is associated with an increase in prevalence of elevated CRP. Obese women are 6.21 times more likely to have elevated CRP levels compared to

their normal weight counterparts. Other studies have also supported this claim stating that excess body fat is associated with higher levels of CRP (Ford, 1999; Danesh et al., 1999; Greenfield et al., 2004). Obese individuals possess more adipocytes, which produce tumor necrosis factor (TNF)-α. TNF-α is associated with increases in IL-6 (Hak et al., 1999). Another related factor that affects CRP levels is physical fitness. It can be inferred that since obesity is associated with high levels of CRP and because exercise can prevent weight gain, exercise can lower levels of adipocytes and therefore, decrease levels of IL-6 and CRP (Ford, 2002). This relationship, observed by LaMonte et al (2002) is contrasted by results found by Kop et al (2008), who showed that CRP levels significantly increase after exercise on a treadmill.

Mental and psychosocial stressors have also been associated with CRP in serum. One study shows that after a mental stressor is initiated and corresponding nor-epinephrine levels increased, CRP levels significantly increased (Kop et al., 2008). Similarly, increased levels of psychosocial stress are associated with increased levels of IL-6 and consequently, increased levels of CRP (Melamed et al., 2004; Jeanmonod et al., 2004). Related to stress, socioeconomic status (SES) is another lifestyle factor that greatly affects CRP serum concentrations. CRP is lower in middle-aged women of a higher SES compared to less affluent counterparts (Owen et al., 2003). This is because low SES populations have poor sanitary conditions and lack of access to healthcare which allow diseases and infections to progress.

Another important factor that can influence CRP levels is diet. A study was conducted in which a randomized trial and patients in the intervention group were instructed to follow a Mediterranean-style diet rich in monounsaturated fat, polyunsaturated fat, and fiber with a low omega-6 to omega-3 fatty acids ratio. Participants consuming this intervention diet have significantly reduced serum concentrations of CRP compared to people who do not consume this diet, suggesting this sort of diet is associated with decreased levels of CRP (Esposito et al., 2004). This diet is primarily low in fat and therefore, there is less IL-6 production and CRP production. Alcohol consumption and smoking can also affect CRP levels. In a randomized diet-controlled intervention study, women who consume three glasses of beer with evening dinner for 3 consecutive weeks have CRP levels decreased by 35% compared to women who did not drink beer for 3 weeks (Sierksma et al., 2002). Alcohol is associated with the suppression of IL-6 production in alveolar macrophages and human blood monocytes (Nelson et al., 1989). Although moderate alcohol consumption is associated with decreased levels of CRP, smoking is associated with increased levels of CRP (Chenillot et al., 2000). Smoking promotes atherosclerotic events and the formation of plaques in blood vessels, which is associated with an increase in inflammatory processes as evidenced by a rise a in CRP levels (Wallenfeldt et al., 2001).

CRP across the Ovarian Cycle

Relationships between levels of individual hormones and levels of CRP have been analyzed, yet only a few studies have been conducted on CRP across the ovarian cycle. In

one study conducted by Wander et al (2008) hormone levels were measured from urine samples and CRP levels were measured from finger-stick blood spot samples. Samples from women living in Seattle show initial levels of estradiol at 34,000 pg/mL, followed by a peak at 80,000 pg/mL by ovulation and a slow decline to 60,000 pg/mL in the luteal phase. Progesterone however, starts at 4,000 ng/mL and remains constant and after ovulation, progesterone levels steadily increase to 16,000 ng/mL and slowly decline to 12,000 ng/mL in the luteal phase. CRP levels are highest during menstruation at 2.8 mg/L and decline to 1.5 mg/L after menses and remain at 2.0 mg/L during the remainder of the cycle (Figure 1 in Wander et al., 2008). As the end of the luteal phase approaches, levels of CRP tend to increase steadily to 2.4 ng/mL. When analyzing CRP in relation to these reproductive hormones, it has been found that when controlling for estradiol and menses, a ten-fold increase in progesterone is associated with a 23% increase in CRP. Meanwhile, when controlling for progesterone and menses, a tenfold increase in estradiol is associated with a 29% decrease in CRP. Finally, when controlling for both estradiol and progesterone, menses is associated with a 17% increase in CRP (Wander et al., 2008). Wander's research also shows that during the follicular phase, the effect of estradiol on CRP is more significant than it is across the entire cycle. During the luteal phase, the effect of progesterone on CRP is significant. There were no findings of an association between FSH and CRP. However, when the highest estradiol values are excluded, there is an overall negative relationship between CRP and estradiol. Other research shows that progesterone could be the main affecter of CRP levels. Bouman et al (2005) states that progesterone levels increase IL-6 production by monocytes. According to this hypothesis, CRP levels should increase as progesterone levels increases. Previous

research shows that when controlling for estradiol and menses, a tenfold increase in progesterone levels is associated with a 23% increase in CRP levels (Wander et al., 2008). Although the exact cause of the fluctuating CRP levels could be attributed to both estradiol and progesterone, more samples and research needs to be done in analyzing CRP across the cycle.

Previous Studies of CRP Across the Ovarian Cycle

As an important factor when analyzing CRP, sex differences such as hormones across the ovarian cycle, are studied to identify correlations between hormone levels and CRP levels. Analyses of CRP fluctuations across the ovarian cycle are limited and most have been conducted in Western populations. Existing findings of CRP across the ovarian cycle are inconsistent. A study conducted in Austria showed that women who have a 10-fold increase in progesterone at midcycle have higher relative increases in CRP levels at midcycle. There is a correlation between the relative increase in CRP levels at midcycle and the relative increase in progesterone levels during midcycle and luteal phase (Jilma et al., 1997). Analyses of American women show that estradiol levels increase during the follicular phase, with a peak at ovulation followed by a decrease in estradiol levels in the luteal phase. Progesterone steadily increases with a peak in the luteal phase followed by a steady decline thereafter. In the American population, CRP varies throughout the cycle. CRP is highest at menses and sharply decreases at the end of menses but slowly increases and remains constant throughout the follicular phase and most of the luteal phase (Wander et al., 2008). However, studies show that a cyclic pattern for CRP is not

observed in Swiss women. Rather, levels of CRP are constant with no significant change throughout the cycle (Wunder et al., 2006).

The CRP assay is used to study relationships between inflammation and degenerative diseases such as atherosclerosis, osteoporosis, and heart disease in Western populations (Danesh et al., 2000; Pradhan et al., 2001). Although CRP is used as an important marker to evaluate health risk in Western populations, CRP as a biomarker has not been used in non-Western populations due to the lack of available data on CRP variation in those populations. Most data on CRP levels have been collected in primarily European or North American populations and very few data have been collected about CRP in various ethnic groups and developing nations (Snodgrass et al., 2007).

Immunity and the Ovarian Cycle

Although, CRP is not a part of the cell-mediated response, it is a marker that can provide a glimpse of quality of immune system functioning. CRP levels increase with increasing levels of IL-6 produced by T_H2 cell responses (Rincon et al., 1997). CRP has also shown to vary across the ovarian cycle . The involvement of CRP in both the immune system and reproductive system suggests not only is there a relationship between both systems, but also that CRP is a possible link between both systems.

Initial findings in the field of reproduction and immunity suggest menstruation as a mechanism for pathogen defense. According to this hypothesis, menstrual blood

removes endometrial tissue that appears as necrotic and sends immune cells to the uterine cavity in order to fight pathogens (Profet, 1993). However, many studies have shown that this is not the case. If this hypothesis were true, uterine pathogens would be more prevalent before menses than after menses, however this is not observed (Strassman, 1996). Recent studies have suggested that menstruation exacerbates infection (Strassman, 1996). This could be due to down regulation of immune cells that fight infection or premeditated increase in cytokines preparing for upcoming infection.

More extensive research shows that communication between the immune and reproductive systems occur through chemical changes, with regards to concentration of T_H1 and T_H2 cells. T_H1 cells are T cells that interact with macrophages and when they receive the proper stimulation, they secrete INF-γ, TNF-β and increase the proliferation of cytotoxic T cells (Fish, 2008). T_H2 cells, however, interact with B cells and when stimulated, secrete IL-4, IL-5, IL-6, IL-10 and IL-13. These two types of T helper cells fluctuate throughout the ovarian cycle. Regulatory T cells (T reg cells) are a specialized population of T cells that suppress activation of the immune system and these cells also fluctuate throughout the ovarian cycle. During menstruation, T_H1 cell responses are high while T_H2 cell responses are low. When the cycle enters the follicular phase and estradiol levels peak, T reg cell responses increase, T_H1 cell responses decrease while T_H2 cell responses increase. When ovulation starts and throughout the luteal phase, when estradiol levels decrease, T reg cell responses decrease, $T_H1$cell responses increase and T_H2 cell responses decrease and both T_H1 and T_H2 cells maintain their initial levels (Fish, 2008).

Prior research finds that the fluctuating levels of estradiol are responsible for fluctuations in helper T cell responses across the ovarian cycle. Estradiol affects many types of cells in the immune system including: CD4+ T helper cells, natural killer (NK) cells, dendritic cells, B cells, macrophages and neutrophils when it binds to receptors on specific estradiol receptors on surfaces of these cells (Figure 2 & 3 in Fish, 2008). Estradiol affects NK cells by decreasing their cytotoxic activity. Activation of estradiol receptors in B cells causes increased survival of autoreactive B cells and increased IgM and IgG antibody production. Estradiol affects macrophages by decreasing cytokine production including production of IL-6 and TNF. When it binds to receptors on CD4+ T cells, T reg cell numbers increase, TNF production decreases. At high levels of estradiol, T_H2 cell responses increase and at low estradiol levels, T_H1 cell responses increase. In dendritic cells, increased levels of estradiol are associated with increased expression of IL-6 and IL-8 by immature dendritic cells. Finally, estradiol is associated with a reduction in chemotactic activity and a gain in anti-inflammatory activity when it binds to receptors on neutrophils (Fish, 2008).

Although estradiol is associated with a decrease in IL-6 production by macrophages, its affects are dominated by increases in T_H2 cell response, which are associated with, increases in IL-6 production, and increases in IL-6 production by dendritic cells. Based on these proposed mechanisms, a positive relationship between CRP and estradiol is expected, such that when CRP is low, estradiol should be low and vice versa (Fish, 2008).

Population of Interest

Of particular interest is studying the influence of reproductive hormones on CRP in women outside the United States. Previous research has shown varying levels of salivary progesterone between women in industrialized populations and women in non-industrialized populations. Women of reproductive age in industrialized populations have higher levels of salivary progesterone compared to women in developed countries (Wilson et al., 1992; Ellison et al., 1993). In developed countries, such as Poland, there are higher salivary progesterone levels than developing countries such as Zaire (Vitzthum et al., 2002). If levels of reproductive hormones differ between industrialized and non-industrialized populations, it is possible that if CRP is affected by reproductive hormones, it too will differ between populations.

