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The Association Between Sex Hormone Binding Globulin And C-Reactive Protein Levels in Premenopausal Women

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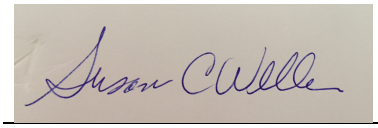

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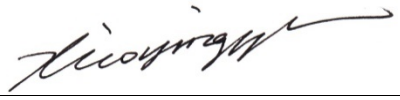

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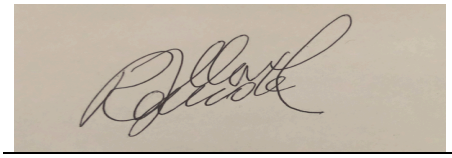

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The Association Between Sex Hormone Binding Globulin And C-Reactive Protein Levels in Premenopausal Women

BY

Rasha Azeez Hammood Al-Lami, MD

Thesis

Presented to the Faculty of the Graduate School of

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DEDICATION

I dedicate this thesis work to my lifesavers, my husband and my mother, who have unselfishly paved my way of success. A special feeling of gratitude to my mother for pushing me forwards and creating a healthy environment for me to focus. I would not have been a working mother without your long-lasting support. Thank you so much Mom

To my son, Mohammed, who is my strength through many trials and tribulations. Although we lived double your age on video calls, you gave me a courage to endure the separation pain and your smile taught me how to transform that pain into accomplishment. Despite the 7000 miles between us, the sense of your wellbeing nourished me through these three years of family separation.

And finally, to my father who is watching from heaven. I know you are still looking at me and our hearts are connected. It is true that you did not have the chance to see your little girl grow up successfully but you showered me with the sense of persistence and dedication through the very few years we lived together.

The Association Between Sex Hormone Binding Globulin And C-Reactive Protein Levels in Premenopausal Women

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Uncertainty remains regarding the effect of female sex hormones on the immune system and inflammation process. Sex Hormone Binding Globulin (SHBG) modulates the relative effects of estrogen and testosterone, specifically promoting estrogen effects systemically. Furthermore, estrogen enhances the synthesis of SHBG and was reported to have a role in immune modulation. Thus, it is believed that SHBG can influence inflammation and associated inflammatory markers such as C-Reactive Protein (CRP). There are few studies on the association between SHBG and CRP levels in premenopausal women. If this association is proven to be true, it could explain the mechanism by which some inflammatory and autoimmune diseases flare up or remit in women during their reproductive years.

This study investigated the association between SHBG and CRP in premenopausal women (age 20-44 yrs) using NHANES national data for the cycle 2015-2016. Women who were pregnant, breast feeding, had hysterectomy or both ovaries removed, had menarche at age > or = 20, had not started menarche yet, or currently had specific comorbidities were excluded. The outcome variable was CRP concentration and the main independent variable was SHBG concentration. Univariate and bivariate analysis were used to describe the sample and test the correlation of independent variables with CRP. Multivariable linear regression analysis was used to test the association between SHBG and CRP while controlling for estrogen and markers of metabolic syndrome (e.g. BMI) following diagnostics analysis.

Although SHBG was significantly and inversely associated with CRP in the unadjusted bivariate analysis (r= -0.14), it was found to have non-statistically significant weak positive association with CRP (r= 0.05) after controlling for multiple covariates and including significant interaction terms. BMI contributed the most to CRP variance in this

study full model (semi-partial R^2= 0.12). The positive association between SHBG and CRP persists after replicating an existing model in the literature. Further research is needed to test this research question in reproductive age-women using nationally representative samples after excluding women using exogenous hormones, controlling serum progesterone and defining the current phase of menstrual cycle.

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LIST OF ABBREVIATIONS

SHBG	Sex Hormone Binding Globulin
CRP	C-Reactive Protein
HbA1C	Hemoglobin A1C (glycohemoglobin)
PCOS	Polycystic Ovarian Syndrome
BMI	Body Mass Index
T2DM	Type 2 Diabetes Mellitus
IL-6,8	Interleukin 6,8
TNF-α	Tumor Necrosis Factor- Alpha
NF-KB	Nuclear Factor Kappa B
R_{SHBG}	SHBG receptor
ER-α,β	Estrogen receptor Alpha and Beta

CHAPTER 1: SPECIFIC AIMS, SIGNIFICANCE AND PROBLEM STATEMENT

SPECIFIC AIMS

Inflammation is a process involved in a variety of autoimmune, chronic diseases and cancers. C-Reactive Protein (CRP) is a sensitive biomarker of inflammation and is used by researchers to assess the inflammatory status. Gender differences in inflammation suggests that reproductive hormones may play a role in regulating the inflammation process. Given that autoimmune diseases have a higher prevalence in women of reproductive age, it is important to shed light on the association between reproductive hormones and inflammation in premenopausal women. Sex Hormone Binding Globulin (SHBG) is a carrier protein for androgen (e.g. testosterone) and estrogen (e.g. estradiol) in the blood stream and regulates the actions of these hormones on target tissues. Previous research suggested that SHBG might have a negative association with CRP; however, results were not consistent. Insulin resistance and metabolic syndrome (obesity) are also associated with higher CRP levels and can inhibit SHBG hepatic synthesis. The objective of this project was to examine the association between SHBG, which functions in the binding of sex hormones, and inflammation in a national sample while controlling for markers of metabolic syndrome, especially BMI. Specifically, this project tested the association between SHBG and CRP in premenopausal women, age 20-44 years, using the National Health and Nutrition Examination Survey (NHANES), for the cycle 2015-2016.

The specific aims are:
Aim#1: To examine the bivariate associations between SHBG, estrogen, testosterone, inflammatory markers, and the metabolic syndrome with CRP. Relationships between SHBG, metabolic syndrome (e.g. BMI, HbA1C), and inflammation (e.g. WBC) with CRP were expected to be significant.

Aim#2: To examine the association between SHBG and CRP in a multivariable model, while controlling for various covariates, e.g., metabolic syndrome (BMI). It was hypothesized that SHBG was associated with CRP. The multivariable model allowed for estimation of the simultaneous effects of reproductive hormones, obesity, and SHBG on CRP.

SIGNIFICANCE AND PROBLEM STATEMENT

CRP is associated with adverse health outcomes, most notably cardiovascular disease (CVD) and Type 2 Diabetes Mellitus (T2DM). Since high circulating SHBG levels enhance the biologic effects of estrogen on target tissues, higher levels of SHBG are expected to increase estrogen effects. [1] Estrogen effects on inflammation and the immune system are complex and dose dependent. [2] In healthy and normally menstruating premenopausal women, endogenous estrogen at physiological level is thought to have anti-inflammatory effects. [3] Consequently, high SHBG levels in the blood are expected to be negatively associated with inflammatory markers such as CRP. However, studies on premenopausal women have yielded inconsistent results. Small clinical studies that followed cohorts of women through their menstrual cycle suggest that CRP is highest during early follicular phase and lowest during the luteal phase, SHBG is highest during the follicular phase and lowest in the luteal phase, and high levels of CRP are strongly associated with lower concentrations of SHBG. [3,4] Two large community samples of healthy premenopausal women both showed a negative correlation between SHBG and CRP. The first study sampled after early follicular phase (n=353) [5], and the second during the luteal phase (n=233). [6] However, some studies related to clinical conditions (e.g., PCOS and non-PCOS[7] or first degree relatives of T2DM patients [8]) found a positive correlation or no correlation between SHBG and CRP. Obesity and insulin resistance are known to decrease the hepatic synthesis of SHBG and increase CRP levels and most analyses have controlled for obesity or BMI. [1] However, it is unclear how much SHBG affects CRP in premenopausal women, especially after controlling for metabolic syndrome measures like BMI and HbA1C. Given the lack of consistency in approaches, reporting, and results, this study proposed to test the association between SHBG and CRP in a national sample of premenopausal women while controlling for metabolic syndrome measures like BMI and HbA1C.

CHAPTER 2: BACKGROUND

C-REACTIVE PROTEIN (CRP)

CRP is a sensitive marker of inflammation and has been widely used as a surrogate in studies of inflammatory status. CRP is an acute phase reactant protein that was first discovered in a patient with bacterial infections. It is mainly synthesized by hepatocytes under the influence of other inflammatory markers, cytokines and interleukins such as IL-6 and TNF-α secreted by lymphocytes and macrophages in response to a current inflammatory state. CRP plays an important role in modulating the immune system and inflammatory processes, and is implemented in many inflammatory diseases like rheumatoid arthritis, lupus, infectious diseases, cancers and endothelium injuries. CRP activates the innate immune system, via activation of the complement pathway, and the adaptive immune system by regulating lymphocyte function. The normal concentration of CRP in the serum of healthy adults is less than 7.48 mg/L. [9] CRP levels tend to increase with age owing to subclinical inflammation induced by normal aging. [10] Inflammation is the basic mechanism in the pathology of many diseases including 1) autoimmune diseases such as rheumatoid arthritis, psoriasis, lupus and inflammatory bowel disease, 2) cancers and 3) other chronic diseases, including cardiovascular diseases. [11]

METABOLIC SYNDROME AND CRP

The metabolic syndrome is a constellation of findings including a state of insulin resistance (with high serum insulin levels), hyperlipidemia, high body fat, high blood pressure and high blood sugar, and occurs especially in obese and overweight individuals. It is thought that the higher body fat in obese and overweight individuals results in higher levels of free fatty acids, which inhibit cellular insulin-dependent glucose uptake, creating a state of insulin resistance. [12] Obesity and insulin resistance, as found in metabolic syndrome, are associated with low-grade inflammation; therefore, CRP is positively associated with markers of metabolic syndrome like BMI, percentage body fat, body and hip circumference, central obesity and other insulin resistance measures. [6 4,13] The low-grade inflammation caused by obesity can be attributed to inflammatory mediators released from the high number of adipocytes, such as TNF-α and IL-6, and these cytokines then augment liver synthesis of CRP. This can explain the association between metabolic syndrome in obese individuals and risk to develop inflammatory diseases and coronary artery disease. [13] In large scale studies in adults, sex differences in inflammatory state are associated with metabolic syndrome markers. Choi et al. reported a systematic review of 51 cross-sectional studies and found that adult women tend to have stronger correlation between BMI and Ln (CRP) than adult men regardless age and ethnicity (overall $r = 0.36$, and $r = 0.53$ for women and $r=0.24$ for men). This higher correlation between BMI and CRP in women was most notable in North American and European women rather than in Asians. The mechanism of sex variation in the association between adiposity and CRP remains poorly understood, but it is suggested

that leptin, which is usually highly expressed among women, is linked to high CRP levels. Furthermore, fatty tissue metabolic activity is thought to be higher in women, thus, causing obese women to have higher CRP levels than men. [14]

SEX HORMONE BINDING GLOBULIN (SHBG)

