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FEMALE SEXUAL FUNCTION

Lack of Evidence for a Relationship Between Salivary CRP and Women's Sexual Desire: An Investigation Across Clinical and Healthy Samples

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ABSTRACT

Background: Inflammation has been linked to a variety of mental and physical health outcomes that disproportionately impact women, and which can impair sexual function; thus, there is reason to expect a link between inflammation and women's sexual functioning.

Aim: To test the hypothesis that higher concentrations of C-reactive protein (CRP), a general biomarker of inflammation, would predict women's lower sexual desire.

Method: As 2 independent research teams, we conducted 3 separate studies (total n = 405) that assessed salivary CRP and various measurements of sexual desire in different women populations.

Outcomes: Female Sexual Function Index, Sexual Desire Inventory-2, Decreased Sexual Desire Screener, and Sexual Interest and Desire Inventory.

Results: Regardless of the way sexual desire was measured (e.g., state vs trait; general desire vs. desire functioning) and the population sampled (i.e., healthy vs. clinically diagnosed with sexual dysfunction), all the studies revealed null results.

Clinical Implications: While exploratory, the convergence of these null results across studies and researchers suggests that if there is an association between inflammation and women's sexual desire, it is likely very subtle.

Strengths & Limitations: Across 2 independent research teams, 3 unrelated studies, and various measurements of sexual desire, results were consistent. These points lend to the generalizability of the results. However, study designs were cross-sectional.

Conclusions: Future research may reveal (i) a non-linear threshold effect, such that inflammation does not begin to impact women's sexual desire until it is at a high level, (ii) inflammatory biomarkers other than CRP might be more sensitive in detecting associations between inflammation and desire, should they exist, or (iii) the mechanisms underlying sexual dysfunction may differ between sexes. **Clephane K, et al. Lack of Evidence for a Relationship Between Salivary CRP and Women's Sexual Desire: An Investigation Across Clinical and Healthy Samples. J Sex Med 2022;19:745–760.**

Key Words: Inflammation; Desire; CRP; HSDD; Women; Sexual Dysfunction

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INTRODUCTION

The effects of inflammation on mental and physical health have been well documented.[1] In men, this research has extended to identifying extensive links between inflammation and sexual dysfunction[2,3] such that clinical recommendations now include routine assessment for sexual dysfunction in assessing risk for inflammation-related conditions, including cardiovascular disease.[4,5] However, to date there has been remarkably little work testing similar effects in women. To begin to address this gap, we explored associations between C-reactive protein, a biomarker that indexes a broad range of inflammatory processes, and women's sexual desire across 3 separate studies.*

Inflammation

Inflammation plays a key role in protecting the body from harm by facilitating the immune response to injury, defense against pathogens, as well as normal cellular and tissue maintenance. The latter function is particularly important in the central nervous system, clearing the brain from debris and metabolic waste as well as supporting neuroplasticity and neurogenesis.[6] Inflammation can also be upregulated in anticipation of potential injury as part of the stress response.[7] While acute inflammatory processes are self-regulating through negative feedback mechanisms, in the presence of ongoing stimulation, acute inflammation can shift into a chronic form. This chronic, "low-grade" inflammation has been implicated in a range of chronic health conditions, including cardiometabolic disease[8–12] and depression.[13] Both acute and chronic inflammation involve complex communication among white blood cells and other tissues via direct contact and via indirect inflammatory mediators such as cytokines. To date, most research on inflammation has measured these inflammatory mediators as biomarkers of the broader inflammatory response system.

C-reactive Protein (CRP)

C-reactive protein (CRP) is one such inflammatory biomarker. CRP is an acute-phase protein secreted primarily from hepatocytes in the liver but also from various other cell types.[14] Clinically, it is a highly sensitive, systemic marker of inflammation and tissue damage. CRP is well known for its role in the innate immune response and has been implicated in several other related physiologic processes such as apoptosis, phagocytosis, NO release, and cytokine production.[14] Circulating serum CRP values are normally less than 3 mg/L,[15] but when activated by cytokines such as IL-6, concentrations can increase up to 1000-fold, reaching a peak within 24–72 hours and returning to baseline within 24 hours postpeak.[14] Because of this reliable response to generalized inflammation, along with its high sensitivity and specificity, CRP is currently the gold standard inflammatory biomarker in outpatient and non-critical inpatient settings (ARUP Laboratories). It is typically used in concert with other biomarkers in the diagnosis and management of a broad range of cardiovascular diseases[10] but can also be employed in the management of several other conditions such as metabolic disease,[16,17] obstructive sleep apnea,[18] and chronic obstructive pulmonary disease (COPD).[19] Moreover, high CRP values have also been associated with several psychiatric conditions such as post-traumatic stress disorder, schizophrenia, bipolar disorder, and depression.[20] In the present study, we examined associations between CRP and sexual functioning not only because of its potential to capture a broad range of inflammation processes, but because of CRP's utility as a clinical test that is already widely available; ergo, if CRP revealed etiologic factors for sexual desire dysfunction, it could quickly become a useful tool for sexual medicine practitioners.

Inflammation and Sexual Dysfunction

Interestingly, many conditions associated with inflammation, including some of the aforementioned health conditions, are also associated with decreased sexual functioning in women.[21–24] There are several mechanisms by which inflammation could potentially impact sexual functioning. Inflammation may elicit its effects directly through vascular damage and changes in blood flow to the genitals, thus impairing the vasculogenic mechanisms necessary to prepare the genitals for intercourse.[21] There is ample evidence to support this mechanism in men with cardiometabolic risk factors.[2] For instance, overweight/obese men with erectile dysfunction (ED) show elevated levels of several inflammatory markers (IL-6, IL-8, IL-18 and CRP) and impaired endothelial function compared to their counterparts without ED.[22] Higher prevalence of ED, reduced markers of endothelial function, and higher levels of CRP have also been detected in men with metabolic syndrome compared to healthy controls.[25]

There is less evidence to support links between inflammation and sexual dysfunction in women, although sexual dysfunction has been associated with women's isolated obesity[26] and diabetes.[27] There have also been some reports of significantly lower sexual functioning and higher levels of CRP in women with metabolic syndrome.[28] Sexual dysfunction in these women likely arises from physiologic processes like those discussed above in men, although there is evidence to suggest that psychosocial and/or neurological factors also play a role.[22]

Inflammation may also impart neuropsychological consequences for sexual functioning through induction of "sickness

*While the link between inflammation and sexual functioning is presumably physiologic in nature, and thus more accurately conceptualized as being linked to biological sex, we recognize that the majority of prior research in this area using human subjects has not differentiated between sex and gender. Moreover, the construct of sexual desire dysfunction is inextricably linked to gendered norms for sexual behavior, and as such likely includes aspects that are both physiological and psychosocial. As such, below, we use the term "woman/women's" to denote people with vaginas/ovaries who are living as women, with the understanding that this reflects a necessarily limited perspective.

behaviors'," a constellation of affective and cognitive behaviors that parallel symptoms of depression (eg, malaise, anhedonia, fatigue, decreased motivation, sleep dysregulation, decreased appetite, increased pain sensitivity, reduced movement, cognitive impairment).[29–31] These behaviors may serve the goal of energy preservation while the body directs resources towards repair and recovery. Studies have shown that the cerebral structures and neural networks involved in regulating sickness behaviors (eg, striatum and amygdala) are sensitive to neuroinflammation.[31] Of note, many of these structures are also integral for reward and motivation processing. Downregulation of such reward-oriented behaviors during sickness and inflammation[32–34] would likely impact sexual functioning, which is intimately reliant on reward processing.[35] From an evolutionary perspective, this is an understandable point of regulation given that illness is not an optimal time for reproduction. Various other mechanisms linking inflammation and sexual problems may also exist and have been discussed elsewhere (eg,[36]). Importantly, these mechanisms are not mutually exclusive and could reasonably be concurrent, affecting both genital and subjective components of sexual functioning.