Previous research conducted outside the U.S. has been restricted to homogenous rural populations (McDade, 2005). It is not known if those populations living in urban areas in less developed countries are similar to their counterparts in other urban settings, or if the presence of the population in a less developed country plays a greater role in affecting reproductive functioning.

To analyze differences in reproductive functioning between developed and less developed countries, Bolivia, one of the poorest countries in Latin America, was chosen as the study site. Citizens of Bolivia have limited access to health care as evidenced by the high levels of morbidity and mortality among women and children (Schuler et al.,

1994). This study will examine urban poor (urban-p) and urban better-off (urban-b) populations in Bolivia.

Results from a study conducted in Bolivia (Vitzthum et al., 2002) showed that salivary progesterone levels are higher in urban better-off compared to urban poor Bolivian women. These differences in progesterone levels may affect CRP levels and may be the cause of differences in CRP levels between these two populations. These differences may be attributed to varying levels of stress since urban poor populations have a decreased caloric intake and fewer energy resources, which impact immune responsiveness and reproductive functioning (Vitzthum et al., 2002).

Purpose

There have been many attempts to explain interpopulational variation in reproductive hormones (Ellison, 1990; Vitzthum, 2001; Vitzthum et al., 2000). However, these studies have been limited by small sample sizes, recently pregnant or lactating women, or women with sexually transmitted diseases which can be a source of skewed results (Vitzthum et al., 2000). Similarly, there have been many studies conducted on CRP levels and associated risks of chronic diseases. Reproduction and maintenance have been studied in great depth as independent body functions, however, very few studies have analyzed the interaction between the reproductive system and immune system in industrialized populations, much less in non-industrialized populations.

The main purpose of this study is to analyze the interaction of reproductive functioning and functioning of the immune system. This study will attempt to understand the tradeoffs between reproduction and maintenance and by studying this tradeoff, the use of CRP as a biomarker will be better understood. In order to utilize CRP as a biomarker more accurately, a better understanding of how CRP fluctuates across the ovarian cycle is necessary. When evaluating health status of women in different populations living in different environments and resources, it is important to understand how reproductive functioning is modulated and the effect of this on maintenance. This will allow for a better understanding of CRP and the interaction between reproduction and maintenance efforts in the body. This study will allow for the comparison of known U.S. samples of ovarian hormone and CRP levels, with Bolivian samples of ovarian hormone and CRP levels. Studying CRP levels across the cycle in non-Western or non-industrialized women will allow for better interpretation of the CRP measure. Studying Bolivian women and understanding how CRP fluctuates across their ovarian cycles will help determine if using CRP as a biomarker to assess health status in non-industrialized populations could be confounded by stage in the ovarian cycle in those women. Furthermore, a better assessment of the relationship between reproduction and maintenance in ancestral populations can be made.

The goal of this study is to analyze CRP across the ovarian cycle to assess if CRP is affected by concentrations of different ovarian hormones across the cycle. Alternatively, CRP levels may not vary throughout the cycle and CRP and ovarian hormone levels may not differ between Bolivian women and women living in the United

States. However, determining this will help assess if CRP is a utilizable marker to assess health status. Amount of CRP in the Bolivian population will be compared to the reported amount of CRP in the U.S. population to suggest relationships between reproductive functioning and maintenance in both populations.

Through accurate analysis of serum levels of reproductive hormones and CRP in this population, more insight into where more energy is being allocated among reproduction and maintenance will be provided. This study will not determine which body function will be compensated or to what degree. Nor will it determine energy allocated toward reproduction or maintenance in the form of calories. This study will simply examine CRP in relation to reproduction to determine broad range of effects and strong relationships, which will be useful for possible in depth analysis in the future.

Hypotheses

Ovarian cycles differ between Western and non-Western populations. Because interpopulational variation exists among ovarian cycles, it can be inferred that these populations allocate different amounts of energy toward reproductive functioning. Because of tradeoffs, these populations must allocate different amounts of resources toward maintenance and immune functioning. Since more energy can be allocated toward reproductive functioning in developed countries, it is hypothesized that concentrations of different hormones in Bolivian women will be lower compared to those countries and more resources can be allocated to maintenance resulting in higher levels of CRP.

Lifestyle differences in activity and diet are also likely to contribute to individual differences in CRP and can be captured by analyzing effects of BMI and socioeconomic status.

1. **Biological factors:**

Hypothesis 1: On average, CRP levels in non-infected Bolivian women will be higher than in Western women.

Vitzhum et al (1994, 2000) shows that progesterone levels are lower in Bolivian women than in Western women. According to life history theory and if this is true, in Bolivian women, less energy is devoted to reproduction, and thus more energy is devoted to maintenance and immune function.

Hypothesis 1b: This difference will remain in both ovulatory and anovulatory cycles.

The previous notion that varying amounts of exogenous estradiol can have effects on CRP (Prestwood et al., 2004) also allows for the hypothesis that if estradiol levels in Bolivian women are lower than those of Western women, then CRP levels in Bolivian women will be lower. However, if estradiol levels of Bolivian women are equal or greater than those of Western women, then CRP levels will be equal or higher than CRP levels of Western women, respectively.

Hypothesis 2: Levels of estradiol in the follicular phase will be lower in Bolivian women than in Western women.

Women in Bolivia survive on low caloric intake and their reproductive functioning has been found to be adjusted accordingly. The Food and Agriculture Organization showed that average energy intake in American women is 15.3 MJ/day, while in Bolivian women, it is 9.2 MJ/day (Jasienska & Thune, 2001). Because there is a lower caloric intake in Bolivian women compared to American women, it is expected that there are less total resources and less resources dedicated to reproductive functioning in Bolivian women compared to Western women.

Hypothesis 2b: This difference will remain when anovulatory cycles are excluded.

If the egg is not fertilized, the corpus luteum does not generate and therefore, an increase in key players of immune functioning are not elicited and CRP does not increase. Because the egg does not release from the ovary, the possibility of an increase in CRP does not occur and therefore, is relatively constant.

Hypothesis 3: Progesterone levels in the postovulatory phase will be lower in Bolivian women than in Western women.

Progesterone is released by the corpus luteum and drives ovulation by preparing the uterine lining for implantation. Because of its key role in ovulation, Bolivian women, with their low caloric intake and decreased investment in reproduction, should also have decreased levels of postovulatory progesterone.

Hypothesis 4: As age increases, CRP levels will increase.

As age increases, women surpass the age in which they can successfully reproduce to have viable offspring. Therefore, according to life history theory, as age increases, women devote less of their total energy resources toward reproduction and more is invested in survival and maintenance. Also, cumulative systemic burden increases and therefore, CRP levels should increase.

2. **Lifestyle factors:**

Hypothesis 5: BMI will be correlated with CRP.

Increased BMI will be correlated with increasing levels of CRP. BMI is a measure of fat based on a given height and weight. Thus, a greater BMI implies more fat stores and greater amounts of fatty tissue or adipocytes. Adipocytes generate IL-6, which is associated with an increase in CRP.

Hypothesis 6: CRP will be higher in urban poor than urban better-off Bolivian women.

In poor Bolivian women, nutritional deprivation can create a stressful environment that causes an increase in resources dedicated to survival and maintenance. This stress may be associated with higher levels of CRP in poor Bolivian women. However, it is possible that ecological factors could confound this relationship. Urban poor and urban better-off populations may have baseline differences in CRP levels due to different histories, work load, diet, stress, and physical activity.

Hypothesis 7: Follicular levels of estradiol will be lower in poor than non-poor women.

Due to a low caloric intake in poor compared to non-poor populations, there are fewer total energy resources and less resources are allocated to reproduction in Bolivian women. This results in lower levels of estradiol.

3. Cycle progression and CRP

Hypothesis 8: CRP levels will fluctuate across the ovarian cycle.

Reproductive hormones, particularly estradiol and progesterone, fluctuate across the ovarian cycle. Previous studies have described the process of ovulation as an orderly

sequence of events similar to an acute inflammatory response (Adashi, 1990). Thus, it is expected that CRP levels will increase as a result of these hormonal changes.

Hypothesis 9: CRP levels will show no systematic change during the mid-late follicular phase (day 6-12).

During day 6-12 of the ovarian cycle, levels of estradiol and progesterone steadily increase but are not drastically different from early follicular phase. If there is a relationship between CRP and estradiol, then CRP should remain relatively constant during the mid-late follicular phase since estradiol does not considerably increase during this time in the cycle.

Hypothesis 10: CRP will be positively correlated with progesterone in the luteal phase.

According to Wander et al (2008), during the luteal phase, progesterone increases while CRP increases. Levels of IL-1, a cytokine positively associated with CRP, increase with increasing levels of progesterone (Jilma et al., 1997). Therefore, the rise of progesterone in the luteal phase will most likely be associated with the rise of CRP in the luteal phase.

Hypothesis 11: At low levels of progesterone (<0.8 ng/mL) on days 16-24, CRP will not differ from follicular levels.

Cycles with low levels of progesterone are anovulatory and thus are not characterized by a rise in progesterone. Since progesterone is associated with IL-1 and CRP, if there are no significant changes in levels of progesterone between day 6-12 to day 16-24, it is hypothesized that there will be no changes in levels of CRP. However, at high levels of P (≥0.8 ng/mL), CRP will be lower than follicular levels.

II. Methods

Populations and study design

Populations and study design procedures have been described in Vitzthum et al (2002). Female participants were recruited in May-June 1995 in La Paz, Bolivia through means including word of mouth and announcements. Informed consent was obtained using an approved protocol (IRB, University of California, Riverside). 31 participants from the national medical school comprised the economically better-off sample while 30 residents from an impoverished neighborhood around La Paz comprised the poorer sample. Women from both samples were administered screening interviews in the native language. Women were only admitted into the study if: they were living at high altitudes (>3500 m) since birth or early childhood, were 23-35 years old, were reporting regular ovarian cycles lasting 25-35 days in length and were free of any known previous or current sexually transmitted diseases or reproductive disorders. These women were not pregnant, lactating, using hormonal contraceptives or medications for the last 6 months, nor were they experiencing any significant gains/losses in weight (+/- 2 kg). Height, weight, and BMI (kg/m^2) were measured and calculated for these women.

Blood Spots

Since levels of CRP offer a glimpse of immune system functioning and because hormonal fluctuations throughout the cycle may influence the immune system, CRP and hormones were measured throughout different points in the ovarian cycle.