SHBG is a testosterone (or androgen) and estrogen binding protein that is mainly synthesized in hepatocytes. SHBG normal values in non-pregnant adult women are 18-144 nmol/L. [15] SHBG regulates the actions of serum estrogen and testosterone on target tissues. In the blood circulation, roughly 38% of estrogen is bound to SHBG and considered inactive according to the "free hormone theory" while the free portions of estrogen and testosterone are considered biologically active. The hormone-SHBG complex acts as an antagonist to the SHBG receptor (R_{SHBG}) which then cannot modulate intracellular signaling. In contrast, free-of-hormone-SHBG can bind to R_{SHBG} but without affecting the intracellular signaling system. If an agonist hormone (e.g. estradiol) encounters a SHBG molecule that is already bound to its receptor (R_{SHBG}), the complex (estradiol-SHBG-R_{SHBG}) will turn on the signaling system inside the cell via the cAMP second messenger pathway (Figure 1). [16]

SHBG has a higher affinity for testosterone (or other androgen) than estrogen which explains the "estrogen amplification phenomenon" in which, high levels of SHBG binds a larger proportion of circulating testosterone than estrogen leaving a proportionately higher free estrogen level compared to free testosterone, which consequently leads to greater biological effects of estrogen relative to testosterone (Figure 2). On the other hand, high levels of estrogen increase liver synthesis of SHBG through a positive feedback mechanism while high testosterone levels inhibit SHBG hepatic synthesis. [1,17]

The properties of megalin, the more recently discovered endocytic receptor for vitamin A, vitamin D and SHBG, contradict "the free hormone theory" in that estrogen and testosterone bound to SHBG can enter the cell and affect intracellular functions. Once inside the cell, SHBG is degraded and the free hormone, that was originally bound to SHBG, is released and acts freely. [18,19] Of note, a recent in vitro study showed that SHBG can affect immune cells and that human immune cells can internalize circulating SHBG using a receptor other than megalin. Furthermore, the study found that prior treatment of lymphocytes with estradiol and SHBG simultaneously at 37 °C enhanced lymphocyte signaling. [20] Thus, SHBG has two mechanisms for mediating the effects of hormones; first, binding directly to R_{SHBG} and then binding its hormones; second, binding to megalin as a SHBG-hormone complex and then entering the cell.

METABOLIC SYNDROME AND SHBG

SHBG serum levels can be affected by various diseases including those of the thyroid, liver and kidneys, diabetes and obesity as well as by normal aging, mainly

through modulation of hepatic synthesis of SHBG. [1] In contrast to the estrogen amplification phenomenon, insulin resistance, characterized by high levels of serum insulin, as seen in the metabolic syndrome, inhibits hepatic SHBG synthesis, which leads to an increase in serum concentration of free testosterone (Figure 2). Obesity is known to alter levels of sex steroid hormones in men and women. Most obese women develop oligomenorrhea, with very light menses and/or long periods between menses, and tend to have high levels of estrogen and testosterone. Adipocytes express aromatase enzyme that can convert circulating androgens, normally released from the ovaries and adrenal glands, into estrogen, creating higher than normal estrogen levels in the form of estrone which is biologically weaker than estradiol. [21] Obese women also suffer from high testosterone level owing to their insulin resistance status and high insulin levels, which inhibit SHBG synthesis and lead to proportionately higher free testosterone levels. For instance, obese women with oligomenorrhea often have Polycystic Ovarian Syndrome (PCOS) with hirsutism due to high free testosterone levels. 40 % of PCOS patients are obese women and most of non-obese PCOS patients have a state of insulin resistance explaining the negative role of metabolic syndrome, and high insulin levels, on SHBG synthesis and consequently high circulating testosterone hormone. Additionally, the combination of high insulin levels and changes in sex steroid hormones can be explained by the role of adipokines, which are cytokines produced by fat cells, and alteration in liver synthesis of SHBG which creates a low-grade inflammation. Furthermore, PCOS patients tend to have high CRP levels regardless of body weight. [22] Most PCOS obese women experience improvement in symptoms and hormone levels after losing weight. [1]

STUDIES ON SEX STEROID HORMONES, SHBG AND CRP IN WOMEN

Although some clinical studies suggest that SHBG and CRP are negatively associated, studies on pre-menopausal women show inconsistent results. All studies I found in literature reported weak correlation between SHBG and CRP regardless the statistical significance and direction of correlation. Given the variation of female reproductive hormones across the menstrual cycle, most of the studies on sex steroid hormones, SHBG and CRP examined postmenopausal women and have evaluated hormone replacement therapy (HRT) and cardiovascular diseases on older women. In general, these studies indicate a negative association between SHBG and CRP. Log-SHBG was *negatively* associated with log-CRP (b = - 0.28) in a cross-sectional study on 513 postmenopausal women with mean age 76 years and not using hormone replacement therapy while serum testosterone and estrogen were positively associated with CRP after adjustment for age, BMI, smoking, insulin, COPD, stroke, CHF, fasting insulin, and physical activity. [23] Another cross-sectional study on 889 healthy postmenopausal women with mean age 59 years and not using HRT found that CRP was positively associated with BMI, estrogen and testosterone but *negatively* associated with SHBG in their bivariate analysis while SHBG was *negatively* associated with CRP (b= -0.11) in a model adjusted for markers of metabolic syndrome and obesity. [24]

SHBG was also negatively associated with CRP and cardiovascular disease index in studies of pregnant women. A prospective study of 291 Caucasian pregnant women

(mean age 30 yrs) with uncomplicated singleton pregnancies who were assessed during the second trimester (mean 26 weeks), found that SHBG at the time of delivery was *negatively* associated with CRP after controlling for markers of insulin resistance and BMI (b = -0.15). [25]

There are few studies of this research question in premenopausal women and the studies that are available have conflicting results (Table 1). In Blum et al., in a small clinical study (n=15) followed two cohorts of women (n=8, BMI<26 and n=7, BMI>27) through their menstrual cycle and found that as CRP increased SHBG decreased. [4] In Bell et al., a cross-sectional representative sample of 353 healthy premenopausal women (age range 25-54 years) who were sampled after the 8th day of their cycle (after early follicular phase) found that SHBG was *negatively* associated with CRP in premenopausal women after adjusting for BMI, exercise, age, smoking status and alcohol intake (b= -0.11, squared semi-partial correlation was 0.01). [5] A strength of the Bell et al study was that they were able to control for phase of the menstrual cycle and exclude any exogenous hormone users as well as relevant co-morbidities such as PCOS. In Nayeem et al., a cross-sectional study that investigated 233 healthy premenopausal women (30-40 years) during their luteal phase of menstrual cycle also found that SHBG was *negatively* correlated with CRP. CRP had a negative unadjusted bivariate correlation with SHBG (r = -0.42) whose effect was reduced when controlling for other metabolic variables (e.g. BMI) in a multivariable model (standardized slope, β = -0.12). Percent body fat (β =0.49), ALP (β =0.15), SHBG (β =-0.12), and WBC (β =0.15) were independent predictors of CRP during the luteal phase of the menstrual cycle. [6] Estradiol and progesterone were neither strongly nor significantly correlated with CRP in Nayeem's bivariate analysis. Studies done on women with specific conditions (obesity, PCOS, and relatives of T2DM) have shown mixed results. Kopp et al., conducted a prospective cohort study on 43 morbidly obese premenopausal women found that after undergoing bariatric surgery, SHBG increased and CRP decreased significantly and that change in SHBG was negatively associated with change in BMI (r= - 0.50). [26] Shen et al., conducted a cross-sectional study of 330 premenopausal women (mean age 27.8 years) with PCOS (n=165) and without PCOS (n=165), who were matched on BMI, and found a *positive* correlation between SHBG and CRP (r = 0.20), BMI and CRP as well (r= 0.40). [7] They reported significantly lower levels of SHBG in higher BMI groups and in PCOS women but non-significant difference in CRP between PCOS and non-PCOS women. [7] In a cross-sectional study of first degree T2DM relatives, Abdella et al. found a *negative* correlation between SHBG and CRP for males (rho=-0.39, p=0.11, n=235), but essentially *no* correlation for females (rho=0.07, p=0.77, n=349). The age range of the premenopausal women subsample was 29-49 years. [8]

The contradictory findings of these studies may be due to differences in sampling, study protocols, statistical analyses, and/or reporting of results. Nayeem et al may have found a smaller effect of SHBG on CRP than did Bell et al., although this may be due to differences in reporting of results. Both Nayeem et al. and Bell et al. sampled healthy premenopausal women, but Nayeem et al limited the sample to the luteal phase while Bell et al. sampled premenopausal women after the early-follicular phase (after day 8) since Bell and colleagues' primary interest was to examine testosterone and androgen

effects (to avoid androgen nadir in early follicular phase). Shen et al. and Abdella et al both studied specialized samples (PCOS and non-PCOS patients and first-degree relatives of T2DM) who might have an unmeasured insulin resistance state. Women with PCOS tend to have insulin resistance even at low body weight. [1] Insulin resistance is known to inhibit hepatic SHBG synthesis.[1] Furthermore, Shen et al. correlated SHBG with CRP in their entire sample consisting of PCOS and non-PCOS patients and this could have attenuated the expected negative association between SHBG and CRP. However, their finding that SHBG was significantly lower in PCOS group compared to non-PCOS group of women supports the negative impact of insulin resistance on SHBG and could have affected the overall relation between CRP and SHBG in their entire sample. Moreover, Shen and colleagues reported significantly higher fasting endogenous insulin levels in PCOS than in non-PCOS groups while fasting and random serum glucose levels were not significantly different between PCOS and non-PCOS women which supports the fact that patients with PCOS have a state of insulin resistance regardless their glycemic control. [7]

WOMEN, ESTROGEN AND INFLAMMATORY DISEASES

Women tend to be at higher risk of developing autoimmune diseases than men. [1] Autoimmune diseases are diseases caused by over-reactive immune responses and intense inflammatory response directed against host tissues and antigens causing tissue destruction. Prevalence of various autoimmune diseases like Systematic Lupus Erythematosus, rheumatoid arthritis, type-1-DM and hepato-biliary related autoimmune diseases are higher in women than in men. [27] The tendency of women to develop autoimmunity hinges on many factors, one of which is linked to female sex steroid hormones. Estrogen may be a main culprit in this pathway. [2] Some autoimmune diseases remit during high estrogen states, but lupus may flare up (up to one-third of cases) in the presence of high hormonal levels during pregnancy. [1] Progesterone reaches very high levels during pregnancy and endogenous progesterone was reported to have pro-inflammatory effects in premenopausal women. [3]

In a normally menstruating female, estradiol is synthesized by granulosa cells of the developing ovarian follicle during the follicular phase of the cycle, the period that extends from the last few days of the previous menstrual cycle until ovulation. The follicular phase is characterized by a high level of FSH hormone, released from pituitary gland, that aids in maturation of developing follicle and activation of aromatase enzyme of the granulosa cells which converts androgen, obtained from nearby thecal cells, into estrogen. [28] 17β-Estradiol (E2) is the main active form of endogenous estrogen hormone and accounts for most physiological estrogen effects. After binding to one of its two receptors, ER-α and ER-β, estradiol forms a compound that act as a transcription factor that modulates the transcription of many genes in the human body like many steroidal hormones. [29]

Estrogen receptors are expressed by variety of tissues apart from reproductive organs. For example, many adaptive and innate immune cells were shown to express

receptors for estradiol, specifically ER-α, through which estradiol can modulate their immunologic functions. [2] Moreover, estrogen plays an important role in the pathogenesis of many cancers including ovarian, endometrial and breast cancers, an effect attributed to increased inflammatory reactions in these tissues leading to carcinogenesis. For example, estrogen receptors and aromatase (estrogen synthesizing enzyme) are found to actively function in a number of cancer tissues. [30] Not only endogenous, but exogenous estrogen can also be implemented in carcinogenesis. For instance, estrogen containing drugs augment the risk of estrogen dependent cancers such as breast and endometrial cancer. [31,32]