Because of the strong evidence linking inflammation with sexual dysfunction in men, and reports of low sexual function in clinical populations of women experiencing elevated baseline inflammation, several research groups have recently posited that inflammation likely plays a role in women's sexual dysfunction.[22,36,37] However, these same researchers have all noted the need for data testing this hypothesis, and to date there has been no study in the literature that has empirically investigated this link in healthy women.

The present set of studies aimed to determine whether CRP is a viable predictor or biomarker of sexual function in women. To fully characterize this possible association, we analyzed data from 3 different studies, including a non-clinical study on stress (Study 1), a non-clinical study on women's sexual desire and arousal (Study 2), and a clinical study on women's sexual function (Study 3). Studies 1 and 2 included samples of healthy women and Study 3 included a sample of women with Hypoactive Sexual Desire Disorder (HSDD).[38] Further reinforcing the independent replication of these effects, Studies 1 and 2 were conducted separately from Study 3 by 2 different research teams (Studies 1 and 2: K.C., M.C.W., A.N.C., & T.K.L.; Study 3: J.I.O., T.S.B., J.T.B.S., J.W., & L.A.B.), and data were only combined after each study had been completed. However, for each study, we hypothesized that higher CRP would predict lower sexual desire and that this association would be amplified in the sample of women with HSDD.

Study 1

Study 1 established effects in a young non-clinical sample of women using self-reported sexual desire in the past month. The primary purpose of the parent study from which the present data were drawn was to compare physical stress during 2 different types of blood collection methods,[39] but the present analysis concerned a secondary aim, which was to characterize inflammatory markers associated with sexual desire and arousal among healthy women. The primary analyses for this study were preregistered on the Open Science Framework (OSF; see https://osf.io/rsg8j for de-identified data supporting these analyses and additional details on research design).

Study 1 Participants. Participants were healthy, premenopausal women (n = 45) recruited via online community boards, social media advertisements, and the online course-credit pool at a university in the Midwestern United States. Potential participants completed an online eligibility screening. Exclusion criteria included poor gum health, use of antibiotics or glucocorticoids within the month prior to study session, menstrual dysfunction, and current pregnancy. Of note and in contrast to Study 3, this sample was not recruited based on reported sexual functioning, nor were participants with sexual functioning concerns excluded. Sample demographics are presented in Table 1.

Study 1 Procedures. Though a host of laboratory procedures were conducted (see OSF registration for details), the present

Table 1. Demographic characteristics for Study 1

	Mean (M)	*St. Deviation (SD)*
Continuous variables		
Age in years	21.93	4.04
Body mass index (BMI)	24.98	5.29
	Number (N)	*Percent (%)*
Race/ethnicity		
European American/White	30	66.7
African American/Black	1	2.2
Asian/Pacific Islander	5	11.1
Latin/Hispanic	3	6.7
Multiracial or other	1	2.2
Sexual orientation		
Exclusively heterosexual	17	37.8
Mostly heterosexual	9	20.0
Bisexual or pansexual	11	24.4
Mostly homosexual	1	2.2
Exclusively homosexual	1	2.2
Relationship status		
Single	17	37.8
Dating nonexclusively	2	4.4
Dating exclusively	22	48.9
Married or cohabiting	3	6.7
Missing/other	1	2.2

For their sexual orientation, participants used 3 sliders ranging from 0 to 100 to independently indicate their attraction to men/masculine people, women/femme people, and nonbinary people. For descriptive purposes, these numbers were coded into categories as displayed in the table above. Relationship duration and highest level of education were not collected.

analysis focused on the baseline saliva sample and self-report questionnaires. Participants were asked to not eat, drink, or chew gum for an hour before their study session. Upon arrival, participants were given bottled water to drink and rinse their mouths while the researcher reviewed the informed consent document. Participants were given time to ask questions and read the informed consent document privately. Then participants completed a passive drool saliva sample into a polypropylene tube. Timing was monitored to ensure that at least 10 but no more than 15 minutes elapsed between the last drink of water and the start of saliva sampling. Participants completed the survey battery at the end of the study session and were compensated with either $20 or course credit for their time. All procedures for this study were approved by the Institutional Review Board at University of Nebraska - Lincoln, and all participants provided written informed consent.

Study 1 Instruments and Measures. Salivary CRP and Cortisol. CRP can be measured in blood or saliva.[40] Blood testing was developed first and is generally accepted as a reliable index of circulating CRP levels, and thus has been more commonly implemented in prior research. However, there are caveats associated with blood sampling — for example, invasiveness, risk of infection, increased anxiety, increased resource requirements, and tedious sample handling — that have driven interest in use of salivary CRP as a surrogate marker. Studies examining the validity of salivary CRP show that it is moderately correlated with serum CRP (mean R^2 = 0.53 ± 0.23, 95% CI = 0.07−0.99),[41] with stronger correlations at higher levels of CRP.[42] Across studies, we used salivary CRP as it allowed for more flexible experimental design and minimally invasive repeated measurements that reduced potential influence on stress measures.[39]

All saliva samples were frozen immediately after collection and stored at -80°C until date of assay. For the present analyses, saliva samples were assayed for CRP and cortisol using commercially available high-sensitivity enzyme-linked immunosorbent assay (ELISA) kits. All assays were conducted according to manufacturer procedural recommendations (Salimetrics, LLC). Inter- and intra-assay coefficients of variance were within acceptable ranges (CRP = 4.6−11.2%; cortisol = 7.7−7.8%), and the minimum detected value for CRP was 68.08 pg/mL.

Self-Report Measures. Participants self-reported their age, height, and weight (from which body mass index [BMI] was calculated). Among others administered during the laboratory session, the following psychological measures were included in the present analyses.

Patient Health Questionnaire (PHQ-9). The PHQ-9 is a widely used 9-item survey measuring depression severity over the last 2 weeks.[43] Response options assess symptom frequency on a scale of 0 ("Not at all") to 3 ("Nearly every day"). Total score was calculated by summing responses across the 9 items, with higher scores indicating more severe depression. Cronbach's alpha for the present sample was 0.786.

Female Sexual Function Index (FSFI). The FSFI is a widely used 19-item questionnaire measuring desire, arousal, lubrication, orgasm, satisfaction, and pain among sexually active women.[44] Items are scored on a 5-point scale, with higher scores indicating better functioning. The present analysis considered the desire subscale, which solicits ratings of 2 aspects of desire (ie, "How often did you experience sexual desire or interest?" and "How would you rate your level [degree] of sexual desire or interest?") over the past 4 weeks. Cronbach's alpha in the present sample was 0.887 for the full questionnaire and 0.750 for the desire subscale items.

Sexual Desire Inventory (SDI-2). The SDI-2 is a 14-item survey measuring sexual desire across both dyadic and solo contexts.[45] Items are divided into 2 subscales concerning the extent to which respondents have been interested in partnered and individual sexual activities during the past month. Total scores range from 0 to 112, with higher scores indicating stronger desire. Cronbach's alpha for the present sample was 0.906.