Blood spots were collected from each woman from day 6 to day 24 of the ovarian cycle, for 2 consecutive cycles, for a total of 5 paired blood spots with collections on day 6-8, 9-10, 11-12, 13-15, 16-19, and/or day 21-24. Spots were assayed for: FSH, LH, estradiol, progesterone, and CRP. Blood spot collection and assays for FSH and LH were obtained from Worthman & Stallings (1997). To collect blood spots, one fingertip was wiped with an alcohol swab and pricked with a lancet pressed firmly to the finger (Unilet Blood Lancets; VWR Scientific Products, Stone Mountain, GA). As blood flow began, the initial bit of blood was wiped away with a tissue since it may contain contaminants. As more blood flowed, the finger was held slightly above the filter paper and drops of blood were placed on each of five preprinted circles on the filter paper, which were standardized to absorb equal amounts of blood (#903; S&S). Subject information was written on this filter paper and the filter paper filled with the five blood spots was left to air-dry at room temperature for 3-4 hours. After the blood spots dried, the card was placed in a zip-lock bag, which was stored in a sealed plastic box in a refrigerator at 2-4°C until it was sent to Emory University . Samples were sent to Emory University within 6 weeks of being collected. Upon receipt, samples were stored at -26°C until analysis took place.

Standards and controls for assays were produced as described in Worthman & Stallings (1997). Quality controls (QC) for each assay are mentioned below. The sensitivity of the assay, or minimal detectable dose (MDD), was derived using the mean +2 SD absorbance of the "zero calibrator" (n = 10 in a single run).

Assay for FSH and LH

Assays were performed in February 2005 using Delfia kits (FSH kit, A017-201; LH kit, A031-10;Wallac, Inc.) to measure FSH and LH in blood spots. In analyzing FSH and LH, hormone levels were detected by using two monoclonal antibodies directed against separate antigenic sites. One antibody was immobilized on the wells of the assay strips and the other antibody was europium-labeled. Through competition between a radioactive and nonradioactive antigen for a given number of antibody sites, concentrations of hormones were determined since they are inversely proportional to the amount of ^{125}I - labeled hormone bound to the antibody (Yalow & Berson, 1971).

Blood spot standards and samples were removed from the freezer and 3 μl whole blood (= 2.5 mm discs using hole punch) were transferred (using tweezers) to assay strips consisting of 12 microtitre antibody-coated wells prewashed with wash solution using an automatic washer (Platewash automatic washer, model 1296-024;Wallac, Inc.). After 30 minutes, 200 μl assay of assay buffer were added to each well and then shook using a automatic plateshaker (Plateshake automaticshaker, model MPS-4; Wallac, Inc), which rotated at room temperature at 150 rpm for 10 min. After rotation for the allotted time

was completed, wells were placed in an air tight container overnight at 4°C. After incubation, the wells were placed on an automatic shaker at 50 rpm for 1 hour at room temperature. Next, using a Pasteur pipette, the filter paper discs were removed from the wells using vacuum aspiration. The strips containing the wells were washed with kit wash buffer two times, using the automatic washer, and 200 µl of diluted europium-labeled tracer solution (1:100 for FSH and 1:150 for LH) were added to the wells. The wells with the tracer incubated for 30 minutes and 15 minutes when measurements of FSH and LH took place, respectively. After that, the strips were placed on the automatic shaker at 150 rpm for 2 min. Each strip was washed six times using the automatic platewasher. 200 µl of kit enhancement solution were then added to the wells on the strips and these strips were rotated on the automatic shaker at 50 rpm for 5 minutes. Next, the wells were incubated at room temperature for 10 minutes and fluorescence was measured using a fluorometer (Arcus time-resolved fluorometer, model 1230; Wallac, Inc.). Concentrations of FSH and LH were calculated from the standard curve using a linear/log data reduction method and converted to plasma equivalents from regression analysis of matched blood spot samples.

The mean values of the FSH QCs for the low, medium, and high controls were 3.89, 7.17, and 18.01 U/L, respectively. The FSH interassay coefficients of variation (CVs) were 7.8%, 9.9% and 9.0%, respectively. The respective intraassay CVs were 5.3%, 7.4% and 9.1%. The FSH assay MDD was .13 U/L. The mean values of the LH QCs were .98, 9.72, and 32.54 U/L for the low, medium, and high controls, respectively.

The LH interassay CVs were 13.5%, 20.8% and 19.0% while the intrassay CVs were 10.94%, 3.64%, and 11.34%, respectively. The MDD for the LH assay was 0.26 U/L.

Assay for Estradiol and Progesterone

The assays for estradiol and progesterone were conducted November 2004-February 2005 and are based on a 17 beta-estradiol and progesterone ^{125}I radioimmunoassay (Shirtcliff et al., 2000) using kit reagents from Diagnostic Systems Laboratories (Estradiol kit, DSL-4800; Progesterone kit, DSL-3400). First, elution buffer (pH 7.4) was prepared by adding 100 mg of gelatin to 100 ml of Dulbecco's buffer (catalog #141-90144, Gibco/Invitrogen, Grand Island, NY). Next, the mixture was heated to 45 °C and stirred to dissolve contents. The solution was titrated with HCl or NaOH as necessary. After the buffer was prepared, standards, controls and samples were removed from the freezer. 12x75 borosilicate glass tubes were then appropriately labeled for standards, controls and samples. Using a hole punch, eight 1/8" spots were punched out from the sample spots. Using tweezers, 4 discs were then transferred to appropriate tubes. After 350 µl of the buffer were added to the tube, tubes were vortexed and rotated on an automatic shaker at 300 rpm for 30 minutes at room temperature. Following that, the rack was rotated 180° and shook for an additional 30 minutes. The tubes were incubated overnight at 4°C and the next day were brought back to room temperature. At this time, the tubes were vortexed and shook at 300 rpm for 30 minutes, rotated 180°, and shook for a final 30 minutes. Using a gamma counter for 5 minutes, concentrations of estradiol and progesterone in the blood spots were calculated.

The mean values of the estradiol QCs are as follows: 13.61, 38.48, and 129.01 pg/mL for the low, medium, and high controls, respectively. The interassay CVs were 34.82%, 15.64%, and 29.66%, respectively. The respective intrassay CVs were 19.83%, 15.23% and 9.24%. The MDD for the estradiol assay was 4.2 pg/mL. The rabbit anti-estradiol serum was the antiserum that cross reacted 6.9% with estrone, and <1% with equilin, equilenin, and 17β-estradiol-3-glucoronide. The mean values for the progesterone QCs for the low, medium, and high controls were 0.61, 4.47, and 12.86 ng/mL, respectively. The progesterone interassay CVs were 28.95%, 17.12%, and 6.05% while the intrassay CVs were 11.66%, 7.60%, and 2.48%, respectively. The MDD for the progesterone assay was 0.17 ng/mL. The rabbit anti-progesterone serum was the antiserum that cross reacted 5% with 5α-Pregnane-3,20-dione and <1% with other related compounds.

Assay for CRP

The CRP assay is a europium labeled biotin-streptavidin system. Streptavidin A (Invitrogen #43-4302) was added to 100 mM citric phosphate buffer (pH 5.0) remaining overnight at room temperature. 100 µl of the Streptavidin A working solution were added to each well for a final concentration of 0.5 µg/well.

To biotinylate CRP monoclonal antibodies, first, sodium azide was removed from anti-CRP monoclonal antibodies (clone C2) using PD-10 equilibrated in PBS (pH 7.4) at

4°C. The purified antibodies were concentrated to 4 mg/ml and labeled with Biotin (Biotin-XX-SSE) for one hour at room temperature and then shook at 400 rpm. These biotinylated anti-CRP monoclonal antibodies were purified by desalting on a PD-10 column and protein content of each fraction was monitored via A280. Fractions with concentrations below 0.1 mg/ml were pooled. After that, labeled antibodies were filtered using a .22 µm syringe filter and biotin was quantified using Pierce's HABA Biotin Quanitation Kit. An average of 7 moles of Biotin per mole of IgG were incorporated.

CRP monoclonal antibodies were also labeled with europium. Anti-CRP monoclonal antibodies (clone C6) were dialyzed overnight using 1 liter of 100 mM Carbonate Buffer (pH 9.3) at 4°C. The concentration of dialyzed antibodies was adjusted to 2 mg/ml. For labeling to occur, 120 molar excess of anti-CRP monoclonal antibodies, clone C6, were reacted with 1200 mmol/L Eu-N^1-ITC chelate in a borosilicate glass test tube for 20-24 hours at 4° C and shook at 400 rpm.

Excess chelate was removed by diluting the reaction in 4 ml of TSA 50 mM Tris-HCl, 0.9% NaCl, and 0.1% NaN_3 at pH 7.8) and concentrated to 500 µl using a centrifugal filter (Amicon). Labeled antibodies were further purified on a PD-10 equilibrated in TSA and collected in 250 µl fractions with protein content estimated via A280. An average of 6 moles of europium per mole of IgG were incorporated.

Briefly, Streptavidin A was coated on a mirotitre plate and bound the biotinylated capture antibody to CRP clone, C2. A second antibody labeled with europium, then

bound to the Streptavidin A Biotin – C2 – CRP complex. Using Delfia enhancement solution, europium was removed and amount of fluorescence was directly proportional to the CRP concentration in each well.

After reagents necessary to carry out the assay were prepared, using a hole punch, one 1/8” spot was punched out from each sample spot. Using tweezers, discs were then transferred to appropriate tubes. 125 µl of the Delfia assay buffer (pH 7.75) were then added to the tube. Tubes were vortexed and rotated on an automatic shaker at 300 rpm for 1.5 hours at room temperature. Next, the Streptavidin coated mirotitre plate was washed two times with 350 µl of the Delfia assay buffer. Paper towel was used to blot the plate to ensure removal of liquid from the wells. To block the plate, 200 µl of the Delfia assay buffer were added to the plate and the plate shook at 400 rpm for 1.5 hours, rotated 180° after 45 minutes. The blocked plate was then washed 3 times using 350 µl of the Delfia wash buffer and wells were blotted with a paper towel. 50 µl of the eluate were pippeted into appropriate wells. Then, the reactant working solution was made by the addition of 12 mL of Delfia assay buffer to C2-Biotin and C6-Europium, both of which were added resulting in a final concentration of 0.5 ng/µl each. This solution was vortexed and 100 µl of it were transferred to each well and left to incubate at room temperature on the automatic shaker at 400 rpm for 2 hours, and the plate was rotated 180° after 1 hour. After incubation, plates were washed 8 times with 350 µl of Delfia wash buffer and 200 µl of Delfia enhancement solution (pH 3.2) were then added to each well. Finally, samples were run through a flourometer to detect concentrations of CRP.

The mean values of the CRP QCs were 0.022, 0.259, 1.208, and 3.271 mg/L for the low, medium, high, and very high controls, respectively. The interassay CVs were 14.4%, 14.9%, 12.3% and 10.9% while the intrassay CVs were 2.0%, 1.2%, 1.6%, and 1.4%, respectively. The MDD for the CRP assay was 0.030 mg/L. CRP monoclonal antibodies did not cross-react with serums from dog, cat, horse, mouse, or rat.