However, estrogen can exert conflicting reactions on immune system depending on its blood concentrations. While most cytokines, (e.g. Il-6, IL-8 and TNF-α), are decreased by periovulatory dosages of estradiol, low levels can increase inflammatory markers, and may put postmenopausal women (who lack high levels of estradiol) at higher risk for cardiovascular diseases. [2]

A possible anti-inflammatory effect of estrogen could be explained by a signaling pathway that involves estrogen receptor mediated inhibition of nuclear factor kapa B (NF-kB) and subsequent downregulation of other inflammatory cytokines that shut down the immune system. [33] In animal studies, activation of ER-α by exogenous estradiol was shown to reduce leukocyte, particularly eosinophils, assembly in non-gonadal tissues. [34] A high estrogen level may reduce influenza infection severity and modulate the inflammatory response. [35] In a vitro study, CRP was shown to be less expressed in human aortic endothelial cells after pre-treatment with estradiol at physiologic dosage. [36] Gaskins et al., did a longitudinal study on the effect of endogenous reproductive hormones on CRP during the normal menstrual cycle in a sample of healthy reproductive age women (18-44 yrs) and found that with higher serum estradiol, levels of CRP would be lower, supporting the anti-inflammatory role of endogenous serum estradiol. Since follicular phase is known to have rising estrogen level secreted from the developing ovarian follicle, CRP level should be low during this phase of the cycle, which is consistent with Gaskins' results. [3] In Blum et al., tested a very small sample of 15 premenopausal women and found that CRP was highest in early follicular phase after 15 measurement. This could be explained by lack of time for the newly developing follicle to secrete enough estrogen to negatively impact CRP levels since Blum classified the early follicular phase as 15 to 9 days before the LH peak, which is very early for the newly developing follicle to secrete sufficient estrogen to attain high levels. [4]

TESTOSTERONE, OR ANDROGEN, IN WOMEN AND INFLAMMATION

Androgen in women is synthesized inside the ovaries by follicular luteal cells under the effect of LH hormone. Testosterone is also synthesized in zona reticularis layer of the adrenal gland cortex. Peripheral 5-alpha reductase enzyme converts testosterone into the most biologically active form, dihydrotestosterone (DHT) while aromatase enzyme can convert testosterone into estrogen in various gonadal and extragonadal

tissues expressing aromatase, making this mechanism the main source of estrogen in postmenopausal women. About 2/3 of circulating testosterone is bound to SHBG and 1/3 to albumin. Testosterone has a positive effect on sexual function and desire in premenopausal women. Testosterone has also been used to treat postmenopausal vaginal atrophy in patients with contraindications for estrogen use and was found to improve cardiovascular diseases by mediating vasodilation. Additionally, there is growing evidence that normal physiologic levels of endogenous testosterone have a protective effect on cardiovascular health in women and that effect is dependent on SHBG. Endogenous testosterone along with estrogen have been reported to have protective and anti-inflammatory effect on brain tissues and may protect from Alzheimer disease in women. More interestingly, the testosterone level inside the brain of premenopausal women seemed to be higher than estrogen and downregulation of testosterone inside the brain was shown to induce neuronal toxicity. However, the role of testosterone in cancers of female reproductive organs and breast cancer is controversial. [37]

POLYCYSTIC OVARIAN SYNDROME (PCOS), SHBG AND CRP [7,38]

Polycystic ovarian syndrome (PCOS), or Stein–Leventhal syndrome, is a hormonal disorder characterized by infrequent menstrual cycles and high androgen index among women of reproductive age. PCOS is one of the most common endocrine disorder that is responsible for infertility among child-bearing age women. Further, patients with PCOS usually have a state of insulin resistance, obesity and are at high risk for adverse health outcomes (e.g. cardiovascular diseases). PCOS is characterized by abnormal LH:FSH ratio that hampers the pituitary-ovarian endocrine axis. For various genetic (including insulin resistance state) and environmental factors, LH tends to be proportionally higher than FSH in women with PCOS but both remain in normal rage. The lower FSH levels are insufficient for granulosa cells in the developing ovarian follicles to mature normally and thus form small cysts inside the ovary without reaching mature size that is ready for ovulation. Without mature follicles, estrogen (in the form of estradiol) levels remain low. The proportionally higher LH levels nourish thecal cells (inside small follicles) to synthesize testosterone which is responsible for high androgen index in these patients causing acne and hirsutism. Testosterone can also be converted peripherally into estrogen (in the form of estrone) which further inhibits FSH levels. The net result is that patients have anovulatory cycles with high testosterone levels. The prevalence of PCOS is about 10 % in the reproductive age women of the U.S. Although the cause of PCOS is not determined, it is postulated that the state of insulin resistance among patients with PCOS play an important role in the disease pathogenesis. Women with PCOS were reported to have lower insulin sensitivity independent of obesity and higher levels of CRP which indicates the presence of an ongoing inflammation. Moreover, women with PCOS were shown to have decreased SHBG levels compared to non-PCOS women. Insulin resistance inhibits SHBG hepatic synthesis and the high levels of testosterone in women with PCOS further inhibits SHBG levels in these patients. Metabolic syndrome, obesity and insulin resistance states are known to be associated with higher CRP levels. In view of long-lasting inflammation (measured by CRP) in women with PCOS, they are at higher risk to develop adverse health outcomes apart from infertility (e.g. Diabetes and cardiovascular disease).

SUMMARY

Inflammation is an important part of the pathogenesis of many diseases, including autoimmune diseases that tend to have higher prevalence in women of reproductive age, and associations have been reported between reproductive hormones and inflammation; therefore, this study tested the relation of surrogate markers of reproductive hormones, SHBG, and inflammation, CRP, in premenopausal women. Furthermore, given the conflicting association between SHBG and CRP in previous studies and small unrepresentative samples, I sought to address this question in a national sample of premenopausal women, age 20-44 years, while controlling for markers of metabolic syndrome and other hormones.

The specific aims of this project were:
Aim#1: To examine the bivariate associations between SHBG, estrogen, testosterone, inflammatory markers, and the metabolic syndrome with CRP. Relationships between SHBG, metabolic syndrome (e.g. BMI), and inflammation (e.g. WBC) with CRP were expected to be significant.

Aim#2: To examine the association between SHBG and CRP in a multivariable model, while controlling for various covariates, e.g., metabolic syndrome (BMI). It was hypothesized that SHBG was associated with CRP. The multivariable model allowed for estimation of the simultaneous effects of reproductive hormones, obesity, and SHBG on CRP.

CHAPTER 3: METHODS

DATA AND DESIGN:

Data came from the National Health and Nutrition Examination Survey, NHANES. NHANES is a national survey performed by the National Center for Health Statistics (NCHS) of Centers for Disease Control and Prevention (CDC). NHANES started in 1960's in a series of surveys then in 1999 NHANES became a continuous cross-sectional study conducted every two years to assess the U.S general population health and nutritional status. NHANES provides prevalence estimates for specific diseases and conditions; tracks the prevalence and behaviors of specific health conditions, exposures, and dietary behaviors. NHANES data are gathered using personal interviews, physical examinations and laboratory investigation results.

NHANES datasets are publicly available. NHANES data are gathered using complex, multistage probability sampling methods in order to obtain a nationally representative sample of civilian and non-institutionalized residents of the U.S from all age groups. NHANES data are gathered according to the following stages: counties or small sections of counties, called primary sampling units (PSUs), are selected first; then blocks or groups of blocks are created by selecting portions of PSUs containing clusters of households followed by selections of specific households from these blocks and finally selecting individuals from these households.

The NHANES cycle 2015-2016 was used since this period contained most of the variables of interest for this study. In cycle 2015-2016, a total of 9,544 participants were examined and total 9,971 participants were screened. Of those screened, 5,079 female individuals were included, from age 0 to 150 years. This cycle oversampled the following subgroups: Hispanic; non-Hispanic black; non-Hispanic Asian; non-Hispanic white and non-Hispanic individuals reported races other than black, Asian or white, and who were at or below 185% of the department of Health and Human Services poverty guidelines or were age 80 years or older.

SUBJECTS:

The sample included reproductive age adult female individuals from 20-44 years. Women who reported a hysterectomy, ovary removal, breast feeding a child, or were pregnant (by positive urine pregnancy test) at the time of the survey were excluded. All of the aforementioned conditions were excluded because they could affect hormone levels and were excluded from our sample. [1,6] Women who had not started menstrual cycle at the time of study, had irregular period or began menarche after the age of 20 were also excluded. Early or late onset menarche can be associated with chromosomal anomalies and hormonal problems. [1] Since patients with asthma, chronic bronchitis, osteoarthritis, psoriatic or rheumatoid arthritis may use steroid or NSAIDs drugs which could affect the CRP levels, those participants were excluded from this study sample. [6]

Participants with thyroid problems at the time of the interview and exam were excluded as well, since thyroid diseases can affect SHBG levels[1] (Table-2: variables used for exclusion).

MEASUREMENT OF VARIABLES:

Analyses focused on the independent variable, SHBG, and the dependent variable, CRP. Anyone with missing values on these variables was omitted from the dataset (Table 3: list of variables).

I. ***Main dependent variable (HS-CRP):*** The outcome variable of interest is high-sensitivity-CRP. It was measured from all participants above the age of one year and was measured on (the Beckman Coulter UniCel DxC 600 Synchron and the Beckman Coulter UniCel DxC 660i Synchron Access chemistry analyzers). The lowest limit of detection for HS-CRP was 0.11 mg/L in 2015-2016 cycle.

II. ***Main independent variable (SHBG)***: SHBG was measured on males and females age 6 to 150 years with a lower limit of detection of 0.800 nmol/L.

III. ***Covariates***: CRP levels may be affected by a current infection, liver and kidney function, and metabolic syndrome. Because CRP can be affected by infections, [11] WBC count is included as a potential covariate. Since the dependent and independent variables are synthesized by liver, liver biomarkers; AST and ALT were included. Sex hormones can be carried by albumin and it was also included as a covariate. Since sex hormones can affect SHBG synthesis and SHBG functions as carrier to testosterone and estrogen, serum estrogen and testosterone were included as covariates. Hormones can be metabolized by kidney as well, [1] thus, BUN and serum creatinine were included as measures of renal function. Values below the lower limit of detection (LLOD) of the used assay were considered invalid and were recoded as missing values. However, LLOD of testosterone and Blood Urea Nitrogen (BUN) in NHANES data files were reported in ng/mL and g/dL, respectively, while in lab methods, LLOD was reported in ng/dL for testosterone and mg/dL for BUN.[39,40] Based on this discrepancy, LLOD values were considered in ng/dL for testosterone and mg/dL for BUN in accordance with lab methods documentation. Previous research found BMI to be strongly correlated with CRP [6,25] and thus, was included in this study. In addition, the following markers were considered in our study as proxies to measure the state of metabolic syndrome which can affect both the main dependent and independent variables; HDL-cholesterol; LDL-cholesterol, triglycerides, total cholesterol; alkaline phosphatase, SBP and DBP and HbA1C. Basic demographic variables were also included like age and race. (table 3).