Study 1 Data Analytic Plan. As continuous salivary CRP values were not normally distributed, raw concentrations were Blom-transformed. Participant age, BMI, and salivary cortisol (controlling for time since waking) were included as covariates across all analyses given their known interactions with immune and/or sexual function.[46–49]

We conducted separate models for sexual desire as measured by the FSFI and SDI-2. We used hierarchical multiple regressions to test whether sexual desire scores over the past month were predicted by inflammation and depression. Covariates were entered at the first step, followed by Blom-transformed salivary CRP values and PHQ-9 depression scores in the second step. To assess directionality of effects, we then ran a second set of hierarchical regressions using the same stepped entry strategy, this time testing whether Blom-transformed salivary CRP values were predicted by sexual desire (as measured by either FSFI or SDI-2 scores) and PHQ-9 depression. Model fit was compared from first to second steps via change in adjusted R^2.

In supplemental analyses, salivary CRP levels (pg/mL) were converted to a predicted measure of serum CRP using an equation provided by Ouelett-Morin and colleagues (2011), where serum CRP = 1553.15 (salivary CRP) − 1413.19. When using a high-sensitivity assay for assessment of cardiovascular disease risk in the North American population, the recognized cut-offs are <1.0 mg/mL indicating low risk (optimally closer to zero), 1.0-3.0 mg/mL for medium risk, >3.0 mg/mL for high risk, and >10 mg/mL for very high risk.[50] Thus, we organized the converted serum CRP data (mg/L) into clinically meaningful risk categories as stratified above. Across studies, we also created a variable that dichotomized the CRP values as "unactivated" at values

less than 1 mg/L or "activated" at values of 1 mg/L and above; these values were chosen to reflect either no evidence of any inflammation processes (unactivated) or evidence of on-demand activation of inflammation (activated). Binary logistic regressions were planned to test if covariates alone (age, BMI, salivary cortisol, and time since waking) at the first step, FSFI/SDI-2 sexual desire at the second step, and PHQ-9 depression at the final step predicted membership in the CRP groups (ie, "unactivated" vs "activated"). Model fit was to be determined by χ^2 likelihood ratio.

Linear multiple regression estimates in G*Power [51] indicated that 40 participants would be sufficient to capture small to medium effect sizes (f^2 = 0.02−0.15) with 80% power. All analyses were performed in IBM SPSS Statistics 26, adopting an α threshold of 0.05 for determining statistical significance.

Study 1 Results. To predict sexual desire over the past month, we first computed a hierarchical regression with FSFI desire subscale scores as the dependent variable. The model that included just covariates (age, BMI, cortisol, and time since waking) was not significant ($F_{(4,21)}$ = 0.160, P= .956, adjusted R^2 = -0.155), and model fit worsened when adding inflammation and depression as predictors at the second step ($F_{(6,19)}$ = 0.184, P= .978, adjusted R^2 = -0.243). Accordingly, no predictors exhibited significant effects (P> .05 for all entered terms). Results were similarly null for regressions where SDI-2 desire scores were entered as the dependent variables (covariates only: $F_{(4,21)}$ = 0.996, P= .432, adjusted R^2 = -0.001; with inflammation and depression as predictors: $F_{(6,19)}$ = 0.856, P= .544, adjusted R^2 = -0.036; P> .05 for all individual terms).

Conversely, to predict inflammation from sexual desire and depression, we calculated hierarchical regressions where Blom-transformed salivary CRP values were the outcome. Including covariates only, the overall model and all predictors were not significant ($F_{(4,21)}$ = 0.970, P= .445, adjusted R^2 = -0.005). Adding FSFI desire and depression resulted in similarly null effects ($F_{(6,19)}$ = 0.684, P= .665, adjusted R^2 = -0.082), as did a separate model where SDI-2 desire and depression were entered as predictors ($F_{(6,19)}$ = 0.718, P= .640, adjusted R^2 = -0.073). These models can be found in Supplementary Materials Tables 1, 2, 3, and 4.

In supplemental analyses, salivary CRP values were converted to serum equivalents and dichotomized into clinically meaningful categories. Of the 37 women yielding complete data, 33 study participants (89.2%) were classified as "unactivated" with CRP values in the low-risk range (ie, <1 mg/L) and only 4 women (10.8%) were classified as "activated" with CRP in the average range (ie, 1−3 mg/L). No women fell into the high or acute risk classifications. Given the extremely low prevalence of women classified as "activated," binary logistic regressions predicting CRP activation group from depression and sexual desire were not computed.

Study 2

Study 2 expanded on Study 1 by examining both sexual desire over the past month as well as changes in sexual desire in response to an erotic stimulus in a controlled laboratory environment. The primary purpose and additional details of this study can be found on the OSF registration page https://osf.io/v49qk. The present analysis investigated bidirectional associations between women's sexual desire and immune system functioning.

Study 2 Participants. Participants were healthy, premenopausal women (n = 91) recruited via physical and online community boards, social media advertisements, and online course-credit pools from 2 universities in the Midwestern (n = 51) and Southeastern (n = 40) United States. Participants were screened via phone and online surveys to determine if they met the inclusion criteria of at least some self-reported sexual activity with a partner within the past month and comfort with all study procedures, including vaginal photoplethysmography (data not included in the present analysis). Exclusion criteria included chronic health conditions, heavy drinking (ie, more than 14 drinks within the past 2 weeks), recreational drug use within the past month, and use of medications known to impact either sexual or immune function (eg, psychotropics, antibiotics). Some women (n = 22) were using hormonal contraception at the time of their study session. Sample demographics are presented in Table 2. Incomplete data were mostly attributable to equipment malfunction (n = 7) during laboratory sessions and potential urinary tract infection (UTI) as identified by urine test (n = 8) at the time of the study session. As with Study 1, participants were not recruited with regard to their sexual functioning.

Study 2 Procedures. Study sessions were scheduled during the luteal phase of participant's menstrual cycles as - approximated from self-reported start date of the current cycle and typical cycle length - of their menstrual cycle. Participants were asked to not eat, drink, or chew gum for an hour before their study session. Upon arrival, participants were led to a private room within the laboratory and given water to drink while the researcher completed informed consent procedures. Timing was tracked to ensure participants waited at least 10 minutes after finishing the water before giving the first saliva sample. Participants were given privacy in the exam room to complete the first saliva sample and pre-film ratings of subjective states, of which the present analysis considered in-the-moment sexual desire. After watching both neutral and sexual stimuli videos, participants completed identical post-film ratings and additional surveys. At the end of the study session, participants were compensated with either $40 or course credit for their time and given the opportunity to ask questions. Procedures were approved by the Institutional Review Boards at Indiana University − Bloomington and University of North Carolina at Charlotte, and all participants provided written informed consent. As in Study 1, the present

Table 2. Demographic characteristics for Study 2

	Mean (M)	*St. Deviation (SD)*
Continuous variables		
Age in years	23.33	5.09
Body mass index (BMI)	25.95	6.91
Relationship duration in months	24.57	36.58
	Number (N)	*Percent (%)*
Race/ethnicity		
European American/White	41	45.1
African American/Black	17	18.7
Asian/Pacific Islander	12	13.2
Latin/Hispanic	2	2.2
Middle Eastern	2	2.2
Multiracial or other	8	8.8
Education		
High school	38	41.8
Associate's or technical degree	14	15.4
Bachelor's degree	19	20.9
Master's degree	10	11.0
Doctorate or equivalent	1	1.1
Missing/other	9	9.9
Sexual orientation		
Exclusively heterosexual	25	27.5
Mostly heterosexual	45	49.5
Bisexual or pansexual	10	11.0
Mostly homosexual	8	8.8
Exclusively homosexual	2	2.2
Relationship status		
Single	6	6.6
Dating nonexclusively	21	23.1
Dating exclusively	45	49.5
Married or cohabiting	13	14.3
Missing/other	6	6.6

Participants reported their sexual orientation as a number between 0 and 100, where 0 was labeled "only heterosexual," 25 labeled "mostly heterosexual," 50 labeled "bisexual," 75 labeled "mostly homosexual," and 100 labeled "only homosexual." For descriptive purposes, these numbers were coded into categories as displayed in the table above.

analysis focused on an initial baseline saliva sample and self-report questionnaires.