Description of Data

61 Bolivian women participated in this study (30 urban poor women; 31 urban better-off women). For each woman, blood spots were collected on each collection day (day 6-8, 9-10, 11-12, 13-15, 16-19, 21-24) for a total of 5-6 CRP measurements per woman per cycle. Blood spots were assayed for CRP for each collection today for two consecutive cycles. Blood spots were to be collected on 5 collection days per cycle for two cycles per woman resulting in 610 expected blood samples. However, only a total of 590 out of 610 samples were collected due to a possible error in collection and/or follow up. In some women, two additional blood spots were collected and assayed for CRP, resulting in 56 more CRP samples for a total of 646 CRP samples. For hormone measurements, blood spots were assayed for FSH, LH, progesterone, and estradiol for each collection day for one cycle only. If each woman contributed 5 hormone data sets (one data set for each day across a given cycle), 305 data sets would be expected. However, data were incomplete for 10 samples resulting in 292 hormone data sets. Therefore, there are only 292 complete sets of reproductive hormone data.

Organization of Data

Initially when considering CRP data, CRP data for both consecutive cycles for each woman were utilized. A CRP measurement was collected for each woman after exercise either once per cycle for both consecutive cycles or twice for a given cycle. Only non-postexcercise CRP values were used for analyses. CRP data were deleted if they were post-exercise values. From these pre-exercise CRP values, CRP values and corresponding hormone values were deleted if an individual was: pregnant, had duplicate data for a given collection window, and if CRP levels were greater than 4 mg/L or were of suspect to effect of infection. Samples with CRP values greater than 4mg/L were eliminated in the study, as CRP values above this show the likelihood of an adverse event to be increased (Pearson et al, 2003). CRP values that showed possibility of infection or post-infection recovery were also eliminated (See Table 1). All together, five complete series were dropped for any of these reasons. Blood spots were only assayed for hormones for one cycle per woman. Thus, only corresponding CRP spots were used for analyses.

Hormone data and corresponding CRP data were organized by collection days into the following categories: day 6-8, 9-10, 11-12, 13-15, 16-19, and day 21-24. If an individual had more than one sample for a given day, the latter duplicate day was deleted. These women were separated as urban better off (urban-b) or urban poor (urban-p). The average, count, and standard deviation for FSH, LH, estradiol, progesterone, and CRP

were calculated for each hormone, each day and phase, for urban poor and urban better off women.

Data, including samples that were considered duplicates for a given collection window, were classified as ovulatory or anovulatory. Cycle series were identified as ovulatory if progesterone values in the luteal phase were greater than or equal to 0.80 ng/mL. The average, count and standard deviation for each hormone, for each day, for ovulatory and anovulatory cycles were calculated.

Data from women with ovulatory cycles were further classified into 3 phases: preovulatory (day 6-12) , periovulatory (day 13-15) and postovulatory (day 16-24) phases. Collection days were considered periovulatory if samples were characterized by a peak in estradiol levels. Preovulatory days consisted of samples collected prior to the periovulatory phase, while the postovulatory phase was classified as days in which samples were collected following the periovulatory phase. The average, count, and standard deviation of the hormones for the 3 phases were calculated.

Table 1. Collected, eliminated, and retained samples of CRP and ovarian hormones.

Total Number of CRP Samples Collected: **646 samples**	Total Number of Hormone Samples Collected: **293 samples**
Omitted from ovarian cycle analysis:	Omitted from ovarian cycle analysis:
samples without hormone data: **353 samples** -no hormone collection nor CRP values for cycle series: **317 samples** -no hormone collection on one of the first five days: **36 samples**	**-** no hormone data for given CRP data: **6 samples**
- Duplicates for a given collection day: **21 samples**	**-** Duplicates for a given collection day: **19 samples**
- CRP > 4.0 mg/L and if CRP series reflected infection or recovery from infection: **33 samples**	**-** CRP > 4.0 mg/L and if CRP series reflected infection or recovery from infection: **29 samples**
- pregnancy: **5 samples**	- pregnancy: **5 samples**
Grand Total Number of Samples of CRP Data Dropped for Ovarian Cycle Analysis: 412 samples	**Grand Total Number of Samples of CRP Data Dropped for Ovarian Cycle Analysis: 59 samples**
Grand Total Number of Samples of CRP Data Retained for Ovarian Cycle Analysis: 234 samples	**Grand Total Number of Samples of CRP Data Retained for Ovarian Cycle Analysis: 234 samples**

Statistical Analysis

CRP, progesterone, LH and FSH data sets underwent a log transformation to conduct parametric analyses. Statistical testing was conducted using statistical software known as STATA 11.0. T-tests were conducted to determine significant differences between SES and variables such as: CRP, ovarian hormones, age, duration, and BMI. A chi-square test was conducted to determine if there was a significant difference in urban-p and urban-b women and the presence of an ovulatory cycle. A one-way analysis of variance (ANOVA) coupled with a Bonferroni test was conducted to determine differences and the extent of these differences through measurements of significance levels in CRP and ovarian hormone values across cycle phases and cycle days. Using a

linear regression model helped to determine significant associations between ovarian hormones, CRP, age, duration, and BMI for the overall data set, and for specific cycle phases and cycle days. Multivariate analyses were conducted by inserting multiple factors into the regression model to determine extent of effects of single factors and combinations of factors on the dependent variable. Finally, generalized linear models (GLM), similar to linear regressions, were used to identify the degree to which these factors caused variation in the dependent variable. Factors ranged from: SES, presence of ovulatory cycle, duration, BMI, age, CRP and reproductive hormones. By generating variables that employ the effect of interaction and inserting the variables into the GLM, this test was used to study the effect of interaction between two specific factors on the dependent variable.

Associations or relationships that were statistically significant had a p-value of less than or equal to 0.05. Those associations with values greater than 0.05 and less than or equal to 0.1 were classified as “trends” that could potentially be significant with a greater sample size.

III. Results

Descriptive Statistics

1. Overall Data

Women who participated in this study had an average age of 28 years and a BMI of 24.02 kg/m^2 with an average ovarian cycle length of 28 days. The average CRP level is 0.577 mg/L. Bolivian women have an average estradiol level of 93.45 pg/mL and an average progesterone level of 2.8 ng/mL. The average LH level is 13.02 IU/L and the average FSH level is 7.82 IU/L.

Estradiol levels increase from day 6-12 and peak at day 13-15, after which they slightly decline. Progesterone continues to steadily increase throughout the cycle while CRP remains mostly constant from day 6-15 and peaks on day 16-19 followed by a decline on day 21-24 (Table 2).

Table 2. Levels of ovarian hormones and CRP across days of the ovarian cycle.

All						
Day	**Day 6-8**	**Day 9-10**	**Day 11-12**	**Day 13-15**	**Day 16-19**	**Day 21-24**
Estradiol (pg/mL)						
n	47	43	49	50	48	2
mean+/- SD	55.92 ± 24.76	81.95 ± 51.14	117.06 ± 75.58	114.44 ± 61.76	108.49 ± 61.09	99.77 ± 17.21
Progesterone (ng/mL)						
n	47	42	49	50	48	2
mean+/- SD	0.43 ± 0.14	0.57 ± 0.64	0.89 ± 1.50	3.56 ± 6.07	8.86 ± 9.70	12.86 ± 5.78
CRP (mg/L)						
n	48	44	50	50	50	29
mean+/- SD	0.53 ± 0.74	0.44 ± 0.46	0.53 ± 0.85	0.51 ± 0.71	0.64 ± 0.86	0.405 ± 0.466

2. Ovulatory and Anovulatory Data

Women who did not have a progesterone value of 0.8 ng/mL on or after day 16-19 were identified as anovulatory. However, because many of these women did not have samples collected on day 21-24 and because their follicular phases might have shifted, these women are not truly anovulatory but are “apparently anovulatory.” There are more women who have ovulatory cycles, than women who have apparently anovulatory cycles. 80% of the women sampled had ovulatory cycles, while 20% had anovulatory cycles. The average age of women is not significantly different between those with ovulatory versus anovulatory cycles. In women with ovulatory cycles, levels of estradiol are almost doubled on day 6-12 and CRP values are less than those in women with apparently anovulatory cycles (Table 3, Table 4). In 4 out of 6 collection days, CRP is greater in women with anovulatory cycles.

Table 3. Levels of ovarian hormones and CRP across days of the ovarian cycle in women with ovulatory cycles.

Ovulatory						
Day	**Day 6-8**	**Day 9-10**	**Day 11-12**	**Day 13-15**	**Day 16-19**	**Day 21-24**
Estradiol (pg/mL)						
n	40	33	40	41	40	2
mean+/- SD	59.20 ± 24.60	93.30 ± 53.22	128.93 ± 76.49	122.79 ± 60.97	103.22 ± 60.95	99.77 ± 17.21
Progesterone (ng/mL)						
n	40	32	40	41	40	2
mean+/- SD	0.43 ± 0.15	0.63 ± 0.73	1.00 ± 1.65	4.26 ± 6.52	10.56 ± 9.78	12.86 ± 5.78
CRP (mg/L)						
n	42	34	41	41	41	24
mean+/- SD	0.48 ± 0.75	0.40 ± 0.41	0.52 ± 0.83	0.52 ± 0.71	0.56 ± 0.59	0.41 ± 0.44

Table 4. Levels of ovarian hormones and CRP across days of the ovarian cycle in women with anovulatory cycles.

Anovulatory						
Day	**Day 6-8**	**Day 9-10**	**Day 11-12**	**Day 13-15**	**Day 16-19**	**Day 21-24**
Estradiol (pg/mL)						
n	7	10	9	9	8	
mean+/- SD	37.18 ± 16.87	44.52 ± 10.10	64.29 ± 43.83	76.37 ± 52.86	134.80 ± 58.39	
Progesterone (ng/mL)						
n	7	10	9	9	8	
mean+/- SD	0.39	0.39	0.39	0.39	0.39	
CRP (mg/L)						
n	8	10	9	9	9	5
mean+/- SD	0.68 ± 0.66	0.58 ± 0.60	0.60 ± 1.00	0.48 ± 0.74	1.02 ± 1.60	0.38 ± 0.63

3. Urban-p and Urban-b Data

When considering ovarian hormones, urban poor (urban-p) women have similar levels of FSH and LH compared to urban better-off (urban-b) women. However, urban-p

women have higher levels of progesterone and CRP than urban-b women (Figure 2, Figure 3). Urban-b women have higher levels of estradiol than urban-p women (Figure 2).