STATISTICAL ANALYSIS

Aim #1: To examine the bivariate associations between SHBG, estrogen, testosterone, inflammatory marker, and the metabolic syndrome with CRP.

First, the sample was defined using the inclusion and exclusion criteria listed above (Table 2), as well as omission of subjects with incomplete data. Anyone with missing values on SHBG or CRP were eliminated from the sample. Variables with many missing values (Table 3) were not considered in the analysis (e.g. LDL, triglycerides, SBP and DBP). Second, univariate distributions were examined. Univariate procedures were used to examine the distribution, presence of outliers, kurtosis, and skewness of each continuous variable. [41] Frequency procedure was used to examine the distribution of the race variable, the only categorical variable in this study dataset. Then bivariate scatterplots were used to show the relation of each continuous independent variable with the outcome (CRP) followed by partial regression plots. CRP, SHBG, estrogen, testosterone, ALT and AST were log transformed to satisfy the assumption of normal residual distribution of multivariable linear regression analysis which is used to test this study hypothesis. The "Mexican-American" and "other Hispanics" levels of the original race variable were combined into a single category named "Hispanics" and other levels were recoded. The final race/ethnicity categorical variable was as the following; White=0; Black=1; Asian=2; Hispanics=3, and Other=4. Finally, bivariate correlations between each variable and CRP was calculated with the Pearson and Spearman correlation coefficients. The statistical analysis of this study was unweighted without consideration for oversampling of racial groups.

Aim#2: To examine the association between SHBG with CRP in a multivariable model, while controlling for various covariates, e.g., metabolic syndrome (BMI).

Multivariable linear regression analysis was used to test the hypothesis that serum log-SHBG was associated with Log-CRP (two-sided hypothesis). Residual analysis was performed to examine the normal distribution of the residuals, equal variance and outliers influence. To detect outliers in residual analysis, Jackknife residuals, Cook's D, Leverage versus Jackknife residual plots, and DFBETAS were used. To examine linearity, equal variance (homoscedasticity) and independence assumption, plots of residuals (Jackknife and raw at a time) versus predicted values and each predictor were performed. Finally, fit diagnosis plots by reg procedure was done including model with all independent variables with raw scale followed by model with transformed scale. Log transformation of CRP, SHBG, estrogen, testosterone, ALT and AST were performed to satisfy the multivariable regression analysis assumptions. Collinearity was tested among the variables in the full model using Tolerence (Tol) and Variance Inflation Factors (VIF). Since this study hypothesis was inference rather than prediction, no sample splitting was conducted. Backward selection was used to select the final main effects model (Model 1) after forcing the main independent variable (SHBG). A second model was then sought (Model 2) to test for possible two-way interactions with SHBG. After centering variables, interaction terms were created between each of 6 selected covariates (log-estrogen, WBC, HDL, BMI, ALP, albumin) and log-SHBG. All tests were two-sided with significance level of 0.05. All analyses were conducted in the department of Preventive Medicine and Population Health using SAS version 9.4. [41]

CHAPTER 4: RESULTS

A cohort of 903 women met the study inclusion criteria and of these, 718 female participants did not have missing values for the main dependent variable (CRP) or the main independent variable (SHBG). LDL, triglycerides, blood pressure (SBP and DBP) variables were dropped due to high number of additional observations with missing values. The sample was further reduced to 687 participants who had complete data on the remaining 15 variables (Figure 3).

AIM#1: TO EXAMINE THE BIVARIATE ASSOCIATIONS BETWEEN SHBG, ESTROGEN, TESTOSTERONE, INFLAMMATORY MARKERS, AND THE METABOLIC SYNDROME WITH CRP.

The average age of women in this study was 32.12 years. 24 % of sample are white, 21.83 % are non-Hispanics black, 13.25 % Asian, 37.12% are Hispanics and 3.78% are of other race groups. Mean CRP was 4.75 mg/L with SD of 6.69 mg/L. CRP median was 2.03 mg/L with interquartile range 4.60 mg/L. Mean SHBG was 69.31 nmol/L and mean BMI was 30.08 kg/m^2 which is in the obesity range while median BMI was 28.50 which is in the overweight range. Data are presented as means and standard deviation (SD) along with median (and interquartile range) for continuous variables on original scale and selected transformed continuous variables in addition to frequency for the lone categorical variable (race) (Table 4). For CRP, 19% of participants had values above 7.4 mg/L which was above the expected 5% cutoff for values outside the normal range for CRP (< 7.48 mg/L). [9] For SHBG values, 8% had values above 144 nmol/L and 2% below 18 nmol/L which is outside the normal range of SHBG for adult non-pregnant premenopausal women (18-144 nmol/L). [15]

All continuous variables were not normally distributed visually and by Shapiro-Wilk test based on univariate procedure results. After performing the multivariable analysis (see aim #2 below) log transformation was conducted on CRP, SHBG, estrogen, testosterone, ALT, and AST. Transformation of other variables (WBC, HDL, total cholesterol, BMI, Alp, albumin, BUN, creatinine, age and HbA1C) did not improve normality, equal variance, or outlier influence of variables' residuals in the multivariable model (see aim #2 below). HbA1C residuals violated the equal variance and normality assumption when plotted against log-CRP but assumptions were not improved after logarithm or square root transformation of HbA1C. However, the multivariable model with log-CRP as the outcome and log transformed SHBG, estrogen, testosterone, AST and ALT along with original scale of HbA1C and remaining covariates in table 4 did not severely violate the multivariable linear regression analysis assumptions. Log transformation was chosen among other possible transformations based previous studies' methodology that tested and/or used same variables or dataset as this study.[6,11]

The Pearson correlation was calculated using log transformed CRP, SHBG, estrogen, testosterone, AST and ALT along with original scale of the remaining variables. Without controlling for any variable, log-SHBG showed weak negative but significant correlation with log-CRP (Pearson r= -0.14). Likewise, log-estrogen, log-testosterone, HDL, and albumin were significantly and negatively correlated with CRP without adjusting for other variables (Pearson r= -0.18, -0.08, -0.36, -0.41, respectively). BMI had a high positive correlation with CRP (Pearson r= 0.61). Similarly, WBC, ALP, ALT, and HbA1C were significantly and positively correlated with CRP. Race, as a categorical variable, was significantly correlated with log-CRP (eta coefficient 0.18). Since the continuous variables were not normally distributed, Spearman correlations were also calculated. Results from the Spearman and Pearson correlations were similar, with the exception of HbA1C where the Spearman correlation coefficient (0.33) was slightly higher than the Pearson correlation coefficient (0.23) but stayed positively and significantly correlated with CRP or log-CRP in both cases (Table 5). Total cholesterol, age, AST, BUN, and creatinine were not significantly correlated with CRP or log-CRP.

The simple straight-line regression showed a weak but significant negative linear relationship between log-SHBG and log-CRP (figure 4) (estimated Log-CRP= 1.96 - 0.28 Log-SHBG, r=-0.14, p= 0.0002 for slope). For each one nmol/L increase in log-SHBG, there was 0.28 mg/L decrease in log-CRP (or 1.32 mg/L decrease in CRP mean). Although there was a significant correlation, the data showed only a very weak linear relationship between Log-SHBG and Log-CRP (R^2=0.02).

AIM#2: TO EXAMINE THE ASSOCIATION BETWEEN SHBG WITH CRP IN A MULTIVARIABLE MODEL, WHILE CONTROLLING FOR VARIOUS COVARIATES, E.G., METABOLIC SYNDROME (BMI).

A backward selection was used to identify the main effects model using the 15 variables in table 4 to test the association between log-SHBG and log-CRP with and without interaction terms of each of the covariates with log-SHBG. Log transformation of CRP, SHBG, estrogen, testosterone, AST and ALT along with raw scale of other variables satisfied multiple linear regression analysis assumptions of normal residuals' distribution, equal variance, linearity and outlier influence which were examined by visual inspection on the diagnostic plots. HbA1C residuals violated the equal variance assumption of multivariable regression analysis that was not satisfied by log or square root transformation of HbA1C plotted against log-CRP. However, no log transformation was conducted on HbA1C or the other remaining continuous variables since their residuals' distribution with log-CRP did not seriously violate normality and equal variance assumptions in a model with all variables. There was no significant collinearity among the variables in the model with all variables (each variable VIF <10 and all Tol >0.1).

Backward selection of the model with 15 independent variables using glmselect procedure [41] with significance level at 0.05 was conducted after forcing log-SHBG into the model without interaction terms. The main effects model consisted of log-SHBG (forced variable), log-estrogen, BMI, WBC, HDL, ALP, and albumin (Table 6, Model 1). In this model, log-SHBG was significantly but positively associated with log-CRP (β= 0.07, P =0.027). Log-SHBG explained 0.4% of the variance in Log-CRP after controlling other covariates in the selected model (semi-partial R^2 =0.004).

Because the slope on SHBG changed from negative in the bivariate analysis (β=-0.14) to positive (β=+0.07) in the multivariable model, a second model was sought by exploring the possible interactions with SHBG. After centering variables, interaction terms between the main independent variable (log-SHBG) and each of the 6 selected covariates from model 1 (log-estrogen, WBC, HDL, BMI, ALP, albumin) were created and the final model was re-selected (from 15 continuous variables in table 4) after forcing log-SHBG only. Only the interactions between log-estrogen and log-SHBG and between ALP and log-SHBG were significant at the 0.05 probability level. In this case, the final model retained the same variables as Model 1, but additionally included the two interaction terms (Table 6, Model 2). The main effects of the independent variables in Model 2 were similar to those in Model 1, except the effect of log-SHBG and log-estrogen were slightly reduced after retaining the two interaction terms. The effect of log-SHBG was slightly lower in Model 2 (β=0.05) than that in Model 1 (β=0.07) and was no longer significantly associated with log-CRP but maintained a weak positive association with log-CRP. The positive effect of log-SHBG on log-CRP was attenuated by the interaction between log-SHBG and log-estrogen. The difference in the slope between the log-SHBG and log-CRP was 0.1 standardized unit decrease in logarithm scale with one standard deviation increase in log-estrogen. Since SHBG is the carrier protein for endogenous serum estrogen[1], it might be expected to find an interaction between SHBG and estrogen. Moreover, endogenous serum estrogen has an inverse relationship with serum CRP in premenopausal women (in other words, higher estrogen can have anti-inflammatory effect) [3], therefore, it may attenuate SHBG main positive effect.

Similarly, the positive effect of log-SHBG on log-CRP was attenuated by the interaction between log-SHBG and ALP. The difference in the slope between the log-CRP and log-SHBG was 0.1 standardized unit decrease with one standard deviation increase in ALP. Although the NHANES data used in this study did not differentiate between hepatic or bone ALP, previous research reported total serum alkaline phosphatase was mostly of hepatic origin. [6] Since both ALP and SHBG are synthesized by the liver, an interaction between both of these markers is expected.

In both models, log-estrogen was negatively associated with log-CRP (β= -0.15, -0.10, respectively) while controlling for other variables. Log-estrogen explained 2% of the variance in log-CRP in the first model and 1% in the second model. HDL and albumin were also inversely associated with log-CRP in both models. BMI had the highest positive association with log-CRP in both models and uniquely contributed to 11% and 12% to log-CRP variation in model 1 and model 2, respectively. WBC and ALP were positively associated with log-CRP in both models.