Study 2 Instruments and Measures. Salivary CRP and Cortisol. As in Study 1, saliva samples were collected via passive drool, stored at -80°C, and assayed for CRP and cortisol using commercially available ELISA kits according to manufacturer recommendations (Salimetrics, LLC). Inter- and intra-assay coefficients of variance were within acceptable ranges (CRP = 4.9−12.7%; cortisol = 3.07−11.6%), and the minimum detected CRP value was 29.67 pg/mL.

Audio-Visual Stimuli. Segments from a documentary from National Geographic and abbywinters.com (a women-oriented erotic film site) were chosen as the neutral and erotic videos, respectively. The neutral video (3 minutes) was presented first and included scenes of humans engaging with nature (eg, walking through the woods), followed by the erotic video (7 minutes), which included scenes of a heterosexual couple engaging in foreplay, oral sex, and vaginal intercourse.

Self-Report Measures. Age and BMI were measured as described in Study 1. Similarly, depression symptoms were self-reported via the Patient Health Questionnaire (PHQ-9), for which Cronbach's alpha in the present sample was 0.839. Sexual desire was again measured with the Female Sexual Function Index (FSFI), yielding Cronbach's alpha of 0.827 for the full 19-item scale and 0.852 for the desire subscale considered in the present analysis. However, Study 2 did *not* include the Sexual Desire Inventory (SDI-2) and instead included the following measure of in-the-moment desire.

Adapted Tape-Film Scale. Before and after the video stimuli, participants completed a 40-item survey assessing a variety of subjective states.[52,53] Sexual desire, emotional reactions (eg, positive and negative affect), and physiological sensations (eg, heart and breathing rates) were rated on a 7-point scale ranging from "not at all" to "intensely." For the purposes of this analysis, only changes in pre- to post-film sexual desire were considered.

Study 2 Data Analytic Plan. As described for Study 1, salivary CRP values were Blom-transformed and all analyses included participant age, BMI, and cortisol (controlling for time since waking) as covariates. Hierarchical regressions tested whether FSFI sexual desire scores were predicted by salivary CRP and PHQ-9 depression, as well as the converse of whether inflammation could be predicted from sexual desire and depression. Model fit was compared from first to second steps via change in adjusted R^2.

In an analysis unique to Study 2, mixed linear models assessed whether inflammation and depression predicted change in subjective sexual desire while watching the sexual film; this analysis was intended to capture responsive desire, a complementary but distinct form of desire from the more traditionally measured spontaneous desire.[54] Long format pre- and postfilm sexual desire ratings were entered as dependent variables. Fixed effect predictors included time point (which was also treated as a repeated measure), Blom-transformed salivary CRP values, PHQ-9 depression scores, and all covariates. A diagonal covariance structure was specified. Model fit was determined by Akaike information criterion (AIC).

For supplemental analyses, we again converted salivary CRP to serum equivalents and classified participants into clinical categories. Stepped binary logistic regressions tested whether covariates alone at the first step, FSFI sexual desire at the second step, and PHQ-9 depression at the final step predicted membership in

the 'unactivated' vs 'activated' CRP groups. Model fit was determined by χ^2 likelihood ratio.

Study 2 Results. Hierarchical regressions with FSFI sexual desire scores as the dependent variable were not significant, whether predicted by covariates alone at the first step ($F_{(4,31)}$ = 1.563, P= .209, adjusted R^2 = 0.060) or after adding inflammation and depression at the second step ($F_{(6,29)}$ = 1.029, P= .330, adjusted R^2 = 0.035). The only significant predictor in the full model was age (standardized β = -0.397, $t_{(6,29)}$ = -2.103, P= .044), whereby older participants reported lower sexual desire. This model can be found in Supplementary Materials Table 5.

Another hierarchical regression predicted Blom-transformed salivary CRP from depression and sexual desire. The model that included just covariates was significant ($F_{(4,31)}$ = 5.882, P= .001, adjusted R^2 = 0.358), and model fit was slightly worsened when adding FSFI sexual desire and PHQ-9 depression as predictors at the second step ($F_{(6,29)}$ = 4.015, P= .005, adjusted R^2 = 0.341). The only significant predictor was BMI (standardized β = 0.736, $t_{(6,29)}$ = 4.538, P< .001), such that participants with higher body mass exhibited greater salivary CRP levels. This model can be found in Supplementary Materials Table 6.

A linear mixed model examined predictors of change in subjective sexual desire while watching a sexual film. There was a significant main effect of time on sexual desire ($F_{(1,61.390)}$ = 136.618, P< .001), such that ratings of desire increased from pre- to post-film (estimated fixed effect = 3.485, standard error = 0.298); in other words, there was evidence that our experimental manipulation of sexual response was successful. There was also a significant main effect of PHQ-9 score ($F_{(1,61.390)}$ = 4.583, P= .036), whereby participants reporting more depressed mood over the past 2 weeks unexpectedly gave higher ratings of sexual desire during the laboratory session (estimated fixed effect = 0.095, standard error = 0.045). Notably, in additional post-hoc models examining interaction effects, model fit was slightly worsened (AIC without interaction terms = 230.311 vs AIC with interaction terms = 231.593−232.039) and there was no evidence that women endorsing higher PHQ-9 scores exhibited greater *change* in desire from pre- to post-film than women endorsing less depressed mood. No other predictors exhibited significant main effects. This model can be found in Supplementary Materials Table 7.

In supplemental analyses, salivary CRP values were again converted to serum equivalents for classification into clinical categories (Supplementary Materials, Table 8). Of the 72 participants yielding complete CRP data, 58 women (80.6%) were classified as "unactivated" with serum CRP estimates in the low-risk range (ie, <1 mg/L), while the other 14 women (19.4%) were classified as "activated" with levels in the average risk category (ie, 1−3 mg/L). No women exhibited CRP in the high or acute ranges. Binary logistic regression tested how well FSFI desire scores, PHQ-9 depression scores, and covariates classified participants into "unactivated" vs "activated" groups. The model including just covariates was not significant ($\chi^2_{(4)}$ = 4.951, P = .292, R^2_N = 0.232). Adding FSFI desire in the second step did not improve prediction of CRP activation group ($\chi^2_{(5)}$ = 5.092, P = .405, R^2_N = 0.238), and the amount of unexplained variance (-2LL) decreased only slightly from 25.993 to 25.793. The model remained non-significant when adding PHQ-9 depression in the final step ($\chi^2_{(6)}$ = 5.412, P = .492, R^2_N = 0.251), which also did not significantly reduce the amount of unexplained variance (-2LL) from 25.793 to 25.473. None of the individual predictors significantly determined CRP activation at any step (P > .05 for all entered terms).