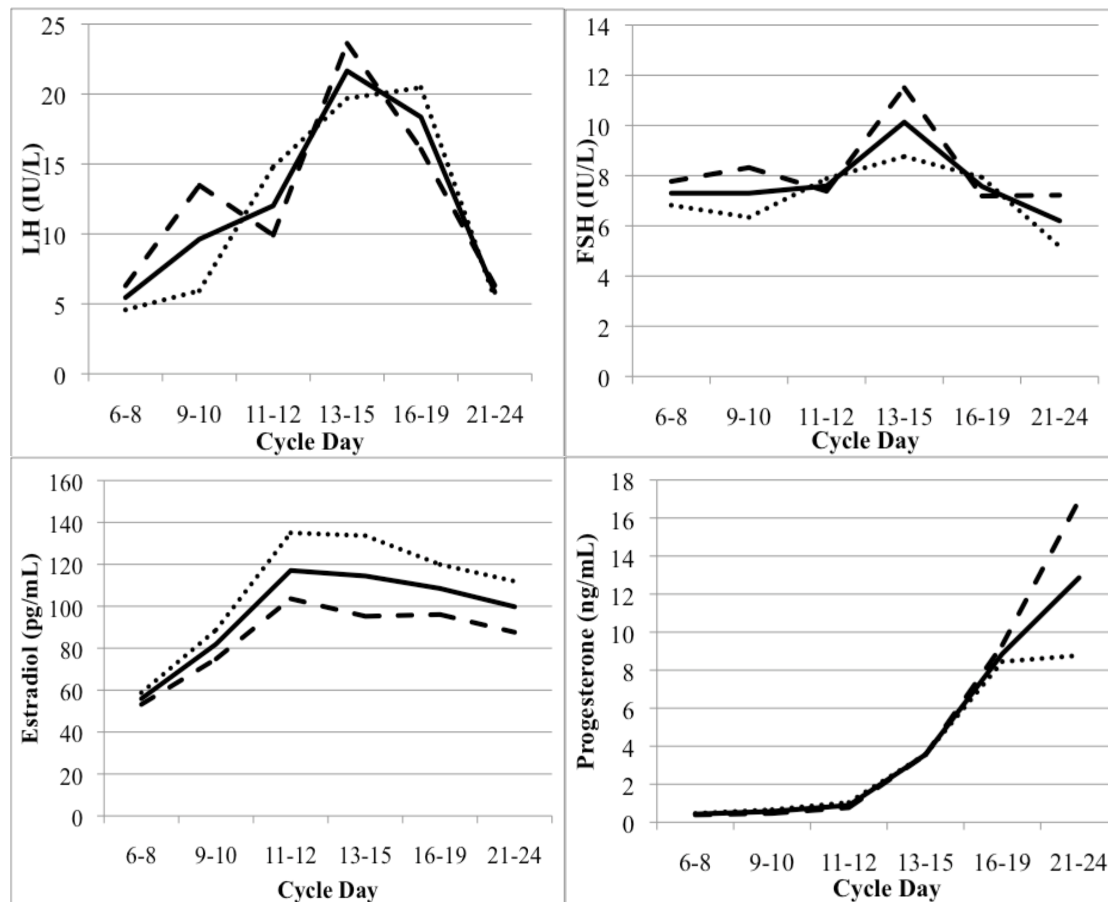

Figure 2. Ovarian hormones across the ovarian cycle in urban poor women (dashed), urban better-off women (dotted) compared to all Bolivian women (solid).

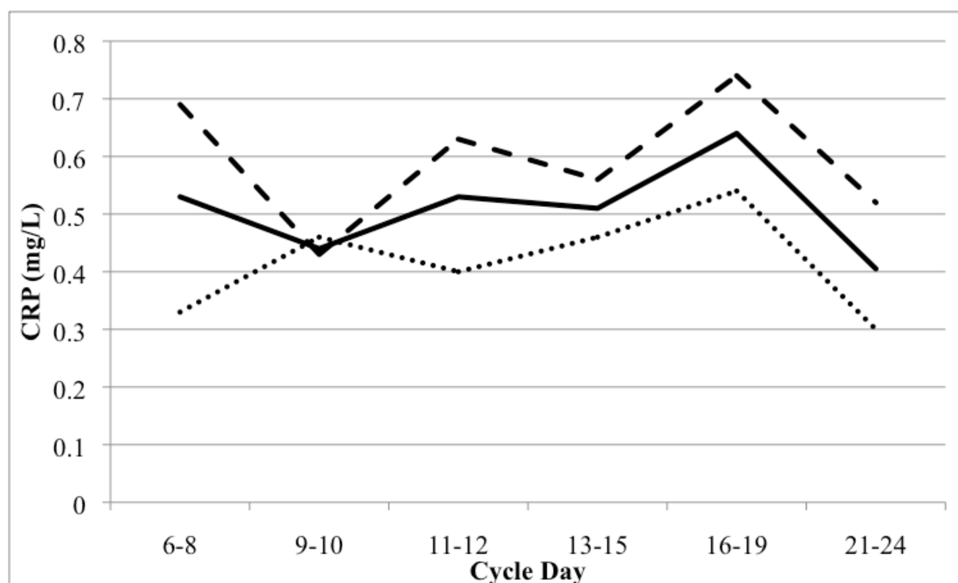

Figure 3. CRP across the ovarian cycle in urban poor women (dashed), urban better-off women (dotted) compared to all Bolivian women (solid).

Reproductive Hormones Across the Ovarian Cycle

Estradiol across Cycle Day and Cycle Phases

Significant differences among estradiol values between preovulatory (day 6-12), periovulatory (day 13-15), and postovulatory (day 16-24) phases exist with estradiol increasing between the preovulatory and periovulatory phases and decreasing between the periovulatory and postovulatory phases. There is a significant relationship between preovulatory estradiol values and periovulatory estradiol values such that as preovulatory estradiol levels increase, periovulatory estradiol levels increase. There is also a significant positive association between periovulatory and postovulatory estradiol levels.

When analyzing estradiol values across cycle days, significant differences in values are observed between day 6-8 and day 11-12, day 13-15, and day 16-19 (Table 5). Trends suggesting a difference in estradiol values are also observed between day 9-10 and day 11-12 as well as day 9-10 and day 13-15. A significant relationship exists between estradiol levels during day 6-8 and day 9-10 as well as between day 9-10 and day 11-12.

Table 5. Significant differences between estradiol levels throughout cycle days and phases.

Estradiol	Day 6-8	Day 9-10	Day 11-12	Day 13-15	Day 16-19	Day 21-24
Day 6-8						
Day 9-10	NSD					
Day 11-12	p = 0.00	p = 0.06				
Day 13-15	p = 0.00	p = 0.1	NSD			
Day 16-19	p = 0.00	NSD	NSD	NSD		
Day 22-24	NSD	NSD	NSD	NSD	NSD	

Estradiol	Preovulatory	Periovulatory	Postovulatory
Preovulatory			
Periovulatory	p = 0.001		
Postovulatory	p = 0.00	p = 0.00	

Progesterone across Cycle Day and Cycle Phases

Progesterone values in women with both ovulatory and anovulatory cycles on cycle days before midcycle (day 6-8, day 9-10, and day 11-12) are not significantly different from one another, but these are significantly different from days after midcycle (day 16-19, and day 21-24) (Table 6). Furthermore, there is a significant difference between progesterone values on cycle day 13-15 and day 16-19.

In those women with ovulatory cycles, analysis shows that there is a significant difference in progesterone levels between preovulatory and periovulatory phases, as well as periovulatory and postovulatory phases. There is also a significant difference in progesterone levels between preovulatory and postovulatory phases.

Progesterone levels are related to cycle duration, BMI and estradiol. Tests analyzing these factors show that duration, BMI and the duration differences in BMI all provide a significant source of variation in progesterone levels.

High levels of progesterone (greater than 0.8 ng/mL) during day 16-19 were analyzed for variation due to BMI, duration and SES and various interactions between factors among them. However, no interactions provided a significant source of differences in progesterone. In addition, cycle length did not significantly differ between the urban-p and urban-b populations.

Table 6. Significant differences between progesterone levels throughout cycle days and phases.

Progesterone	Day 6-8	Day 9-10	Day 11-12	Day 13-15	Day 16-19	Day 21-24
Day 6-8						
Day 9-10	NSD					
Day 11-12	NSD	NSD				
Day 13-15	p = 0.00	p = 0.00	p = 0.00			
Day 16-19	p = 0.00	p = 0.00	p = 0.00	p = 0.00		
Day 22-24	p = 0.00	p = 0.00	p = 0.00	p = 0.033	NSD	

Progesterone	Preovulatory	Periovulatory	Postovulatory
Preovulatory			
Periovulatory	p = 0.00		
Postovulatory	NSD	p = 0.00	

CRP across the cycle

Estradiol and progesterone vary throughout cycle day and cycle phase. CRP is not significantly associated with progesterone or estradiol and thus, did not vary throughout the ovarian cycle. Progesterone levels are significantly different between the preovulatory phase (day 6-12) and the postovulatory phase (day 16-19). Similarly, estradiol levels are significantly lower in the preovulatory phase (day 6-12) compared to the postovulatory phase (day 16-19). Though the two main reproductive hormones vary throughout the cycle, CRP levels do not vary significantly between preovulatory and postovulatory phases.

Factors associated with CRP

The only two factors that appear to be associated with CRP are estradiol and BMI. There is a negative association between CRP and estradiol. Estradiol appears to be affected by many variables including: progesterone, presence of an ovulatory cycle, cycle duration, BMI, and SES. Estradiol has a significant positive association with progesterone. Presence of an ovulatory cycle is significantly positively associated with increased levels of estradiol. Estradiol also has a significant negative association with duration and BMI. This hormone is affected by SES such that urban-p women have significantly lower values of estradiol compared to urban-b women. In preovulatory, periovulatory and postovulatory phases, estradiol levels do not vary between urban-p and urban-b women. In summary, both BMI and SES influence levels of estradiol, however,

BMI alone is the main factor that alters estradiol, not the BMI difference in SES. Duration also plays a significant role in impacting estradiol and the interaction between BMI and duration provides a small source of variation for differences in estradiol.

There is a significant positive association between CRP and BMI (Table 7). BMI is affected by: levels of estradiol, progesterone, and SES. Estradiol levels are significantly negatively associated with increases in BMI. To a lesser extent, progesterone values are significantly negatively associated with increases in BMI. Urban-p women have significantly greater BMI than urban-b women.

Although CRP is associated with BMI, and BMI is associated with SES, CRP is not significantly associated with SES. CRP values do not differ between urban-p and urban-b women. Urban-b women have an average CRP value of 0.285 mg/L while urban-p women have an average CRP value of 0.318 mg/L. Therefore, when studying BMI and SES, BMI is the main factor affecting CRP levels.