CHAPTER 5: DISCUSSION

Among the 15 tested covariates, only six were significantly associated with CRP in model 1 and model 2 (estrogen, BMI, WBC, HDL, ALP, and albumin). SHBG was negatively associated with CRP in the bivariate analysis but positively associated with CRP in the multivariable linear regression analysis that controlled for the six covariates. Higher levels of estrogen, HDL, and albumin were associated with decrease in inflammation as measured by CRP, while higher levels of BMI, WBC and ALP were associated with increased inflammation as measured by CRP. Sex hormone studies that tested the association between SHBG and CRP have yielded inconsistent results (table 1). This national cross-sectional study of reproductive-age women found a weak negative correlation between SHBG and CRP in the unadjusted bivariate analysis and a weak positive association in the adjusted multivariable linear regression analysis. It was especially important to control for serum estrogen in all models of the regression analysis of this study because women in this sample could have been at various phases of their menstrual cycles. [1,3] In addition, NHANES data did not have information on (1) current menstrual cycle phase; (2) endogenous progesterone, and (3) use of exogenous hormones including contraceptive methods.

Among the very few studies on premenopausal women that tested the relationship between SHBG and CRP, Nayeem's and Bell's studies were the most relevant to this study.[5,6] In contrast to Nayeem et al. and Bell et al., this study found that SHBG was positively associated with CRP while controlling for covariates of serum estrogen, inflammation (e.g. WBC), metabolic syndrome (e.g. BMI, HDL, and total cholesterol), liver function (e.g. AST and ALT) and other confounders that could affect the SHBG and/or CRP (e.g. ALP, testosterone, HbA1C and albumin). Nayeem and colleagues controlled for serum progesterone, excluded women who were currently using exogenous hormones (e.g. contraception users) and tested women (mean age 36.3 yrs) in their luteal phase of menstrual cycle. Bell and colleagues tested women (age range 18-54 yrs) after early follicular phase (after day 8 of the menstrual cycle) and excluded women on exogenous hormone use. Although women in all phases of the menstrual cycle were included in this study, serum estrogen was controlled for in all models of the regression analysis.

Another difference among the Bell et. al., Nayeem et. al studies and this study was the range of values for the main variables SHBG and CRP, as well as for estrogen. In this study, CRP ranged between 0.2-49.1 mg/L with mean of 4.75 mg/L (median of 2.03 mg/L) with 19% of participants above the normal range. [9] Nayeem et al. did not report their range of CRP values, but their CRP mean was 6.5 mg/L with SD of 6.9 mg/L. suggesting that their upper 95% limit was approximately 20 mg/L. Bell et al., reported CRP mean of 2.79 mg/L and their CRP 90th percentile of 6.9 mg/L, suggesting that almost all of the observations were in the normal range. Also, in this study the SHBG mean was 69.3 nmol/L and ranged from 7.29 to 313 nmol/L. This study had 2% of observations below and 8% above the normal range of SHBG serum levels (18-144

nmol/L) [15] In Nayeem's study the SHBG mean was 101 nmol/L . There was no univariate descriptive information about SHBG in Bell's study. Oral contraceptive methods usage was associated with high CRP levels. [42] Because this study could not exclude some of the chronic inflammatory diseases (e.g. cancers) and women using contraceptive agents, it is likely to have CRP values above the normal range of serum CRP. These differences in serum levels of CRP and SHBG among this study and other studies on the same research question could have affected the relationship between SHBG and CRP in the multivariable regression analysis of this study.

Estrogen range in this study was 3.02 - 2120 pg/mL and mean 104.32 pg/mL which are equivalent to estrogen range of (11.08 - 7780.4) pmol/L and estrogen mean 382.85 pmol/L.[43] While Nayeem and colleagues did not report the range of estrogen values, their total estrogen mean was 293.2 pmol/L which was in the lower normal range for total estrogen in the luteal phase of adult non-pregnant women (normal estradiol level in luteal phase 110-1652 pmol/L) which might explain the positive, but non-significant, association between estrogen and CRP in their unadjusted bivariate analysis.[43] Moreover, Nayeem and colleagues recruited their sample of premenopausal women during the luteal phase of menstrual cycle during which progesterone (which is usually high in luteal phase) was reported to have positive association with CRP while controlling other hormones that might explain the positive association between estrogen and CRP in Nayeem's unadjusted bivariate analysis. [3] There was no information on serum estrogen in Bell's study.

In the bivariate results of this study, log-SHBG correlation with log-CRP (r=-0.14) was lower than Nayeem et al. finding (r= -0.42). Results were also different for estrogen. In this study the bivariate correlation for log-estrogen with log-CRP was -0.18 while in Nayeem et al. it was +0.11. Although Nayeem et al. excluded women on exogenous hormones, they recruited women in luteal phase of menstrual cycle during which endogenous progesterone is expected to be high and could affect estrogen and CRP association in unadjusted bivariate analysis.

Log-AST in this study was not significantly but positively associated with log-CRP (Pearson r= 0.06) in contrast to Nayeem's findings (Pearson r for log-AST and log-CRP = -0.04 ns). Log-testosterone was negatively but non-significantly associated with log-CRP (Pearson r= - 0.08) in contrast to Nayeem's study (Pearson r for testosterone and log-CRP = + 0.1 ns). Results for BMI cholesterol, HDL, albumin, ALP, log-ALT, WBC were not substantially different from Nayeem's findings. There was no bivariate information in Bell's study.

In model 1 and 2, log-SHBG was positively associated and log-estrogen was negatively associated with log-CRP which is consistent with Gaskins' et al. who also reported a negative association between endogenous estrogen and CRP in premenopausal women across all phases of menstrual cycle; however, this study did not differentiate between endogenous estrogen or serum estrogen that could have been originated from exogenous source. [3]

Similar to Nayeem et al. multivariable results, BMI contributed the most to log-CRP variation and WBC and ALP were positively associated with log-CRP while HDL was negatively associated with log-CRP (table-6-A). BMI was reported to have higher correlation with log-CRP in adult women than men. [14] The high levels of free fatty acids, formed from high fat cells in obese individuals, induces proinflammatory serine kinase cascades that subsequently increases hepatic synthesis of CRP. [14] Similarly, high levels of free fatty acids can phosphorylate serine residues of insulin receptor substances rendering insulin receptors non-responsive to serum insulin which creates a state of insulin resistance. [1,44] Therefore, in obese and overweight individuals, especially women, it is likely to find a positive association between BMI and CRP and a state of insulin resistance. Nayeem et al. final model did not contain albumin which was negatively associated with log-CRP in this study main effects and final model. The interaction term between serum estrogen and SHBG was significantly associated with log-CRP which means that SHBG main positive effect on CRP was attenuated by serum estrogen, this is also true for ALP and SHBG interaction (table 6). Bell and colleagues reported negative association between SHBG and CRP in their multivariable linear regression analysis but they excluded women on exogenous hormone usage. However, Bell and colleagues tested women across wide range of age (18-75 yrs), recruited premenopausal women (age 18-54 yrs) after early follicular phase of menstrual cycle (after day 8), and only controlled for BMI, age, smoking, alcohol, and exercise in their multivariable regression analysis with ln-CRP as the outcome. Of note, Bell's study full model R^2 (0.47) was close to this study final model R^2 (0.48). [5] Nayeem's final model R^2 was 0.59 with log-CRP as the outcome and 0.6 with BMI as the outcome which are higher than this study full models R^2 (~0.48 in both models).[6]

Differences in sample selection criteria and variables choice could explain the variability across this study and other studies' results. While Nayeem et al. controlled for progesterone, this study did not control for serum progesterone as a potential confounder since NHANES dataset did not have information on current exogenous progesterone usage or endogenous serum progesterone levels. Serum progesterone was shown to have a positive association with CRP regardless estrogen levels, [3,45] which could have drawn SHBG and CRP association into the opposite direction in this study. This is particularly important since NHANES 2015-2016 dataset did not have information on current menstrual cycle phase. Bell et al. did not have information on progesterone.

Moreover, insulin resistance inhibits hepatic synthesis of SHBG and that insulin resistance and metabolic syndrome are associated with high CRP levels. [1,5,6] Although metabolic syndrome assessment is difficult, this study did not control for other markers of metabolic syndrome and insulin resistance like LDL, triglycerides, endogenous serum insulin levels, and blood pressure measurements. NHANES cycle 2015-2016 did not have information on endogenous serum insulin levels. It is possible that this study sample contained a significant proportion of women with insulin resistance status which might explain the positive association between SHBG and CRP in this study multivariable linear regression analysis. Positive association between SHBG and CRP was found in special samples of premenopausal women with unmeasured insulin resistance status. [7,8] Shen et al. finding that SHBG was *positively* associated with CRP in the bivariate

analysis and was significantly lower in PCOS women compared to non-PCOS women supports the fact that insulin resistance, commonly seen in PCOS patients regardless BMI, has an inverse relationship with SHBG and positive association with CRP. [7] Furthermore, Abdella et al. reported *positive* association between SHBG and CRP in their sample of participants with relatives of diabetes supports the possibility of finding a positive association between SHBG and CRP if the sample contained participants with unmeasured insulin resistance state. [8] It is important to consider the positive association between SHBG and CRP in specific patients known to have insulin resistance states (like PCOS patients). Given that PCOS prevalence is high in the U.S. [38] and it is one of the most common endocrine abnormality in reproductive age women, finding high CRP and SHBG in reproductive age woman signifies further investigation for PCOS.

I also tried to replicate the final model of Nayeem et al. with log-CRP as the outcome (table 7). By reg procedure,[41] a model with log-CRP as the outcome and log-SHBG, BMI, ALP, WBC, log-ALT, log-AST, HbA1C, HDL, log-SHBG*log-estrogen and log-SHBG*ALP as predictors were created. In a model with log-CRP as the outcome (table 7-A), log-SHBG maintained a *positive* and significant association with log-CRP (β= 0.08, p=0.009) which supports that not controlling endogenous progesterone, exogenous hormones intake and/or current phase of menstrual cycle could have affected this study SHBG and CRP association results. In the model with BMI as the outcome (table 8), log-SHBG was significantly and negatively associated with BMI (β= -0.16, p <.0001) which supports that markers of metabolic syndrome are inversely related to SHBG.