Study 3

The primary objective of Study 3 was to examine the relationships among several physiological and psychological correlates of sexual desire (https://clinicaltrials.gov/ct2/show/NCT01706406). The present analysis explored whether increased levels of salivary CRP were associated with sexual desire in a clinical sample of women who either met criteria for HSDD as per the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV-TR)[38] or who had no sexual desire concerns.

Study 3 Participants. For the current analyses, a subset of women (n = 269) were included based on completion of all necessary procedures (eg, questionnaires, saliva collection) and did not report any factors (such as immune conditions) associated with acutely elevated CRP levels. Participants were recruited via advertisements in the community and in primary care clinics. Eligibility was determined via a telephone interview. Eligibility criteria included being between the ages of 19 and 65 with no known medical illnesses or medications associated with sexual or hormone functioning (eg, hormone replacement therapy, hormonal contraceptives). Participants were also required to be non-smokers/non-drug users, not pregnant, have no current diagnosis of depression, and have a BMI between 18.5 and 29.9.

The current analyses were conducted using a group of healthy women with no indication of sexual dysfunction (control group; n = 134) and a group of women who met criteria for HSDD (low sexual desire group; n = 135). To be included in the low sexual desire group, prospective participants were required to report whether their lack of interest in sexual activity and sexual thoughts/fantasies were associated with significant personal or interpersonal distress. If women reported low desire of less than 1 year's duration, or attributed their low desire entirely to daily stress, severe relationship discord, or pain during penetrative sex that was not alleviated by an external lubricant, they were not included in the study. Prospective participants with low desire concerns that did not fully meet DSM-IV-TR criteria for HSDD were not included. Demographics were similar across the 2 groups, as summarized in Table 3.

Table 3. Participant demographic characteristics for study 3

	Low sexual desire (n = 135)		Control (n = 134)	
Continuous variables	*M*	*SD*	*M*	*SD*
Age	32.99	11.74	31.61	11.90
Relationship duration (months)	84.61	89.32	71.04	90.85
Race/Ethnicity	*N*	%	*N*	%
Euro-Caucasian	83	61.5	80	59.7
East Asian	24	17.8	33	24.6
South Asian	8	5.9	9	6.7
First Nations	2	1.5	-	-
Middle Eastern	5	3.7	3	2.2
African-Canadian	2	1.5	1	0.7
Other	11	8.1	8	6.0
Education	*N*	%	*N*	%
High school	13	9.6	25	18.7
College/Technical/Trade School	24	17.8	19	14.2
Undergraduate degree	55	40.7	53	39.6
Master's degree	27	20.0	23	17.2
Doctoral degree	6	4.4	7	5.2
Other	10	7.4	7	5.2
Employment status	*N*	%	*N*	%
Full-time	54	40.0	49	36.6
Part-time	21	15.6	20	14.9
Self-employed	6	4.4	8	6.0
Unemployed	2	1.5	1	0.7
Retired	5	3.7	1	0.7
Student	38	28.1	52	38.8
Homemaker	3	2.2	-	-
Other	6	4.4	3	2.2
Sexual orientation	***N***	**%**	***N***	**%**
Heterosexual	113	83.7	111	82.8
Lesbian	4	3.0	4	3.0
Bisexual	12	8.9	14	10.4
Other	6	4.4	5	3.7
Relationship status	*N*	%	*N*	%
Single	27	20.0	39	21.9
Dating	29	21.5	44	32.8
Married/Cohabitating	70	51.9	42	31.3
Divorced	2	1.5	2	1.5
Widowed	2	1.5	1	0.7
Other	5	3.7	6	4.5

No significant group difference on any of these demographic variables was observed (all *P*'s > .05).

Study 3 Procedure. At a laboratory session, participants who met inclusion criteria completed the informed consent process and were screened for HSDD and depression using clinician administered diagnostic screeners. They were also provided with a saliva sampling kit along with detailed verbal and written instructions for saliva collection procedures and a link to an instructional video. Participants were instructed to provide a passive drool saliva sample as in Studies 1 and 2, however, participants in Study 3 collected their samples at home. Saliva samples were collected at 4 time points in the diurnal cycle, on 3 separate (not necessarily consecutive) typical weekdays. The saliva sample collected at the 60-minute post-waking time point was used to determine CRP and cortisol levels (saliva samples collected at other time points were used in analyses published elsewhere).

In order to minimize sample contamination, participants were asked to avoid eating, brushing or flossing teeth, consuming beverages (other than water), using mouthwash, chewing gum, or eating a large meal within an hour of collecting a saliva sample. Participants were also asked to perform a cold-water rinse prior to saliva sample collection. For sample collection days, they were

instructed to avoid using any non-steroidal anti-inflammatory medications or eating any food known to disrupt cortisol levels (eg, chocolate, alcohol, coffee). Saliva vials were briefly stored in participants' freezers at approximately -15°C until all samples had been collected. Samples were then transferred on ice to a university laboratory for storage at -20°C until analysis.

In addition to in-person assessments for HSDD and depression, participants completed a battery of self-report questionnaires at a time of their choosing. Each participant received $100 for completion of questionnaires, interviews, and at-home saliva collection. All study procedures were approved by the Clinical Research Ethics Board at British Columbia Centre for Sexual Medicine and University of British Columbia.

Study 3 Instruments and Measures. Salivary CRP and Cortisol. As in Studies 1 and 2, assays for CRP and cortisol were conducted using commercially available ELISA kits according to manufacturer recommendations (Salimetrics LLC). For the present study, participants' salivary cortisol measures were represented by the average, across 3 days, of saliva samples collected 60 minutes post-awakening, which is widely considered to be the true morning cortisol level.[55] A separate aliquot of saliva collected 60 minutes post-awakening was used to measure CRP. The minimum detectable level of CRP was 94 pg/mL. Inter- and intra-assay coefficients of variance were within acceptable ranges (CRP = 3.9-7.5%; cortisol = 4.6-6.0%).

Self-Report Measures. In addition to the screening criteria for low sexual desire (as described above), prospective participants were asked a series of demographic questions (eg, age, relationship history, degree of relationship satisfaction, etc.) during their telephone screening interview. The following psychological measures were also included in analyses.

Decreased Sexual Desire Screener. The Decreased Sexual Desire Screener (DSDS) is a 5-item clinician administered diagnostic brief screener for generalized acquired HSDD in women.[56] Initially, the clinician presents participants with a set of 4 "yes" or "no" questions relating to sexual desire (ie, level of, satisfaction with, etc.), where "yes" answers indicate symptoms of HSDD. For cases wherein all 4 items were endorsed by the participant, a fifth question was administered to rule out potentially confounding causes for decreased desire (ie, medical illness, relationship factors, medications, or stress and/or fatigue). In cases wherein the respondent answered "yes" to the first 4 questions and 'no' to all factors in question 5, she was placed in the HSDD group. Respondents who answered "yes" to all items were placed in the HSDD group only if endorsed factors did not point to another primary diagnosis. The DSDS shows 85.2% diagnostic accuracy and high sensitivity and specificity (with point estimates of 0.84 and 0.88, respectively).[56]

Sexual Interest and Desire Inventory-Female. The Sexual Interest and Desire Inventory-Female was administered to obtain a continuous measure of sexual desire.[57] As a portion of the scale's items exclude individuals who are not currently in a relationship, other items measure sexual arousal, and yet other items are possibly intercorrelated with depressive mood (ie, frequency of positive thoughts about sexual activities), we identified the 2 items that represent the dimensions of clinically significant low sexual desire as per the diagnostic criteria for HSDD[38]: (i) *desire* (ie, "Over the past month, how frequently and how strongly have you wanted to engage in some kind of sexual activity, either with or without a partner?") and (ii) *distress* (ie, "Over the past month, when you thought about sex or were approached for sex, how distressed [worried, concerned, guilty] were you about your level of desire?"). The 2-item composite score had a possible range of 0-9, with lower scores indicating a lower level of sexual desire. Cronbach's alpha for the 2 items in the present sample was 0.60. Past studies[57] have indicated that these 2 items sensitively distinguish individuals with HSDD from women with no sexual dysfunction, as well as women with HSDD from women with Female Orgasmic Disorder. Both items showed high correlations with the total SIDI score.