Table 7. Relationship between CRP, ovarian hormones, and lifestyle factors (NA = no association, NSD = no significant difference).

Factors	**CRP**	**Estradiol**	**Progesterone**	**FSH**	**LH**	**SES**	**BMI**	**Age**	**Duration**	**Presence of Ovulatory cycle**
CRP										
Estradiol	β=-0.0072, p=0.08									
Progesterone	NA	β=11.17, p=0.002								
FSH	NA	NA	NA							
LH	NA	β=16.09, p=0.014	NA	β=0.310, p=0.00						
SES	NSD	Urban-b greater, p=0.02	NSD	NSD	NSD					
BMI	β=0.106, p=0.009	β=-3.84, p=0.004	β=-0.084, p=0.086	NA	NA	Urban-p greater, p=0.0009				
Age	NA	β=1.90, p=0.095	NA	NA	NA	NSD	NA			
Duration	NA	β=-4.50, p=0.016	β=-0.270, p=0.00	NA	NA	NSD	NA	NA		
Presence of Ovulatory cycle	NSD	ovulatory greater, p=0.0003	ovulatory greater, p=0.00	NSD	ovulatory greater, p=0.001	NA	NSD	NSD	ovulatory longer, p=0.00	

Hypothesis Testing

1. **Biological Factors:**

Hypothesis 1: On average, CRP levels in non-infected Bolivian women will be higher than in Western women.

The average CRP level in Bolivian women is 0.577 mg/L, while the median level of CRP is 0.281 mg/L. Median CRP levels in Western women are higher with a value of 2.20 mg/L (Ford et al., 2004).

Hypothesis 1b: This difference will remain in both ovulatory and anovulatory cycles.

CRP levels are lower in non-infected Bolivian women with ovulatory cycles. On average, CRP levels at the periovulatory phase are 0.45 mg/L (± 0.65 mg/L). In a sample of Western women, however, CRP levels during ovulation are about 1.5 mg/L (Wander et al., 2008).

Hypothesis 2: Levels of estradiol in the follicular phase will be lower in Bolivian women than in Western women.

In ovulatory Bolivian women, the average estradiol level in the follicular phase (day 6-12) is 83.17 pg/mL, while in Western women, it is 78.73 pg/mL (Sehested et al., 2000).

Hypothesis 2b: This difference will remain when anovulatory cycles are excluded.

In ovulatory Bolivian women, the average estradiol level in the follicular phase is 83.17 pg/mL while in anovulatory Bolivian women, the average estradiol level is 40.85 pg/mL during that particular time.

Hypothesis 3: Progesterone levels in the postovulatory phase will be lower in Bolivian women than in Western women.

Results from this study show that Bolivian women with ovulatory cycles have an average postovulatory progesterone level of 11.71 ng/mL from day 16-24. In anovulatory women, the average progesterone level from day 16-24 is 0.39 ng/mL. In Western women the average progesterone level found in ovulatory women in the postovulatory phase is 14.47 ng/mL. These values are not notably different from one another.

Hypothesis 4: As age increases, CRP levels will increase.

No significant association between age and CRP is detected.

2. Lifestyle Factors:

Hypothesis 5: BMI will be correlated with CRP.

BMI is positively associated with CRP ($\beta = 1.14$, $p = 0.009$).

Hypothesis 6: CRP will be higher in urban poor than urban better-off Bolivian women.

There is no significant difference in CRP levels between those two groups. Similarly, across the three phases of ovulatory cycles, progesterone levels do not differ significantly between urban-p and urban-b women.

Hypothesis 7: Follicular levels of estradiol will be lower in urban poor than urban better-off women.

Follicular levels of estradiol are consistently lower in urban-p women compared to urban-b women. When considering both ovulatory and anovulatory cycles, urban-b women have a level of 94.16 pg/mL of estradiol in the follicular phase, while urban-p women have 77.22 pg/mL. In women with ovulatory cycles alone, urban-b women have an estradiol level of 99.76 pg/mL while urban-p women have a level of 88.03 pg/mL during day 6-12. When categorizing by cycle phase, urban-b women with ovulatory cycles have an estradiol value of 74.29 pg/mL while urban-p women have a value of 70.07 pg/mL in the preovulatory phase.

3. Cycle Progression:

Hypothesis 8: CRP levels will fluctuate across the ovarian cycle.

CRP shows very little systematic change between phases during the entire cycle. Although CRP values of previous cycle days influence CRP values of upcoming cycle days, there is no significant difference between CRP levels in the preovulatory and postovulatory phases or between preovulatory and periovulatory phases. The only trend shown is a slight change seen in CRP values that occurs between the periovulatory and postovulatory phases, in which CRP values in the postovulatory phase are greater than those in the periovulatory phase. CRP values toward the end of the cycle (day 16-19) are associated with CRP values at the beginning of the cycle (day 6-8).

Hypothesis 9: CRP levels will show no systemic change during the mid-late follicular phase (day 6-12).

There are no significant differences in CRP levels between cycle days. Conducting a paired samples t-test between CRP values and cycle days shows a trend that CRP values in day 16-19 tend to be higher than CRP values day 6-12. Estradiol levels steadily increase in the mid-late follicular phase, but no relationship is observed between CRP and estradiol in the preovulatory or follicular phase. However, a trend is shown between CRP and estradiol in the periovulatory phase. There is a significant relationship

between CRP and estradiol in the postovulatory phase such that when estradiol levels increase, CRP levels decrease.

Hypothesis 10: CRP will be positively correlated with progesterone in the luteal phase.

Data analyses do not show a trend between CRP and progesterone values in the preovulatory or periovulatory phases. However, there is a trend between CRP and progesterone levels in the postovulatory or luteal phase. Another trend exists between progesterone values in the preovulatory phase and CRP values during ovulation. A similar trend is observed between progesterone values during ovulation and CRP values in the postovulatory phase. But, levels of CRP do not differ significantly between day 6-12 and day 16-19 in those women with higher progesterone or lower progesterone values on day 16-19.

Hypothesis 11: At low levels of progesterone (<0.8 ng/mL) on days 16-24, CRP will not differ from follicular levels.

This hypothesis states that in anovulatory cycles, CRP values will not differ between day 6-12 and day 16-24. Results show that values of CRP are not significantly different on day 6-12 or 16-24 between those who have progesterone levels greater than 0.8 ng/mL and those with progesterone levels less than 0.8 ng/mL.

Multivariate Analyses

Multiple factors were inserted into the linear regression model to determine extent of factors and combinations of factors on dependent variables. A multi-variable regression was conducted on CRP by looking at the effect of BMI and age. BMI seemed to drive the variation in CRP ($\beta = 0.106$, $p = 0.010$, $R^2 = 0.13$). BMI is significantly associated with CRP and when age is added into the model, BMI remains significant but the statistical power is weakened. Therefore, it can be stated that age moderated the relationship between BMI and CRP. BMI and age account for 13% of the variation in CRP. Next, age and SES were put into the regression model and both were found to be not significantly associated with CRP. A stepwise comparison was conducted between CRP, BMI, age, and SES. BMI is significantly positively associated with CRP. SES is not significantly associated with CRP and there is an even weaker association between age and CRP. However, when SES and age are added to the regression model, the statistical power of the relationship between BMI and CRP weakens yet is still significant. Analyses suggest that there is too much variation in BMI and CRP that it is hard to sort out.

Another stepwise comparison was conducted between CRP, BMI, estradiol and SES. When looking at data from women with both ovulatory and anovulatory cycles, BMI is significantly positively associated with CRP and estradiol and SES are not significantly associated with CRP. However, when conducting analysis on women with only ovulatory cycles, both BMI and SES are significantly associated with CRP, such

that BMI has a negative relationship with CRP and SES has a positive relationship with CRP and is more significant than BMI.

IV. Discussion

Summary of Findings

Results from this study show a significant difference in BMI between urban-p and urban-b women such that urban-p women have higher BMI values. Although there is no significant difference in CRP levels between urban-p and urban-b Bolivian women, there is a significant positive association between BMI and CRP. BMI drives variation seen in CRP levels, and not the interaction of BMI and SES. There is no significant difference between CRP levels in the preovulatory and postovulatory phases or among cycle days. CRP levels in each phase (preovulatory, periovulatory, and postovulatory phases) are not different between urban-p and urban-b women. Although CRP did not vary between phases, CRP levels in the preovulatory phase are significantly associated with CRP levels in the postovulatory phase. Furthermore, CRP levels on a given cycle day are significantly associated with CRP levels on the day before the given cycle day. Tests attempting to detect CRP lag in the cycle show a positive trend between progesterone levels in the preovulatory phase and CRP levels in the periovulatory phase, as well as progesterone levels in the periovulatory phase and CRP levels in the postovulatory phase. The latter trend is also observed with levels of estradiol.

Hormone data were obtained from Bolivian women with normal ovarian cycles as evidenced by expected hormonal changes. As expected, levels of estradiol and progesterone vary significantly across cycle days and cycle phases. 80.4% of cycles were

ovulatory while 19.6% of cycles were anovulatory. As expected, biological and ecological variations between urban poor and urban better-off populations can explain differences in estradiol values between urban-p and urban-b women such that urban-b women have higher levels of estradiol. BMI, a biological factor, and SES, an ecological factor, are significantly related to estradiol. More specifically, BMI (not SES) drives variations in levels of estradiol. Progesterone levels also vary significantly across the cycle. As expected, BMI and SES both affect progesterone. BMI is inversely significantly related to progesterone throughout the cycle and BMI, duration and the positive effect of the interaction between BMI and duration are all significant sources related to variation in progesterone levels. These findings show that ovarian cycles are normal and that endocrine changes are influenced by biological and ecological factors. Because of this, it is likely that CRP will fluctuate throughout the ovarian cycle.

Data from this study, however, shows that CRP does not vary significantly throughout the ovarian cycle and there is little association between reproductive hormones and CRP. There is a slight negative association between overall values of CRP and estradiol across the cycle, but especially seen in the periovulatory and postovulatory phases. The study found a negative trend between values of progesterone and CRP in the postovulatory phase.

These very slight associations can be explained by biological factors, such as BMI and duration and ecological factors, such as SES. BMI and duration are both inversely significantly related to levels of progesterone throughout the cycle, and BMI, duration,

and the positive effect of the interaction of BMI and duration are all significant sources that cause variation in progesterone levels. There is also a significant negative association between estradiol levels and BMI is observed. Urban-p women have higher levels of progesterone in the postovulatory phase (day 16-24) than urban-b women CRP and SES are related such that estradiol values are higher in urban-b women than in urban-p women.