The positive association between SHBG and CRP in this study adjusted multivariable regression analysis was inconsistent with studies on postmenopausal women. Maggio and colleagues reported a negative association between log-SHBG and log-CRP in their multivariable linear regression analysis while controlling for age, BMI and few of comorbidities in older women (mean age 76) not using exogenous hormones. [23] Maggio's mean CRP was 2.7 mg/L (close to Bell's) and mean SHBG was 117.8 nmol/L (close to Nayeem's). Maggio et al. reported negative correlation between SHBG and CRP in their bivariate analysis which is consistent with this study bivariate result. [23] SHBG was also negatively associated with log-CRP in bivariate and adjusted regression analysis in Stork's study that was conducted on postmenopausal women not using exogenous hormones. [24] In studies conducted on pregnant women, SHBG was also negatively associated with CRP in Torrent's bivariate and regression analyses. [25]

This study has limitations. NHANES datasets for the 2015-2016 cycle did not have clear information on 1) serum progesterone, FSH and LH to isolate women with hormonal disturbance (e.g. premature ovarian failure) and/or specify the current phase of menstrual cycle. 2) current usage of exogenous hormones including contraception. 3) the current usage of anti-inflammatory drugs; 4) use of immunosuppressants and anti-cancer drugs like corticosteroids, NSAIDS, methotrexate…etc.; 5) current medical conditions such as cancers and autoimmune diseases including celiac, SLE, T1DM…etc that could affect the outcome variable. Chronic inflammatory diseases (e.g. obesity, autoimmune diseases and cancers) could augment CRP level and affect its association with SHBG which might

explain the above normal range CRP values in this study in comparison to Nayeem's study, which excluded cancers (e.g. breast cancer) and medical conditions (e.g. cardiovascular diseases). I believe that the main limitation of this study were the followings; 1) not excluding women who were using exogenous hormones (e.g. contraception); 2) not controlling for serum progesterone, and 3) not controlling the current phase of the menstrual cycle. Oral contraception usage could not be excluded in this study. Oral contraceptive agents are metabolized by the liver and can augment levels of biomarkers synthesized by the liver (e.g. SHBG).[1,46] Furthermore, oral contraceptives are associated with increased CRP levels. [42,47,48] According to national data, oral contraception usage (including types that contain estrogen) was common among reproductive age women for the period 2015 to 2017 [49] which coincided with the same period of this study (NHANES 2015-2016). Thus, it was likely to have a large proportion of women who were using oral contraceptives in this study sample. Additionally, this study could not control markers of metabolic syndrome (e.g. LDL and triglycerides) and insulin resistance (e.g. endogenous serum insulin levels). LDL and triglycerides were not considered in the regression analysis because of high numbers of missing values (>5%). Homeostatic Model Assessment of Insulin Resistance **(**HOMA-IR) is the most widely used insulin resistance estimate in research. [50,51] However, I could not calculate HOMA-IR because NHANES 2015-2016 cycle did not have information on endogenous serum insulin levels and C-peptide. Nevertheless, HbA1c was considered in my analysis. NHANES 2015-2016 oversampled minority racial/ethnic groups (e.g., Hispanics, non-Hispanic Blacks, non-Hispanic Asians). However, the analysis of this study was unweighted and so estimates may not be representative of the population. Despite these limitations, this study was the first in literature to test wide range of biomarkers in clearly defined and relatively large national sample. There is paucity of research regarding sex hormones and CRP relationship in reproductive age women and I tested this research question using meticulous research design and adjusted statistical analysis in addition to replication of available models in the literature.

In conclusion, this study demonstrated that SHBG might have a pro-inflammatory role in premenopausal women as measured by CRP, regardless of serum estrogen levels and other markers of metabolic syndrome (e.g. BMI). It is important to examine this association in future nationally representative population samples after excluding women on exogenous hormones, while controlling for serum progesterone levels and considering the phase of menstrual cycle at the time of sampling.

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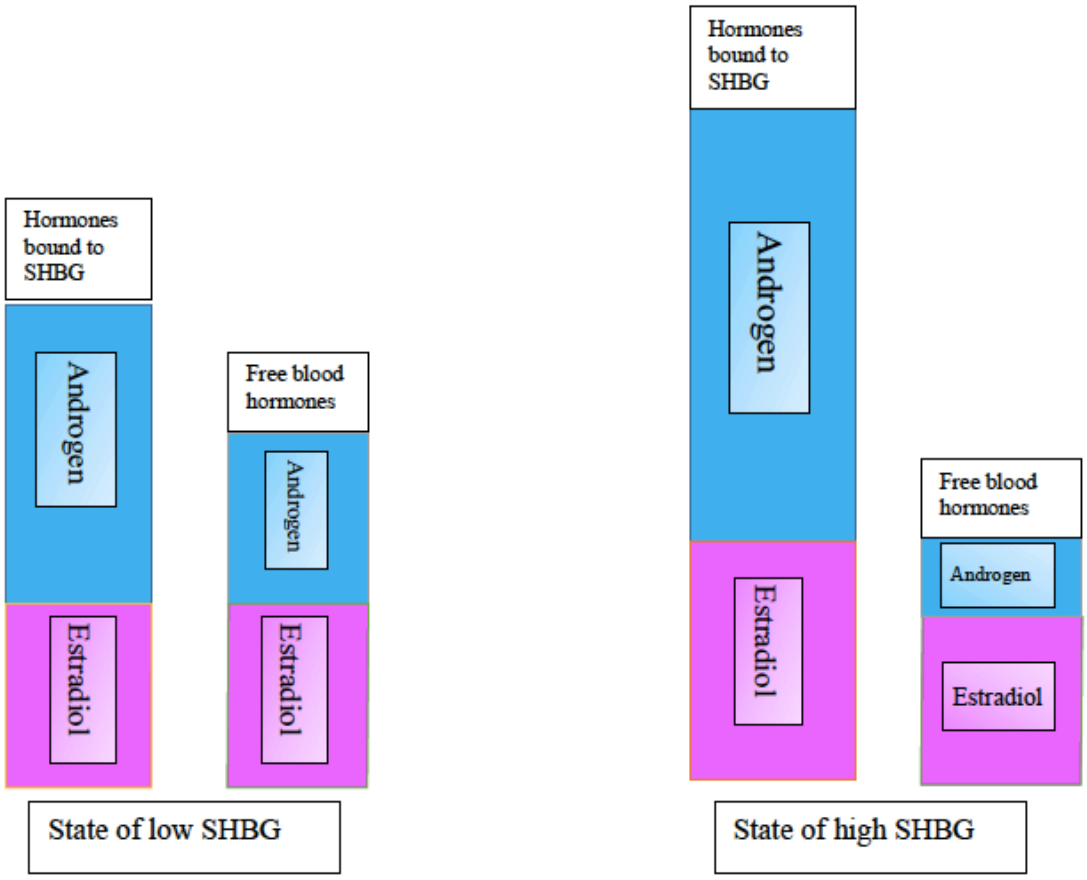

Figure 1. SHBG regulates levels of sex steroid hormones in the blood circulation. SHBG favors androgen binding. Therefore, with higher levels of SHBG, a higher proportion of androgen, compared to estrogen, will bind to SHBG leaving a lower proportion of freely circulating androgen compared to estrogen. Therefore, higher levels of SHBG tend to amplify the effects of circulating estrogen (estrogen amplification phenomenon).

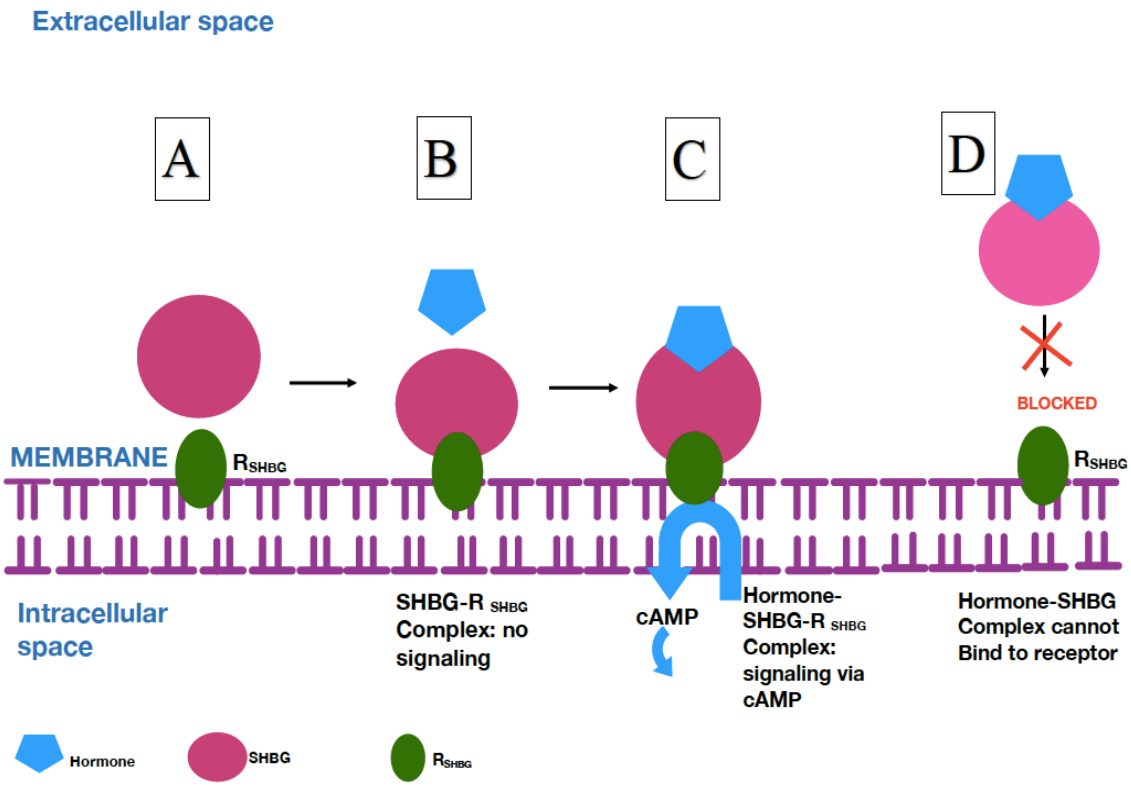

Figure 2. SHBG signaling pathway.

A: SHBG in extracellular space and its receptor on cell membrane. B: Free-of-hormone-SHBG can bind its receptor but without inducing any signaling intracellularly. C: Hormone-SHBG-RSHBG complex can induce cAMP signaling pathway inside the cell. D: Hormone-SHBG complex formed in extracellular space cannot bind to R_{SHBG} or induce intracellular signaling.

*SHBG: Sex Hormone Binding Globulin, $R_{SHBG:}$ receptor of SHBG.

