Partnered sexual activity moderates menstrual cycle–related changes in inflammation markers in healthy women: an exploratory observational study

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Objective: To examine differences in inflammation markers in sexually active versus abstinent women and observe changes in inflammation markers across the menstrual cycle. Cycle-related immune fluctuations may have evolved to reduce interference with conception. If so, reproductively active (i.e., sexually active) women should show the most variability in cytokine expression.
Design: Participants provided serum samples at menses and ovulation (from which cytokines were assayed) and saliva samples at menses and during follicular, ovulation, and luteal phases (from which C-reactive protein [CRP] was assayed). Participants self-reported intercourse frequency during the study.
Setting: Academic research laboratory.
Patient(s): Thirty-two healthy, naturally cycling premenopausal women (sexually active, n = 15; abstinent, n = 17).
Intervention(s): Observational study.
Main Outcome Measure(s): Levels of proinflammatory cytokines (interleukin-6 [IL-6], interferon γ [IFN-γ], tumor necrosis factor-α [TNF-α]), an anti-inflammatory cytokine (interleukin-4 [IL-4]), and a marker of total inflammation (CRP).
Result(s): Sexually active women had higher levels of all of the immune markers measured, including both pro- and anti-inflammatory cytokines, than abstinent women. Relative to sexually active women, abstinent women had less change across the menstrual cycle in levels of CRP. Among sexually active women, higher intercourse frequency predicted greater midcycle decreases in CRP, IL-6, and IFN-γ and midcycle increases in IL-4.
Conclusion(s): Sexual activity may stimulate a complex interaction between pro- and anti-inflammatory cytokines that subsequently drives midcycle declines in inflammation. (Fertil Steril® 2017;107:763–73. ©2016 by American Society for Reproductive Medicine.)
Key Words: Inflammation, sexual activity, menstrual, C-reactive protein, interleukin-6, cytokine, interferon-γ, tumor necrosis factor-α, interleukin-4

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Inflammation is a critical process in the immune response, as it is the first-line defense against pathogens, tissue healing or remodeling, and toxin containment and removal. Variations in inflammation drive variations in symptoms such as fatigue (1), pain (2), and depression (3). In premenopausal women, inflammation varies significantly across the menstrual cycle (4). Several studies have documented midcycle decreases in markers of inflammation, with higher levels at menses (5, 6) and a nadir around ovulation (4, 7). Such variation has important implications for research and the clinical interpretation of inflammation biomarkers. One study examined values of C-reactive protein (CRP), a marker of inflammation commonly used to index heart disease risk, and found that failure to account for menstrual cycle–related variability

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doubled the chances of misclassification of heart disease risk in a large sample of healthy women (8).

The curvilinear pattern in CRP has been attributed to a fundamental trade-off between reproduction and immune response: for conception to occur, the female immune system must provide a conducive environment for sperm and conceptus. Local inflammation can impair sperm motility (9) and make the uterine environment more hostile to implantation (10). More broadly, inflammation in general circulation can interfere with conception in two significant ways. First, systemic inflammation can signal a potential infection or injury, which would divert energetic resources from reproduction to somatic maintenance or defense (11–13). Second, the female reproductive tract uses inflammatory signals such as cytokines to coordinate ovulation and implantation (14); in the presence of high systemic inflammation, however, these small, local inflammation signals fail to appropriately trigger the processes that lead to ovulation or implantation (10, 15)—potentially leading to poorer rates of conception.

Thus, it is argued, a midcycle decrease in systemic inflammation—corresponding to peak fertility—may have evolved to reduce potential disruption of reproduction (11). If so, it would follow that these effects would be most critical (and most subject to evolutionary selective pressure) in individuals who are reproductively active, that is, regularly engaging in sexual activity. Indeed, several studies suggest that immune parameters, including inflammatory cytokines, differ in sexually active versus abstinent women (16–18), with sexually active women showing more variation in immune markers across the menstrual cycle than abstinent women (13, 19, 20). Thus, we would expect that inflammation would differ significantly across the menstrual cycle in sexually active but not in abstinent women.