Hypotheses Testing

a. Comparison Between Bolivian Women and Western Women

Many studies, including some of Bolivian women, have found lower levels of ovarian hormones than in Western women. However, that does not seem to be the case in this study. The average progesterone level in the follicular, luteal and midluteal phases for this given assay is 0.4 ng/mL, 10.5 ng/mL and 14.9 ng/mL (DSL-#3400). Bolivian women and Western women have similar progesterone values throughout the cycle, however, the progesterone levels are higher in the Bolivian women than Western women (Ellison, 1990). The average estradiol level in the follicular and luteal phases reported for the assay we used in Western populations is 44.73 pg/mL and 48.96 mg/mL, respectively (DSL-#4800). These assays were conducted on sera sampled from Western populations. On average, Bolivian women show higher estradiol values than Western women.

This study showed that progesterone levels are higher in Bolivian women than in Western women. Average progesterone levels in Bolivian women are 0.608 ng/mL and 15.69 ng/mL for the follicular and luteal phases, respectively. In the Nurses Health Study II that surveyed Western women, progesterone levels in the luteal phase are 14.47 ng/mL, which is not notably different from progesterone levels in the luteal phase in Bolivian women (15.69 ng/mL) (Eliassen et al., 2006). Therefore, the hypothesis that progesterone levels in the postovulatory phase are lower in Bolivian women than Western women is not supported for this data set. Because progesterone levels between Western and Bolivian women are not significantly different, a bigger sample size is necessary to really to explore this hypothesis further. Previous studies conducted in Bolivian populations show that Bolivian women, both urban-p and urban-b, have significantly lower salivary progesterone levels than Western women. In a study conducted by Vitzthum et al (2002), it is shown that in women with ovulatory cycles, on the day of peak progesterone levels, urban-p Bolivian women had 208 pmol/L while urban-b women had 232 pmol/L compared to a population of women from Chicago who had a mean peak progesterone level of 330 pmol/L.

Estradiol levels in Bolivian women are higher than those in Western women. Average estradiol levels in all Bolivian women in the follicular phase are 85.89 pg/mL and 108.14 pg/mL in the luteal phase. The hypothesis stating that levels of estradiol in the follicular phase are lower in Bolivian women than in Western women appears to be incorrect. This study found that when anovulatory cycles are excluded, estradiol levels in the follicular phase in Bolivian women are 83. 17 pg/mL (305.32 pmol/L) while a study

conducted in Maryland women (with an average age of 29 years) showed average estradiol levels in the follicular phase to be 141 pmol/L (Dorgan et al., 1994). Bolivian women have higher estradiol levels in the ovulatory phase as well. However, estradiol levels in the luteal phase are similar between both populations. If both ovulatory and anovulatory cycles are considered, estradiol levels in Bolivian continue to be higher than estradiol levels in Western women. The Nurses Health Study II showed 394 women (with an average age of 42 years) to have an average estradiol level of 44 pg/mL in the follicular phase (Elliasen et al., 2006).

Estradiol levels in Bolivian women tend to be higher than in Western women for many reasons. One reason is that there are higher levels of stress among women in Western populations. Stress can originate from the work place or psychosocial stress. Other differences could be due to less physical work load which decreases effects of physical stress on the body, allowing the body to better prepare for reproduction and have increased estradiol levels.

Due to differences in reproductive hormones between populations, if CRP is associated with reproductive hormones, CRP should also vary between populations. CRP levels in non-infected Bolivian women have a median value of 0.281 mg/L. In a study conducted by Ford et al (2004), blood samples were collected from U.S. women and CRP concentrations were determined. The study sampled 393 women 20-29 years of age and 30-39 years of age and found CRP concentrations at the 50th percentile to be 2.20 mg/L and 2.50 mg/L, respectively. Relative to women in industrialized countries, it can be

inferred that non-infected Bolivian women in this study have lower values of CRP. Therefore, the hypothesis is incorrect. In a study conducted in urban areas of Turkey, mean CRP levels for women age 30-39 were measured as 1.05 mg/L (Onat et al, 2001). 87% of Children and adolescents in urban New Delhi had CRP levels of less than 2.1 mg/L (Misra et al., 2006).

Western populations may have higher values of CRP than Bolivian populations for many reasons. One major reason is due to higher BMI in Western populations. BMI has consistently been associated with increases in CRP. BMI is significantly associated with CRP and therefore, the proposed hypothesis is correct. This study demonstrated that those with high BMI values have high levels of CRP. A high BMI is associated with a high caloric intake and obesity. In obesity, concentrations of TNF-α are increased (Hotamisligil et al., 1995) and can stimulate the production of CRP (Warren et al, 1987). In addition, obesity can increase the production of macrophage migration inhibitory factor (Hotamisligil et al., 1993), a proinflammatory cytokine, as well as IL-6, a key promoter of CRP (Hirokawa et al., 1998). In the NHANES III study conducted among the U.S. population, individuals with BMI less than 25 kg/m^2 had CRP levels of 2.5 mg/L while those with BMI between 25 kg/m^3 and 30 kg/m^2 had CRP levels of 2.9 mg/L (Ford, 1999).

Another reason why CRP could be higher in Western populations is due to the use of oral contraceptives. In 2002, 62% of American women age 15-44 were using an oral contraceptive (Mosher et al., 2004) while in Bolivia, only 19% of women age 15-24 were

(Ali & Cleland, 2005). Most studies have shown that the use of oral contraceptives tend to elevate CRP levels (Dreon et al., 2003). Also, CRP may vary due to differences in prevalence of smoking among women. Smoking is associated with increases in CRP levels (Chenillot et al., 2000), however, in 1992-93, smoking rates among women in the United States (22.5%) were comparable to smoking rates among women in Bolivia (21.4%) (WHO, 1997). Sedentism and lack of exercise could also be associated with increased levels of CRP in Western populations. Also, a poor diet, high in fat and sugars, could also increase levels of CRP. CRP levels may be lower in Bolivian women if Bolivian women receive lower amounts of exogenous estradiol. Prestwood et al (2004) states that varying amounts of exogenous estradiol can affect CRP such that CRP levels are higher in Western women who have higher levels of estradiol. This suggests that if estradiol levels in Bolivian women are low, then they should have low CRP levels.

In this study, 20% of the samples from the study showed evidence for the presence of an infection during the cycle. If CRP values greater than 4 mg/L would have been retained for analysis, the average CRP level would be 0.821 mg/L and the difference in magnitude between CRP levels in the Bolivian population compared to the Western population would have been slightly less distinct.

CRP and SES

The expectation that CRP will be higher in urban-p women was not substantiated by the results found in this study. These results conflict with results in American women

(Owen et al., 2003) who showed that CRP is lower in women of a higher socioeconomic status. Socioeconomic differences could lead to differences in inflammation and CRP levels for a variety of reasons. Low SES individuals may have greater exposures to infections (Cohen, 1999) or lack of access to health care and treatments (Adler et al,, 1993). They might also have more psychosocial stress (Siegrist & Marmot, 2004) or engage in risky health behaviors (Pincus & Callahan, 1995) all of which have been associated with increased inflammation. But, CRP is not significantly different between urban-p and urban-b women. Environments that house poor populations may promote diseases, leading to higher CRP concentrations in those low SES individuals. Although this is possible, this is not observed in the urban-p Bolivian population. In a study conducted in the U.S, serum CRP levels were measured in women who were categorized as poor or non-poor according to family income. Results showed that differences in CRP among poor and non-poor are only observed at high CRP levels (>10 mg/L) indicative of infection. CRP levels of lower income populations have been shown to be higher than levels observed in higher income populations in the U.S (Alley et al., 2006).

Energetics

Earlier, it was hypothesized that differences in available energy stores in Bolivian women could result in a decreased estradiol level in Bolivian women since there are less total resources and thus less energy to allocate to reproductive functioning. This study shows that women in the urban-p population are characterized by higher levels of BMI than women in the urban-b population. There is also a strong positive association

observed between BMI and CRP. Therefore, it would be expected that since urban-p women have higher levels of BMI, they should have higher levels of CRP compared to urban-b women. However, in this study, no significant difference in CRP levels is found between urban-p and urban-b women. This suggests that not only does socioeconomic status play a role in the variation of CRP levels, but other factors also play a role. If the strong positive relationship between CRP and BMI is universal, that is, apparent in other populations, it can be inferred that Western populations have a greater proportion of individuals with high BMI such that the average CRP level in Western populations is greater than that in non-Western populations. Although the difference in CRP level could be attributed to caloric intake, it could also be due to diet quality. Given the results from this study, it is possible that urban-p women have a higher BMI because they have a poor quality of diet compared to urban-b women. Differences in micronutrient intake, stress levels, and exercise could also be potential factors in causing possible variation between these two urban Bolivian populations. Also, differences in early histories of populations could also result in current populational differences in immune function. Findings suggest that intrauterine and early postnatal environments may have long-term consequences for infectious disease risk and risk of other diseases, reflecting in possible interpopulational variations in CRP (McDade et al., 2001).

CRP and Age

Given the narrow age range, this study shows no association between age and CRP and the hypothesis that as age increases, CRP levels increase, was not supported.

One study collected CRP measurements from boys and girls, age to 2-15 year olds, from the rural Tsimane' population across 12 villages in Bolivia (McDade et al., 2005). The median bloodspot CRP concentration was 0.73 mg/L. Age was the strongest predictor of CRP with highest concentrations among young children. In the study conducted on a Western population, Bermudez et al (2002) showed that CRP had a strong, positive association with age. These results may be due to increased states of disease encountered due to increasing age or could be due to effect of cumulative pathogen exposure.

CRP and Ovarian Hormones

The hypothesis (hypothesis 8) that suggested that CRP would fluctuate throughout the ovarian cycle was not supported. Results from this study indicate that CRP has no association with estradiol and are similar to findings stated in Wunder et al (2006). Other studies have reported a negative trend between values of CRP and estradiol in the periovulatory and postovulatory phases. The negative relationship between CRP and endogenous estrogen was observed by Blum et al (2005). This is similar to results found in Wander et al (2008), however Wander showed that increases in estrogen are associated with decreases in CRP throughout the entire cycle ($p = 0.05$). Evidence suggests that in vitro, low estrogen doses are associated with higher levels of IL-6 production (Rogers & Eastell, 2001). Regardless, women in this study had robust ovarian cycles and samples were collected from mid-follicular to early luteal phase so there were many opportunities to observe the possible relationship between estradiol and CRP. Therefore, it is concluded that there is a lack of cycle variation in CRP.