Beck Depression Inventory-II. The Beck Depression Inventory-II (BDI-II) is a brief criteria-referenced instrument consisting of 21 questions designed to measure the degree of severity of cognitive, affective, and somatic depressive symptoms.[58] All scale items range on a Likert scale from 0 to 3. Responses are summed to create a total score with a possible range of 0-63, where higher scores represent greater severity of depressive symptomatology. The BDI-II is positively correlated with other measures of depression, such as the PHQ-9 ($r = 0.84$[59]), and shows strong 1-week test-retest reliability ($r = 0.93$[58]). Cronbach's alpha for the BDI-II in the present sample was 0.88.

Study 3 Data Analytic Plan. Outliers (n = 6) were removed only in cases where there was a discernible reason for higher CRP levels. In our sample, these exclusions consisted of participants with hypothyroidism, celiac disease, raised liver enzymes, type 1 diabetes, and polycystic ovarian syndrome. As in Studies 1 and 2, CRP values were Blom-transformed and age, BMI, and salivary cortisol (collected 60-minutes postawakening across all participants) were included in all analyses as covariates.

Hierarchical multiple regression was performed to examine whether inflammation (CRP level) was predicted by clinical diagnosis (ie, HSDD vs control group), a continuous measure of sexual desire (ie, abbreviated SIDI score), and BDI-II depressive symptoms. Covariates were entered in the first step, followed by the sexual desire and depression measures. Model fit was compared from first to second steps via the change in adjusted R^2. Next, a binary logistic regression was performed to examine how well inflammation, depressive symptoms, and covariates classified participants by diagnosis (ie, HSDD vs control). An *a priori* power analysis was computed using G*Power. To achieve a large effect size in the multiple regression model ($f^2 = 0.35$) with 90%

the total sample size was estimated at 45 individuals (actual sample size n = 269).

For supplementary analyses, salivary CRP levels were converted to serum equivalents and participants were classified into both clinical categories and dichotomous "unactivated" vs "activated" groups, as described for Studies 1 and 2. In the first supplemental analysis, binary logistic regression examined the degree to which membership in the "unactivated" vs "activated" CRP groups (ie, the dependent variable) was predicted by diagnosis (ie, HSDD vs control group), SIDI sexual desire, and BDI-II depression symptoms. Variables were entered sequentially in 4 blocks, starting with covariates (ie, age, BMI, cortisol), then group membership (ie, HSDD vs control), then the continuous measure of sexual functioning, and finally depression symptoms. Using the same analytic plan described above, the second supplemental analysis examined the degree to which membership in the HSDD vs control group was predicted by CRP group (ie, "unactivated" vs "activated;" block 2) and depression symptoms (block 3). Model fits were compared on the basis of the χ^2 likelihood ratio test and the significance of individual regression coefficients was assessed according to the associated Wald test.

Study 3 Results. To establish whether composition was similar across groups, groups were compared on several demographic variables including age, ethnicity, highest education level, sexual orientation, relationship status, and relationship duration. No significant group difference in these demographic variables was observed (all P's > .05; Table 3).

Results of the first hierarchical multiple linear regression indicated that CRP levels were not predicted by either diagnosis (HSDD vs control), sexual desire, or depressive symptoms. Specifically, the model that included just the covariates (age, BMI, cortisol) was not significant ($F_{(3,\ 243)}$ = 2.52, P = .058, adjusted R^2 = 0.018), with model fit worsening when HSDD diagnosis, sexual desire, and depressive symptoms were included as predictors in the second step ($F_{(5,\ 243)}$ = 1.62, P = .143, adjusted R^2 = 0.012, R^2 change = 0.003).

Next, the binary logistic regression tested how well inflammation, depressive symptoms, and covariates classified participants by diagnosis (ie, HSDD vs control). Though covariates alone were not significant (p = 0.226) the full model including inflammation and depression symptoms was significant ($F_{(5,\ 261)}$ = 8.14, P< .001, adjusted R^2 = 0.118, R^2 change = 0.117). Of note, however, the only significant predictors in the full model were depression symptoms (standardized β = 0.35, $t_{(261)}$ = 5.90, P < .001) and cortisol levels (standardized β = -0.12, $t_{(263)}$ = -1.97, P = .050), such that higher depression and lower cortisol predicted membership in the HSDD group. CRP levels (untransformed), across a continuous measure of sexual desire (SIDI score) and categorized by BDI-II score (low-moderate depression [BDI-II score 0−18], moderate-severe depression [BDI-II score 19−63]) are shown in Figure 1.

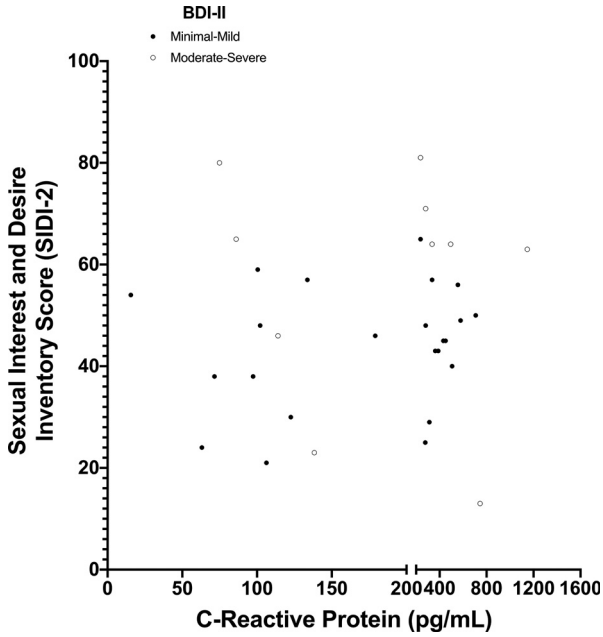

Figure 1. Interaction between untransformed salivary CRP and sexual desire by depression scores. Figure1is available in color online at www.jsm.jsexmed.org.

Using the serum-equivalent values of CRP, a majority of participants (n = 243; 90.3%) had CRP levels in the low-risk range (ie, <1 mg/L); of these participants, n = 120 were in the control group and n = 123 were in the low sexual desire group. Another n = 15 (5.6%) participants showed CRP levels in the average risk range (ie, 1−3 mg/L); n = 7 of these participants were in the control group and the other n = 8 were in the low sexual desire group. Another n = 11 (4.1%) participants exhibited CRP levels in the high range (ie, 3−10 mg/L); n = 7 of these participants were in the control group whereas the other n = 4 were in the low sexual desire group. Finally, no participant in either group demonstrated a level of CRP indicative of acute activation (ie, >10 mg/L). Thus, when dichotomized based on CRP classification, n = 243 (90.3%; n = 120 controls; n = 123 low sexual desire) of the sample fell into the "unactivated" group whereas n = 26 (9.4%; n = 14 controls; n = 12 low sexual desire) of the sample fell into the "activated" group, with no differences between sexual functioning groups.