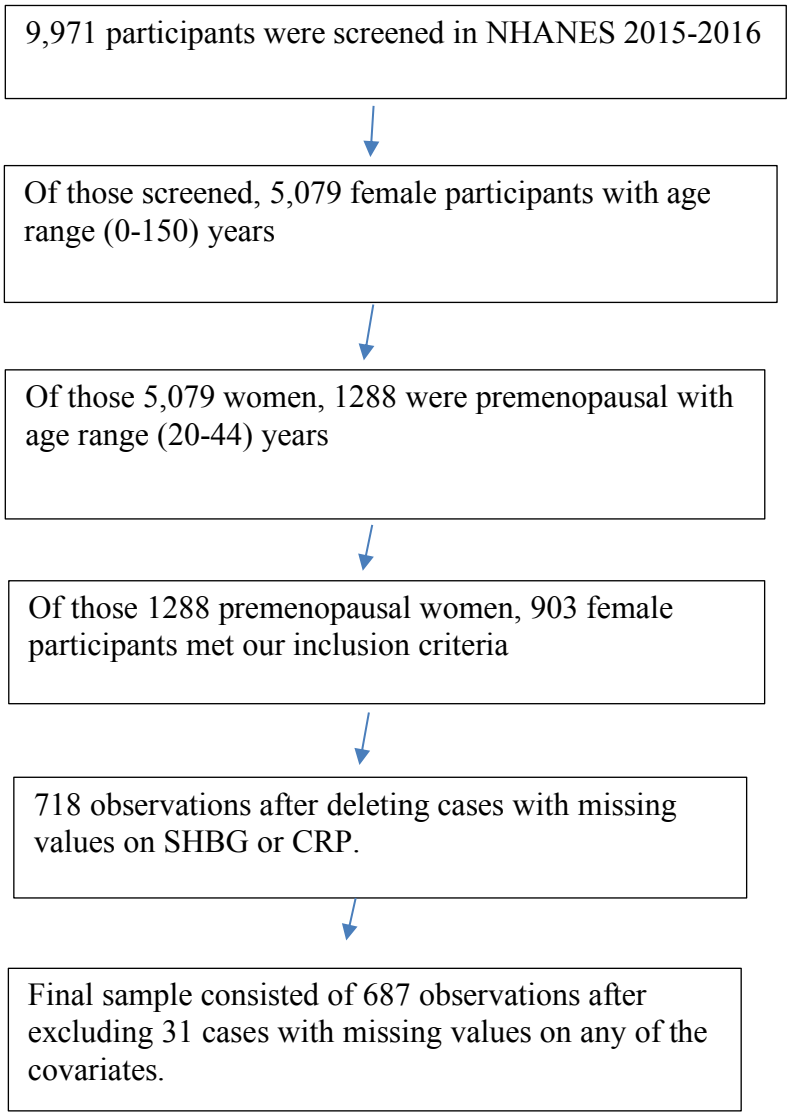

Figure 3. Scheme of sample selection

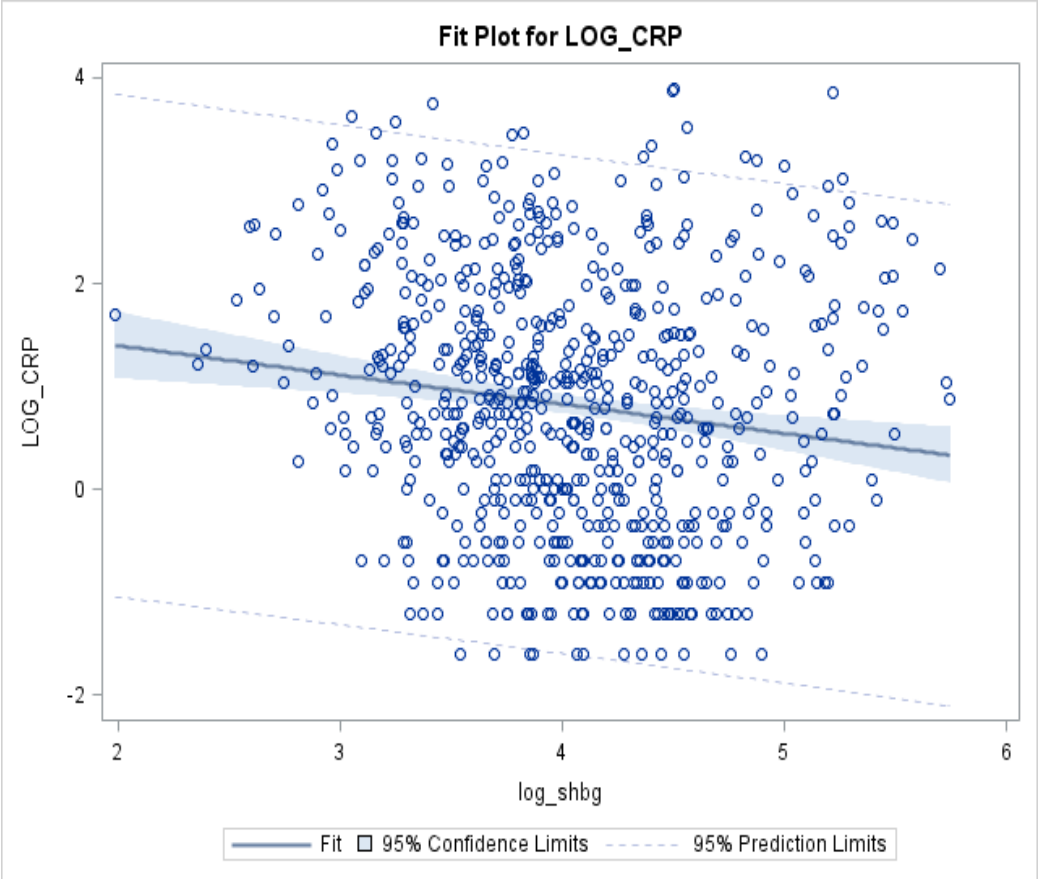

Figure 4. Log-CRP and log-SHBG relationship.

Table 1. Studies that examined the association between SHBG and CRP in premenopausal women.

Author name (date)	**Study purpose**	**Study design & Sample**	**IVs**	**DV**	**results**
Bell (2007)	Test the relation between endogenous androgen and SHBG with CRP and lipds in women	Cross-sectional study. Representative community sample pre- (n=353) & post-men (n=234) healthy women (18-75 yrs). During *mid-follicular* phase (8+ d of cycle) Excluded hormone use, PCOS, etc.	Total T, free T, SHBG, adrenal androgens, Covariates: Age, smoking, alcohol, BMI, exercise	Hs-CRP and lipids (HDL, LDL, TGs)	1.Because menp status correlated with outcomes, PRE & POSTmenp analysed separately 2.BMI strongest predictor of CRP 3.SHBG negatively associated with hs-CRP (slope = -0.11, partial correlation r= - 0.69; sqd semi-partial <0.01) in premenopausal group after adjusting for covariates 4. Free T similarly affected CRP, but 4.Low SHBG and risk of CVD not due to endogenous androgens, but likely SHBG is marker for insulin resistance.
Nayeem (2010)	Association between obesity, metabolic markers (liver function), and sex steroids with CRP (&ALP) in premenopausal women.	Cross-sectional N=233 premen healthy women (30-40 yrs) during ***luteal*** phase of cycle	Obesity (BMI, % fat, etc Liver (ALP, ALT, AST) SHBG, estradiol, progesterone, WBC	CRP (separate model for ALP)	Predictors of CRP: Bivar ***r*** MultVar (slope) Body Fat 0.70 0.49 (standardized slope) ALP 0.44 0.15 SHBG -0.42 -0.12 WBC 0.45 0.25 ALP: Bivar ***r*** MultVar (slope) CRP 0.44 0.27 Insulin 0.36 0.18
Blum (2005)	1-Changes of inflammatory and insulin resistance biomarkers across menstrual cycle in premenopausal women. 2- association of hs-CRP with SHBG estradiol, body fat and insulin resistance markers.	Cross-sectional. N=15 female patients (20-36 yrs).	Estradiol, progesterone, TNF-alpha, HOMA-IR, ProCT, BMI, waist circumference, tissue fat.	Hs-CRP SHBG	1-SHBG was higher in luteal than follicular phase and hs-CRP was highest in early follicular phase regardless participant weight (but CRP was higher in overweight than normal weight). 2-CRP was negatively associated with SHBG (slope= -0.83) after adjusting for body weight.
Shen (2015)	Correlation of inflammatory and metabolic	Cross-sectional. N=330; PCOS (n=165) and non-PCOS (n=165) women	BMI	Biomarkers for insulin resistance,	1-Higher BMI was sig associated with lower SHBG and higher CRP (Table 1), but they report

	biomarkers in PCOS vs non-PCOS premenopausal female patients	(14-45 yrs) Matched on BMI.		HOMA, SHBG, CRP, IL-6, leptin, etc.	CRP was *positively* correlated with SHBG (r= 0.20, Table 2). 2-SHBG was sig. lower in PCOS pts compared to non-PCOS, while CRP was not sig different between the two groups.
Abdella (2017)	To test whether the association between SHBG and Diabetes (T2D) is mediated by metabolic syndrome markers including CRP and BMI in first degree relatives of T2DM	Cross-sectional Clinical sample of first-degree relatives of T2D Age 19-49 Females premenopausal (n=349)	DM2 status Males Normal (n=95) PreDm (n=100) Diab (n=40) Females Normal (n=171) PreDm (n=137) Diab (n=41) Other IVs: CRP, lipids, Age, BMI, etc	SHBG	In female group: 1-SHBG decreased from normal to prediab to diab ($p<0.02$); & bivariate correlation with HbA1C (r= -0.29) and BMI (r= - 0.354). 2. CRP increased from normal to prediab to diab ($p<0.03$) 3-SHBG and CRP bivariate correlation was rho = -0.39 for males (p=0.11) rho = +0.07 for females (p=0.77) 4- They did not include hs-CRP in multiple regression analysis predicting SHBG because it was ns.
Kopp (2006)	Effect of weight loss on various markers including SHBG and CRP	Prospective-cohort. N=43 obese women (mean age=41, mean BMI=48)	Bariatric surgery (weight loss)	SHBG, hs-CRP, T, androgen, insulin, cortisol, IL-6 and TNF-alpha	After bariatric surgery, CRP decreased and SHBG increased in the entire cohort of participants. Change in SHBG was negatively associated with change in BMI.

Table 2. Variables used to satisfy the exclusion criteria (N=903).

	SAS Variable name	Variable label & Exact question asked in NHANES	Responses/measures
1.	RIAGENDR	Gender	**1=male (0)** 2=female = 903+all inclusion criteria
2.	MCQ035	Still have asthma (Do you/Does SP) still have asthma (az-ma)? *Both males and females 1 YEARS - 150 YEARS	Categorical: 1="yes" **(0)** 2="no" (59) 9="don't know" (2) Missing=842
3.	MCQ170k	Do you still have chronic bronchitis (Do you/Does SP) still . . . have chronic bronchitis? *both males and females age 20-150	1= yes (**0)** 2="no"(13) Missing=890
4.	MCQ195	Which type of arthritis was it? "Has a doctor or other health professional **ever** told that (you/s/he) . . .had arthritis (ar-thry-tis)?"	Categorical: 1=OA=(0) 2=RA (0) 3=Psoriatic (0) 4=Other (0) 9=don't know=24 Missing=879
5.	MCQ170m	Do you still have thyroid problem *both males and female age 20-150 (Do you/Does SP) still . . . have another thyroid problem?	Categorical: 1=yes (**0**) 2=no (18) 9=don't know (8) Missing=877
6.	RHQ010	Age when first menstrual period occurred	Range=7 to 19 Code 20=Started at age 20 or older (0) Code 0=Has not started yet=(0) Code 777=refused (0) Code 999=don't know (3) Missing (197)
7.	RHQ031	Had regular periods in past 12 months *females age 12-150	Categorical: 1=yes (706) 2=no (0) Missing (197)
8.	RHD043	Reason not having regular periods *females age 20-150	Categorical: 1=pregnancy (0) 2=breast feeding (0) 3=hysterectomy (0) 7=menopause/ change of life (0) 9=other (0) 77=refused (0) 99=don't know (0) . missing (903)
9.	RHD280	Had a hysterectomy? *females age 20-150	1=yes (0) 2=no (706) 7=refused (0)

		9=don't know=0 .missing (197)
10. RHQ305	Had both ovaries removed? *females age 20-150	1=yes (0) 2=no (706) 7=refused (0) 9=don't know =0 . missing (197)
11. URXPREG	Urine Pregnancy Result	1=positive (0 obs) 2=negative (840) 3=not done (22) 4=invalid (zero obs) Missing= 41
12. RHQ200	Now breastfeeding a child? *females age 20-44	Categorical: 1=yes (0 obs) 2=no (94) 7=refused (0) 9=don't know (0) . missing (809)

Table 3. Variables list with no missing values in SHBG or CRP (N=718).