We examined this hypothesis by comparing changes in pro- and anti-inflammatory cytokines as well as CRP across a menstrual cycle in women who were abstinent and women who were sexually active with a partner. We assessed four primary cytokines: interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-4 (IL-4). The first three are commonly measured as an index of inflammation signaling (21, 22), while IL-4 is considered an "anti-inflammatory" cytokine (23). We included an anti-inflammatory cytokine as there is some work suggesting sexual activity may influence inflammation through shifting T-helper cell profiles (20). In addition to their important roles in coordinating inflammation, IFN-γ, TNF-α, and IL-6 interact with the central nervous system, supporting a suite of behavioral effects related to sickness (e.g., loss of appetite, decreased motivation for social interaction) (24). More specifically, IFN-γ is predominantly produced by natural killer cells and cytotoxic T cells (25) and is a potent activator of macrophages, which in turn induce local inflammation (26). TNF-α is produced primarily by activated macrophages and mast cells and stimulates the release of histamines and other inflammation-causing agents (27). Both TNF-α and IFN-γ have been identified as particularly predictive of early pregnancy loss (28, 29) owing to their role in stimulating natural killer cells in the uterus (28). Thus, suppressing these proinflammatory cytokines could improve the chances of sexual activity leading to offspring. IL-6 is widely used by a variety of immune actors such as T-cells and macrophages and is an important mediator of inflammation and acute phase response (see, however, Schindler et al. [30] for discussion of IL-6's anti-inflammatory effects via suppression of IFN-γ-producing cells). Finally, CRP is an acute phase protein induced by the liver in response to cytokines such as IL-6 and has a relatively short half-life ($\sim$48 hours); as such, it is a good index of total current inflammation across the body (13).

There have been several studies investigating menstrual variations in cytokines and CRP. In healthy nonpregnant women, higher levels of IL-4 have been observed in the luteal phase relative to the follicular phase (20, 31). Studies of IFN-γ, TNF-α, and IL-6 have had more mixed findings, with some studies documenting decreases from early to mid- or late cycle (32–34) and others increases (35, 36) or no change (37). Similarly, studies of menstrual cycle variations of CRP have been mixed, with some showing a midcycle nadir and others a midcycle peak or no change (4, 6, 7, 38, 39). The variability in findings may be due to lack of consideration of the timing (or even occurrence) of ovulation. The largest and best-controlled study to date, which accounted for differences in luteal phase progesterone (P_4) in ovulatory and anovulatory cycles, found that markers of inflammation were lowest at ovulation and rose during the luteal phase (4). Similarly, confusion across studies may occur due to lack of consideration of sexual activity. A recent study found that among women with ovulatory cycles, being sexually active (dichotomized as yes/no) was associated with a U-shaped pattern of serum CRP, while sexual abstinence was associated with no significant change across the cycle (13); unfortunately, this study did not measure the frequency of sexual activity or cytokine concentrations.

In the present study, our primary analysis compared women by sexual activity status (currently sexually active vs. abstinent). As a secondary analysis, we also considered the potential effect of frequency of sexual activity (within sexually active women only), as this may reflect how the immune system interprets sexual activity as a reflection of reproductive state and, by extension, the need for flexibility in how it interprets the "self." One of the most puzzling questions in immunology is how the immune system adapts its responsiveness over time, maintaining vigilance while also learning tolerance of elements that are different from the original self but not harmful (e.g., commensurate microbes, the body's own cells as it grows and changes over time). One recent theory addresses this problem by introducing the concept of the "liquid self," a flexible state that adapts the distinction between self and nonself over time. The individuals' life history and reproductive state are particularly important in how the immune system considers the self, increasing the dynamicity of potential responses (40). For example, a fetus is nonself and thus could potentially trigger an inflammatory response; however, these cells are tolerated by the maternal immune system owing to the flexible immune response associated with the reproductive state of pregnancy. Similarly, there may be different responses during the fertile window of the menstrual cycle versus nonfertile times. Finally, immune responsivity includes regulatory processes;

a stimulus can induce either a pro- or anti-inflammatory response. For example, glucocorticoids such as cortisol are thought to be anti-inflammatory; however, as predicted by the above theories, the anti-inflammatory response to glucocorticoids in one reproductive phase would be expected to differ from that experienced in other phases.