In supplemental analyses, binary logistic regression tested how well categorical and continuous measures of desire, BDI-II depression scores, and covariates classified participants into "unactivated" vs "activated" CRP groups (Supplementary Materials, Table 9). The model including just covariates was not significant ($\chi^2_{(3)}$ = 5.20, P= .158, R^2_N = 0.05). Adding the categorical indicator of diagnostic group (ie, HSDD or control group) did not improve prediction of CRP activation ($\chi^2_{(4)}$ = 5.50, P= .240, R^2_N = 0.05), and the amount of unexplained variance (-2LL) decreased only marginally from 133.17 to 132.87. Adding a continuous measure of sexual desire in the

third step did not improve predictions of CRP activation ($\chi^2_{(5)}$ = 5.99, P= .307, R^2_N = 0.06), and the amount of unexplained variance decreased only slightly from 132.87 to 132.38. Finally, the addition of BDI-II depression scores to the model did not improve prediction of CRP activation ($\chi^2_{(6)}$ = 6.80, P= .340, R^2_N = 0.06), and the amount of unexplained variance (-2LL) decreased only marginally from 132.38 to 131.57. None of the individual predictors significantly determined CRP activation at any step (P> .05 for all entered terms).

Results of the second binary logistic regression predicting group membership (ie, HSDD vs control) from CRP group (ie, "activated" vs "unactivated") and BDI-II depression symptomatology indicated that CRP status did not significantly determine group membership (P= .902), but depression scores did (P< .001; Supplementary Materials, Table 10). The model including just covariates was not significant ($\chi^2_{(3)}$ = 4.41, P= .220, R^2_N = 0.02). Adding CRP status in the second step did not improve prediction of group membership ($\chi^2_{(4)}$ = 4.42, P= .352, R^2_N = 0.02), and the amount of unexplained variance (-2LL) decreased only marginally from 357.38 to 357.37. With the addition of BDI-II scores in the final step, the model was shown to significantly predict group membership step ($\chi^2_{(5)}$ = 36.39, P< .001, R^2_N = 0.17). The amount of unexplained variance (-2LL) reduced from 357.37 to 325.40.

DISCUSSION

Previous research has suggested that elevated levels of inflammation may be a viable predictor of sexual desire in women (eg, [36]). The present set of analyses used salivary CRP and information regarding sexual desire in clinical and non-clinical samples using various experimental paradigms to investigate whether CRP was significantly associated with women's sexual desire Figure 2. contains a summary of results across studies.

Null Results

Despite applying several analytic models and using various measurement tools for assessing sexual desire, we did not find that CRP predicted trait or state sexual desire in any of the studies. The lack of association is notable, as there has been ample evidence in animal models and human men that inflammation plays a role in sexual function.[36] Perhaps the lack of association here is related to the fact these studies involved physically healthy samples, as demonstrated by the limited range of CRP values. Significantly elevated CRP is a useful indicator of acute inflammation (eg, during infection, [14]), but even moderately elevated CRP has been shown to reflect chronic inflammation.[60] As such, CRP may be a useful biomarker for determining sexual function in populations with greater physical pathology and associated inflammatory burden (ie, chronic low-grade inflammation) marked by elevated baseline CRP. Moreover, these null findings could indicate the existence of a higher threshold that needs to be met before inflammation interferes with healthy sexual desire, which we did not capture using our study samples.

Alternatively, CRP may not be a sensitive enough measurement. While CRP is a good index of a wide variety of inflammatory processes throughout the body, it is perhaps too nonspecific to capture the inflammatory signals that interact with the neural and endocrine systems governing women's sexual desire. It is also possible that a more proximate temporal marker would better reflect ongoing fluctuations in sexual desire. For example, while CRP does not predict sexual desire over the last month (as would be indexed by the FSFI), inflammatory cytokines may suppress activation of sexual reward pathways on a more time-

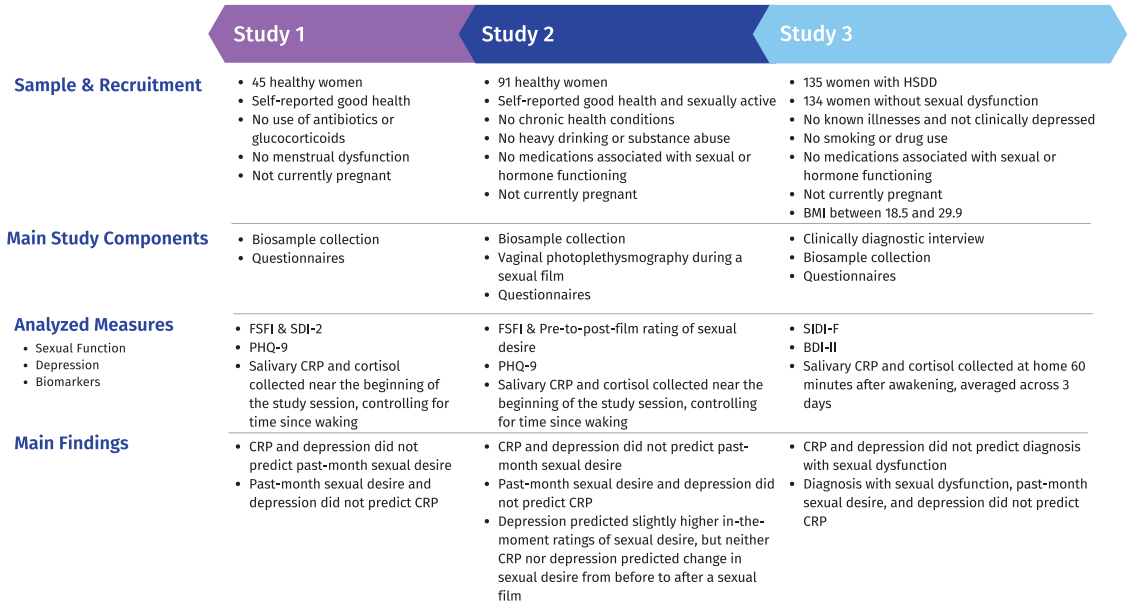

Figure 2. Summary of studies. Figure 2 is available in color online at www.jsm.jsexmed.org.

limited basis. Moreover, future investigations should consider exploring whether a more sensitive biomarker of neuroinflammation predicts sexual desire in women.

Clinical Vs Nonclinical Findings

Although we anticipated an observable and possibly amplified relationship between salivary CRP and sexual desire in our clinical sample (ie, women meeting diagnostic criteria for HSDD), our results did not reveal such an association. The levels of salivary CRP were relatively similar between the clinical and nonclinical samples and were not predictive of altered sexual desire regardless of whether they were measured continuously or categorically. These null findings may reflect the fact that our clinical sample was otherwise healthy in that women with diagnosed depression and inflammatory-mediated diagnoses were excluded from participation. Had we included women with conditions associated with elevated CRP in our sample, it is possible we would have seen more variability and impact on sexual desire. This not being the case, we may conclude that CRP itself has little, if any, direct impact on HSDD in otherwise healthy women. It is possible, however, that higher levels of CRP may contribute to clinically significant low sexual desire through sequelae of inflammatory-mediated health conditions.