SAS Variable name	Variable label & Exact question asked in NHANES	Responses/measures
OUTCOME VARIABLE		
LBXHSCRP	HS C-Reactive Protein (mg/L) LLOD=0.11	Continuous Range: 0.2 to 62.3 Missing=0
MAIN INDEPENDENT VARIABLE		
LBXSHBG	SHBG (nmol/L) LLOD=0.8 nmol/L	Continuous Range: 7.29 to 475.8 Missing=0
COVARIATES		
1. LBXEST	Estradiol (pg/mL) LLOD=2.994 pg/mL	Continuous variable Range: 3.02 to 2120 Missing=21
2. LBXTST	Testosterone, total (ng/dL) LLOD=0.75 ng/mL	Continuous Range: 5.3 to 198 Missing=0
3. LBXWBCSI	White blood cell count (1000 cells/uL)	Range: 2.5 to 19.1 (1000 cell/uL) Missing=4
4. LBDHDD	Direct HDL-Cholesterol (mg/dL) LLOD=3 mg/dL	Range: 16-165 Missing=1
5. LBXTC	Total Cholesterol (mg/dL) LLOD=4 mg/dL	Range: 99-335 Missing=1
6. BMXBMI	Body Mass Index (kg/m**2)	Range: 16.6-64.6 Missing=4
7. LBXSAPSI	Alkaline Phosphatase (ALP) (IU/L) LLOD = 5 U/L	Range: 27 to 153 Missing=1
8. LBXSASSI	Aspartate Aminotransferase (AST) (IU/L) LLOD = 5 U/L	Range: 8 to 169 Missing=1
9. LBXSATSI	Alanine Aminotransferase (ALT) (IU/L) LLOD= 5 U/L	Range: 8- 208 Missing=1
10. LBDSALSI	Albumin, refrigerated serum (g/L) LLOD=1 g/dL (10 g/L)	Range: 30-52 Missing=1
11. LBXSBU	Blood Urea Nitrogen (mg/dL) LLOD = 1 g/dL	Range: 3 to 26 Missing=1
12. LBXSCR	Creatinine, refrigerated serum (mg/dL) LLOD = 0.1 mg/dL	Range: 0.33-1.63 Missing=1
13. LBXGH	Glycohemoglobin (%)	Continuous (HbA1C) Range: 4.2 -14.4 Missing=1
14. BPXSY3	Systolic: Blood pres (3rd rdg) mm Hg	Range: 82-170 Missing=41

15. BPXDI3	Diastolic: Blood pres (3rd rdg) mm Hg	Range: 40-108 Missing=41
16. RIDAGEYR	Age in years at screening	Range: 20-44
17. RIDRETH3	Race/Hispanic origin w/ NH Asian *Recode of reported race and Hispanic origin information, with Non-Hispanic Asian Category	1=Mexican-american **(167) obs** 2=other Hispanic (100**)** 3=non-hispanic white (175**)** 4=non-hispanic black (156**)** 6=non-hispanic Asian (94**)** 7=other race-including multi-racial (26**)** Missing=0
18. LDL mg/dL		Range: 23-245 Missing = 431
19. Triglycerides mg/dL		Range: 20-378 Missing = 431

Table 4. General characteristics of study subjects (20-44-year-old premenopausal women, N=678)

Variable	Range	Mean (SD) or n (%)	Median (IQR)
HS C-Reactive Protein (mg/L) LLOD=0.11	Range: 0.2 to 49.1 Missing=0	4.75 (6.69)	2.03 (4.60)
SHBG (nmol/L) LLOD=0.8 nmol/L	Range: 7.29 to 313.5 Missing=0	69.31 (47.63)	55.38 (47.43)
Estradiol (pg/mL) LLOD=2.994 pg/mL	Range: 3.02 to 2120 Missing=0	104.32 (121.60)	71.00 (103.40)
Testosterone, total (ng/dL) LLOD=0.75 ng/mL	Range: 5.3 to 198 Missing=0	25.89 (13.70)	23.40 (14.40)
White blood cell count (1000 cells/uL)	Range: 2.5 to 19.1 (1000 cell/uL) Missing=0	7.68 (2.24)	7.40 (2.90)
Direct HDL-Cholesterol (mg/dL) LLOD=3 mg/dL	Range: 16-165 Missing=0	56.26 (15.92)	54.00 (21.00)
Total Cholesterol (mg/dL) LLOD=4 mg/dL	Range: 99-335 Missing=0	177.16 (33.89)	174.00 (43.00)
Body Mass Index (kg/m**2)	Range: 16.6-64.6 Missing=0	30.08 (8.22)	28.50 (10.30)
Alkaline Phosphatase (ALP) (IU/L) LLOD = 5 U/L	Range: 27 to 153 Missing=0	62.97 (19.38)	60.00 (24.00)
Aspartate Aminotransferase (AST) (IU/L) LLOD = 5 U/L	Range: 8 to 169 Missing=0	22.26 (11.00)	20.00 (5.00)
Alanine Aminotransferase (ALT) (IU/L) LLOD= 5 U/L	Range: 8- 208 Missing=0	20.57 (14.64)	17.00 (7.00)
Albumin, refrigerated serum (g/L) LLOD=1 g/dL (10 g/L)	Range: 30-52 Missing=0	42.48 (3.16)	42.00 (4.00)
Blood Urea Nitrogen (BUN) (mg/dL) LLOD = 1 g/dL	Range: 3 to 26 Missing=0	11.63 (3.31)	11.00 (5.00)
Creatinine, refrigerated serum (mg/dL) LLOD = 0.1 mg/dL	Range: 0.36-1.63 Missing=0	0.68 (0.14)	0.67 (0.18)
Glycohemoglobin (%) (HbA1C)	Range: 4.2 -14.4 Missing=0	5.46 (0.93)	5.30 (0.50)

Age in years at screening	Range: 20-44	32.12 (7.07)	32.00 (12.00)
Race, n (%)	White	165 (24.02 %)	
	Black	150 (21.83 %)	
	Asian	91 (13.25 %)	
	Hispanics	255 (37.12 %)	
	others	26 (3.78 %)	

Table 5. Unadjusted bivariate analysis results for CRP as the outcome variable (N=687).

Variable	**Pearson r (P values) ***	**Spearman r (P-value)**
SHBG	-0.14 (.0002)	-0.17 (<.0001)
estrogen	-0.18 (<.0001)	-0.17 (<.0001)
Testosterone	-0.08 (0.04)	-0.09 (.02)
WBC	0.29 (<.0001)	0.29 (<.0001)
HDL	-0.36 (<.0001)	-0.37 (<.0001)
Total cholesterol	0.03 (0.362)	0.04 (0.33)
BMI	0.61 (<.0001)	0.61 (<.0001)
ALP	0.39 (<.0001)	0.41(<.0001)
AST	0.06 (0.14)	0.01 (0.78)
ALT	0.21 (<.0001)	0.21(<.0001)
Albumin	-0.41 (<.0001)	-0.41 (<.0001)
BUN	-0.03 (0.45)	-0.02 (0.52)
Creatinine	0.04 (0.272)	0.05 (0.22)
HBA1C	0.23 (<.0001)	0.33 (<.0001)
Age	0.07 (0.067)	0.07 (0.08)
Race**	0.18	

*for Pearson correlation, log-CRP correlated with log-transformed scale of SHBG, estrogen, testosterone, ALT and AST along with original scale of remaining independent variables to satisfy the bivariate normal distribution of Pearson correlation.
**eta coefficient for race categorical variable

Table 6. Standardized slopes and squared semi-partial correlation with log-CRP as the outcome variable (N=687).

Variable	**⊥Model 1 Standardized estimate β**	**Squared Semi-partial correlation**	**⊥Model 2 Standardized estimate β**	**Squared Semi-partial correlation**
Log-SHBG^	0.07*	0.004	0.05	0.002
Log-estrogen	-0.15****	0.02	-0.10**	0.01
BMI	0.42****	0.11	0.43****	0.12
WBC	0.11 ***	0.01	0.10***	0.01
HDL	-0.12 ***	0.01	-0.12***	0.01
ALP	0.19 ****	0.03	0.20****	0.03
Albumin	-0.15****	0.02	-0.15****	0.02
Log-SHBG x log-estrogen			-0.10**	0.01
Log-SHBG x ALP			-0.10***	0.01
TOTAL R^2		0.48		0.49

^ Forced into the model

⊥ Model 1 was selected from 15 independent variables without interaction terms while model 2 was selected from 15 independent variables with interaction terms.

*p<0.05

** p<0.01

*** p<0.001

**** 0<0.0001

Table 7. Standardized slopes and squared semi-partial correlation with log-CRP as the outcome variable.

Final model R^2 =0.45 (adjusted full model R^2=0.44), N=687.

Variable	**Standardized estimate β (P value)**	**Squared Semi-partial correlation**
Log-SHBG	0.08 **	0.005
BMI	0.49 ****	0.18
ALP	0.21 ****	0.03
WBC	0.06 *	0.004
Log-ALT	0.04 (0.39)	0.0006
Log-AST	-0.04 (0.40)	0.0005
HBA1C	0.05 (0.07)	0.002
HDL	-0.14 ****	0.02
Log-SHBG x estrogen	-0.14 ****	0.02
Log-SHBG x ALP	-0.10 ***	0.01

*p<0.05
** p<0.01
*** p<0.001
**** 0<0.0001

Table 8. Standardized slopes and squared semi-partial correlation with BMI as the outcome variable.

Final model R^2 =0.48 (adjusted full model R^2=0.48), N=687.

Variable	**Standardized estimate β (P value)**	**Squared Semi-partial correlation**
Log-SHBG	-0.16 ****	0.02
Log-estrogen	0.01 (0.66)	0.0001
Log-CRP	0.43 ****	0.12
WBC	0.10 ***	0.01
Log-ALT	0.13 **	0.006
Log-AST	-0.06 (0.21)	0.001
ALP	-0.01 (0.99)	0.0001
Albumin	-0.26 ****	0.05
HBA1C	0.05 (0.11)	0.002
Log-SHBG x estrogen	0.07 *	0.004
Log-SHBG x ALP	0.03 (0.36)	0.0006

*p<0.05
** p<0.01
*** p<0.001
**** 0<0.0001

VITA

Dr. Rasha Al-Lami was born in Baghdad, Iraq to Ilham Jafaar and Azeez Hammood Al-Lami. She completed her medical degree from Almustansiriyah College of Medicine before moving to the United States on Fulbright scholarship to study at UTMB. During her time at UTMB, she published numerous scholarly articles and worked as a reviewer in renowned journals like *BMJ, Obstetrics and Gynecology and Mayo Clinic Proceedings.* She also won the National Society of Leadership and Success Academic Excellence scholarships for Spring and Fall 2019 and became a member of Sigma-Xi the Scientific Research Honor Society. Dr. AL-Lami research interest lies in women health and Obstetrics and Gynecology. After graduating from UTMB, Dr. Al-Lami will pursue a residency in Obstetrics and Gynecology.

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