CRP was hypothesized (hypothesis 9) to show no change during the follicular phase (day 6-12) and changes in CRP throughout the follicular phase were not observed. In this study, CRP levels ranged from 0.44-0.53 mg/L in the follicular phase. Wander et al (2008) found that levels of CRP are highest during menstruation (2.8 mg/L) and decline to 1.5 mg/L after menses. Throughout the follicular phase, CRP values were 2.1 mg/L and slowly decreased to 1.7 mg/L by the end of the phase. This small decrease in concentration is significantly associated with an increase in estradiol levels throughout the follicular phase. Similarly, in a study conducted in a Swiss population (Wunder et al., 2006), CRP levels in the follicular phase decreased from 2.3 mg/L to 1.7 mg/L. However, different from the study conducted by Wander et al (2008), this study did not find changes in follicular phase CRP levels to be significant, which could be due to the lack of data collected in the early follicular phase.

Differences in the postovulatory phase show that increased progesterone values are associated with decreased CRP values. Therefore, hypothesis 10 that suggests a positive association between CRP and progesterone in the postovulatory phase, was not supported. The negative relationship of progesterone on CRP is consistent with the idea that progesterone provides an overall anti-inflammatory effect by decreasing NK cell activity and TNF-α (Roberts et al., 2001). Many studies, however, show a positive association between progesterone and CRP (Wander et al., 2008). Studies that suggest a positive relationship between these two variables attribute it to progesterone's effects on

promoting activity of neutrophils and increasing production of IL-6 (Bouman et al., 2005).

The hypothesis (hypothesis 11) that at low levels of progesterone (<0.8 ng/mL) on days 16-24, CRP does not differ between follicular and luteal phases, is supported. Similarly, at high levels of progesterone (≥0.8 ng/mL), CRP is not different between follicular and luteal levels. This further indicates that CRP is not associated with progesterone levels and despite the progesterone increase in the luteal phase, CRP levels remain similar to those found in the follicular phase.

Interpretation of Findings

A comparison of BMI, CRP and estradiol between Western women from the U.S. and Bolivian women is shown in Table 8. This table shows data from the luteal phase in American and Bolivian women who had ovulatory and anovulatory cycles and who did not use oral contraceptives. Data from the U.S. women were collected in 2003, while the data from Bolivian women were collected in 1995. Although the table portrays a valid comparison, with more updated data and a comparison of data across all cycle phases, a broader and more relevant assessment of the relationship between reproduction and maintenance in American and Bolivian woman can be obtained.

Overall CRP levels across the cycle are lower in Bolivian women compared to American women, and Bolivian women have higher levels of estradiol than reported for

American women. The CRP value for the Western population is the median value of CRP found from sera from women who were a part of the Nurses Health Study II. Samples were obtained from the luteal phases of ovulatory and anovulatory women with an average BMI of 24.4 kg/ m^2 (Pischon et al., 2003). The median estradiol value for the luteal phase in cycles of Western women is 125 pg/mL. The slight difference between urban-p and urban-b women in CRP exaggerates the CRP difference between Bolivian and American women. Women living in the U.S. have BMI, CRP, and estradiol values more similar to those found in urban-p Bolivian women than urban-b women. This suggests that BMI could be driving these similarities, and a greater caloric intake or poor diet quality could be a strong predictor of CRP and/or estradiol levels. However, BMI is not the only factor driving differences in CRP between urban-p and urban-b women. The variation in both BMI and CRP is very large to sort out and other baseline ecological factors could explain differences in CRP levels.

Table 8. BMI, CRP and estradiol levels in Bolivian women (1995) and American women (2003) in ovulatory and anovulatory cycles in the luteal phase.

	Urban-p	**Urban-b**	**Median Bolivian**	**Median U.S.**
BMI (kg/m^2):	24.92	22.26	24.1	24.4
CRP (mg/L):	0.310	0.239	0.259	1.6
Estradiol (pg/mL):	71.41	82.55	77.27	82

Life History

Life history theory would predict a tradeoff between reproductive functioning and maintenance, at low levels of energy reserves. It was hypothesized that in this situation,

the body would allocate more resources toward survival efforts than reproductive efforts. However, data presented from this study shows that women with a lower BMI are associated with higher levels of estradiol and lower levels of CRP.

By applying life history theory, it can be stated that the body first allocates resources towards reproductive functioning and then allocates resources to maintenance. However, if this explanation were true, women with a high BMI would have higher levels of estradiol and that is not the case. However, if a higher quality diet in urban-b women is ingested, it does not institute as high of an inflammatory response as a possible lower quality diet in urban-p women. Thus, less energy can be spent in anti-inflammatory efforts and maintenance, while more energy can be spent on reproductive functioning, explaining the inverse relationship between both variables.

Another mechanism to explain this observed trend is not related to life history, but is due to the idea that an increased BMI interacts physiologically with the body to increase CRP levels. The role between adipocytes and CRP and the direct relationship between them can explain the direct relationship found between BMI and CRP.

Finally, it is important to note that ecological factors and not life history theory, are playing a significant role in influencing estradiol levels and CRP levels. There are many ecological factors that may account for a decrease in estradiol levels with higher BMI, such as stress and heavy world loads. Also, populations of urban poor and urban better-off women may have different ecological histories and differences in histories of

stress, diet quality, and pathogen exposure. Ecological conditions early in life may influence current estradiol levels and CRP levels. However, due to our limited data set, it is hard to assess this notion.

Limitations

A few limitations exist in this study that, if eliminated, could strengthen results from this study. The study sampled 30 residents from an impoverished neighborhood around La Paz while 31 residents were sampled from a national medical school in La Paz. An increased sample size for both groups of women would provide increased precision in the study design as well as statistical power for a possible association between two variables. An increased sample size and more sample points across the entire ovarian cycle in which data would be collected might strengthen some associations and resolve ambiguous trends. For example, the positive trend between BMI and CRP might be stronger with a greater sample size or a difference in CRP levels between urban-p and urban-b women may be detectable with a greater sample size.

Another limitation is that in this study, samples were collected during day 6-24 of the ovarian cycle and there was no data collected measuring CRP or hormone levels before day 6 (early follicular phase) or after day 24 (late luteal phase) where CRP may vary the most. By collecting and analyzing data from these phases in the ovarian cycle, a possible relationship between reproductive hormones and CRP may emerge.

Similarly, a wider age range of subjects would be beneficial in detecting if a true relationship does exist between age and CRP levels in urban Bolivian women. Participants in this study were age 23-35 years old, an age in which the quality of eggs and the ovarian cycle is similar for each age within the age range. However, if there were other age groups, such as 40-50 years old, there is more of a clear distinction in ovarian cycles (since they prepare for menopause) and could provide a source for possible variation in CRP levels.

Another limitation is lack of other demographic, lifestyle and development data. Initially, interviews with the women from both samples were conducted in the native language to detect characteristics for inclusion in the study. It would be useful to know place of birth, household size, marital status, number of children, education level, blood pressure level/presence of hypertension, and diabetes to provide a better context for understanding levels of reproductive hormones and CRP. If a survey on food intake, not only amounts of food but also diversity in food items consumed for time before, after, and during blood spot collections, it would be beneficial in hypothesizing a link between diet and CRP levels. Similarly, a qualitative analysis on exercise/physical activity or measures of stress and mental health might provide insight to other sources that may affect levels of CRP between populations. One important piece of demographic, lifestyle data would be early histories of individuals and populations. There may be population differences in immune exposure throughout history which could reflect current interpopulational differences in immune function. In this study, only the current immune

status of women was known. By obtaining early histories of women, experiences that may affect immune function can be noted.

Finally, findings from this study suggest that SES is significantly associated with BMI and BMI is significantly associated with CRP. Given these relationships, it is expected that the relationship between SES and CRP should also be significant, however, this is not observed. This is a limitation because it is not known how powerful these relationships are and if there are other intervening variables to weaken the relationship between SES and CRP. Thus, it is difficult to make a claim that BMI and SES are the only factors that may influence CRP.

Significance

Before the advent of widespread treatment for infectious diseases, Western populations had greater access to vaccines and treatment for bacterial infections. The difference in infectious pathogen exposure provided a source for differences between CRP levels between both populations. However, in modern times, less infectious diseases affect Western populations and Bolivia. Thus, when examining CRP levels between both populations, differences emerge not primarily due to differences in prevalence of disease, but due to baseline differences in ecological factors.

Results from this study suggest that CRP might be a good marker of inflammation and that it may not change throughout the cycle and apparently was not influenced by

concentrations of ovarian hormones. Individual differences for CRP levels are known to be related to different factors (such as: histories, physical activity, diet quality, stress, family history, ecology, and work load). These factors could cause individual differences in CRP, which makes CRP a good marker to assess personal risk to cardiovascular disease, and identify infection. More importantly, researchers conducting epidemiological studies may not have to worry about the ovarian cycle affecting CRP levels.

Differences in life history strategies are associated with interpopulational differences in levels of estradiol and CRP between Bolivian women and women living in Western countries. Life history theory states that an increase in caloric intake is associated with more total resources that can be allocated to different functions. This would suggest, after allocating resources to cover basic survival needs, the body would allocate resources to reproduction. Thus, with higher energy reserves, one would expect high levels of CRP as well as high levels of estradiol. However, this is not observed. Instead, the study shows women with higher energy reserves to have higher CRP levels and lower levels of estradiol compared to those with a fewer energy reserves. This suggests that in times of high nutrient availability, the body devotes more resources to survival and less to reproduction and in times of low nutrient availability, the body devotes more resources to reproduction and less to survival. If BMI captured all the variation in CRP levels between urban poor and urban better-off populations, life history theory may hold stronger weight. However, this does not seem to be the case and

although women may be poor and have fewer energy reserves, their levels of CRP and reproductive hormones are still affected by many other ecological factors.

Due to differences between the urban poor and urban better-off populations in histories, diet, physical activity and other ecological factors that may play a role, life history predictions do not apply in this study. Instead, differences in CRP can be primarily explained by ecological factors. This thesis attempted to explain tradeoffs between reproduction and maintenance. It is not life history theory, but rather ecological complexities that are important to sort these issues and understand the relationship between these functions.

V. Conclusion

Urban-p women with high BMI values have high CRP levels and low estradiol levels while urban-b women with low BMI values have low CRP levels and high levels of estradiol. Although socioeconomic status is slightly associated with CRP levels, individual differences between women exist that provide variation in levels of CRP. Because it can be shaped by individual differences and not by hormones throughout the ovarian cycle, CRP is a good biomarker of inflammation and health risk. Through further analysis of diet, stress, and exercise, further conclusions about the usefulness of CRP as a biomarker can be determined.

C-reactive protein's usefulness to research is growing. Studies that utilize this marker range from investigations in biological anthropology to infectious disease, to cardiovascular risk to life history evolution. Human biological variation across populations is prevalent and using CRP as a biomarker may be adequate since values of CRP do not fluctuate throughout the ovarian cycle. Analyzing CRP levels and reproductive hormone levels in Bolivian women provides a glimpse of the biological profiles of women living in a non-Western or non post-industrial population that is usually the focus of most research.

VI. References

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