Depression

While higher depression scores on the BDI-II were found to be an important predictor of sexual desire in the HSDD sample, depression scores (PHQ-9 and BDI-II) in the non-clinical samples did not predict sexual desire. Though none of our samples included overtly depressed participants, it is possible the depression symptoms that the HSDD participants endorsed mimicked the distress and behavioral patterns associated with HSDD (eg, decreased interest in/responsiveness to typically rewarding activities, low energy, etc.). Depression has been linked to altered immune function as a manifestation of sickness behaviors, particularly in regards to somatic and vegetative symptoms such as decreased appetitive response.[61] Women tend to exhibit more sickness behaviors and greater detrimental psychological effects in response to increased inflammation than men,[31] suggesting that the stronger links between depression and sexual dysfunction observed in women[62,63] may be mediated by greater susceptibility to inflammation-related sickness behaviors. However, in our samples, we observed effects of depressive symptoms on sexual desire only at relatively higher levels, further supporting the possibility that our null results are driven by low levels of observed pathology. If so, this would suggest that the effects of both inflammation and depressive symptoms on women's sexual desire are non-linear, and instead follow a threshold model. In this case, it is likely not the *direct* effects of inflammation and/or depressive symptoms, but the *indirect* effects of either on broader pathological processes (such as allostatic load or interference with intimate relationships) that would impair women's sexual desire function.

Strengths and Limitations

Collectively, these studies have several strengths. First, the current studies used different analytical methods as well as different measures of depression and sexual desire. Because we used 2 different measurements of depression, there is an increased likelihood that the results reflect more general aspects of depression rather than specific symptoms. Similarly, the studies used 4 different measures of sexual desire spanning from in-the-moment to over the past month, with consideration of responsive desire (Study 2) and distress related to low desire (Study 3). The assessment of state and trait desire as well as the assessment of distress allows for our results to be more generally applicable to sexual desire as a construct.

Very few studies have examined the effects of inflammation on women's sexual functioning despite sexual problems being a prominent complaint among women with health conditions associated with inflammation.[21–24] To the best of our knowledge, the current set of studies is the first to focus on sexual desire in a sample of healthy women. While inflammation, as marked by CRP, does not appear to significantly impact sexual desire across our 3 samples, evidence from studies in men with ED[25] highlights an opportunity to investigate whether sexual *arousal* may similarly be modulated by inflammation in women. Future studies should aim to explore sexual arousal in women and its possible relationship to inflammatory markers.

Another limitation of this work is that our studies were retrospective and cross-sectional in design. All 3 studies employed standardized questionnaires to examine sexual desire over the previous month, with collection of saliva for quantification of CRP on the date of study. In-the-moment sexual desire was also assessed in Study 2. The former methodology allowed us to make conclusions about the ability of current salivary CRP levels to predict past sexual desire based on the assumption that CRP values are constant over time. We recognize that this assumption does not account for the fact that baseline CRP values can fluctuate depending on several factors, given its role as an acute phase protein.[14] Ergo, relying on a single value of CRP to correlate with measures of sexual desire over the past month may not perfectly capture the relationship between desire and CRP. We also recognize that the FSFI and SDI-2 are retrospective questionnaires that rely on individual recall of events and can thus result in under- or over-estimation of the magnitude of sexual desire complaints. Collecting in-the-moment subjective sexual desire ratings in Study 2 circumvented the aforementioned limitations by enabling comparison of sexual desire and CRP values acquired at the same time, although we did not observe a significant correlation or predictive capacity between these 2 variables using our models. Moreover, evidence from studies examining men with erectile dysfunction shows inflammation modulates both current and long-term sexual functioning.[64] A longitudinal study design may mitigate the effects of recall bias and enable a more holistic understanding of how inflammation impacts sexual functioning in women over time (eg, recording sexual desire using written

journal entries and quantifying inflammation using multiple samples over the same time period).[65]

Additionally, we note that there are still gaps that need to be addressed before salivary CRP can be validated as a surrogate for serum CRP.[41,66] While a moderately positive correlation between salivary and serum CRP has been identified by systematic review,[41] the empirical data are mixed, with some studies finding a moderately positive correlation between salivary and plasma CRP [42,67–73] and others not.[15,74,75] When a correlation was identified in these studies, it was only at higher concentrations of salivary and serum CRP, with either weak or null correlations at lower concentrations.[42,69,70,72] These findings have implications for our sample of otherwise healthy women as the majority had very low or low salivary CRP levels. This implies that our sample would be expected to have low levels of serum CRP, thus impacting the sensitivity of our salivary CRP measurements. In turn, this may explain why we were unable to detect differences using the raw salivary CRP values or when converting to serum equivalents. That said, we can conclude that the actual circulating CRP levels in these samples were low, because although salivary measures have lower levels of sensitivity than blood measures, they can still reliably detect significant elevations of CRP.

Clinical Implications

As CRP is among the most commonly available clinical tests of inflammation, it holds great promise as an accessible way to index immune contributions to many chronic conditions, including potentially sexual dysfunction. However, our findings cast doubt on the clinical utility of CRP as a diagnostic or prognostic for women's sexual desire concerns. Specifically, across 3 studies, salivary CRP was not found to be a reliable metric for assessing aspects of inflammation that contribute to low sexual desire in physically healthy women. That said, although salivary CRP may not be a useful metric of women's sexual desire, this does not rule out the clinical utility of measuring other markers of inflammation. There are several avenues by which inflammation may play a role in sexual desire; further investigations are warranted.

Conclusion & Summary of Recommendations for Future Directions

The link between inflammation and sexual functioning in men is well documented, such that several consensus documents now recommend assessment of erectile functioning in young healthy men as a means of estimating disease risk for inflammation-related conditions such as cardiovascular disease (eg, [76–78]). As inflammation-related diseases are also among the top causes of disability and mortality in women, if there are similar links between inflammation and sexual dysfunction in young healthy women, measurement of women's sexual function could prove to be incredibly valuable not only within sexual medicine but in women's health more broadly. However, to date, there is extremely limited data on if and how inflammation may relate to women's sexual desire, and none examining these associations in pre-morbid, non-clinical samples. We analyzed data from 3 studies that spanned across multiple sites, samples of women, and measures of sexual desire, but we found no evidence for a robust association between CRP and women's sexual desire. Most immediately, these findings suggest that salivary CRP — a widely used biomarker of both chronic and acute inflammation — does not predict women's sexual desire and as such, would likely not be a useful biomarker of inflammatory contributions to women's sexual desire dysfunction. More broadly, taking our findings together with prior work suggesting lower sexual desire among women with inflammatory pathologies, we posit there is likely a non-linear relationship between inflammation and women's sexual functioning, such that a threshold must be crossed before inflammatory processes have a detectable effect.

As such, we see value in investigating this relationship further to understand the dimensions of this threshold, and the mechanisms by which inflammatory conditions exert an effect on women's sexual function. We recommend longitudinal study designs that also include measures of sexual arousal to examine the effects over time and across multiple aspects of sexual functioning. We also recommend quantifying CRP from serum alongside saliva, as well as measuring representative markers across inflammatory systems (such as cytokines and other acute phase proteins) to obtain multiple measures of systemic inflammation. Finally, we suggest broadening the inclusion criteria to avoid exclusion of depressed or otherwise unhealthy populations, thus enabling comparisons between different demographics.

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