We conceptualized that sexual activity may suppress inflammation to reduce immune interference with conception. Sexual activity may provide a signal of potential to reproduce to the immune system, which responds by down-regulating inflammatory responses around ovulation. Liquid self theory would predict that the degree of immune response to sexual activity (to the extent that it occurs at all) would indicate something about how the immune system interprets that stimulus. One possibility is that the transition from sexual inactivity to activity would be interpreted as moving from one reproductive state to another. As long as a woman remains sexually active to some degree, the immune system continues to provide a stable degree of response. If so, we should expect *all* women who have been consistently sexually active to have similar patterns of immune response, regardless of sexual frequency. This would further suggest that the effect of sexual activity on immune response is tied to a discrete mechanism (such as changing the likelihood of ovulation). Another possibility is that each act of sexual activity is considered separately. Each act of intercourse would further amplify the immune system's interpretation of the reproductive state as potentially conceptive, increasing its flexibility across the menstrual cycle. In this case, we should expect different immune responses among women who are frequently versus infrequently sexually active. This would further suggest that the effect of sexual activity on immune response is graded, such as endocrine changes associated with each act of intercourse. In short, testing whether the frequency of sexual activity changes the degree of immune response (rather than simply sexual activity status) may shed light on how the immune system interprets sexual activity and possible mechanisms for these effects.

Taking together the findings on the association between sexual activity and immunity in women, and the changes in cytokines and CRP across the menstrual cycle, we hypothesized that, in a sample of women with confirmed ovulation, sexual activity would moderate cycle-related variations in inflammation. Specifically, women reporting a higher frequency of sexual activity should show greater midcycle decrease in proinflammatory cytokines and CRP as well as greater midcycle increase in anti-inflammatory cytokines. In contrast, abstinent women should show less change over the menstrual cycle in all markers of inflammation.

MATERIALS AND METHODS

Participants

Participants were recruited from the community via flyers and online advertisements and screened over the phone. General inclusion criteria included self-reported good health and regularly cycling (menses every 26–34 days with no more than one missed period in the past 6 months). General exclusion criteria included use of hormonal medications (including oral contraceptives), self-reported use of anti-inflammatory or immunoactive medications, current illness or allergies, history of medical conditions known to impact immune response (e.g., cancer), cigarette use, and pregnancy or lactation within the last 6 months. Currently sexually active participants were restricted to women reporting regularly engaging in intercourse at least once per week with a one and only one partner. Women taking hormonal contraception were excluded, and thus sexually active participants were included only if they reported either consistent condom use or nonhormonal intrauterine devices as contraception. As further confirmation, all participants completed a commercially available pregnancy test (OneStep hCG Test, BlueCross Biomedical) at intake and at the midstudy lab session; no woman was found to be pregnant at any point during the trial. Women who reported trying to conceive were excluded. Abstinent participants were restricted to participants reporting no partnered genital sexual activity in the last 4 months; however, individuals reporting lifetime sexual history were included. All participants provided informed consent, and study procedures were approved by the Indiana University Institutional Review Board.

Data Collection

General procedures. Participants (n = 32; sexually active, n = 15; abstinent, n = 17) were scheduled for two lab sessions, one within 2 days of menses onset and one within 2 days of ovulation (periovulatory). Ovulation was estimated from the participant's onset of menses and typical cycle length via backwards counting (41) and confirmed via commercially available urine test (OneStep Urine Ovulation Test, BlueCross Biomedical) performed 2 days before and 2 days after the expected date of ovulation. At both study visits, participants were measured for body composition with a floor scale (FitScale 585F, Tanita Corporation).

Blood sampling. Each woman provided two blood samples, at menses and at ovulation (i.e., during lab visits). Blood was collected via standard venipuncture techniques, drawing blood from the anterior cubital fossa (a vein in the inner elbow). Whole blood was collected into uncoated glass tubes, allowed to coagulate at room temperature for 45 minutes, and spun down. Serum was drawn and aliquoted into individual cyrovials and stored at −80°C until analysis. Serum was used for cytokine assays.

Saliva sampling. Each woman provided four saliva samples, at menses and during the follicular, ovulation, and luteal phases. The menses and ovulation samples were collected at lab visits. In addition, participants collected two saliva samples at home during their mid-late follicular phase (7–10 days after onset of menses) and luteal phase (7–10 days after ovulation). At-home samples were frozen in the participant's home freezer immediately after collection and transported frozen to the laboratory in Styrofoam boxes lined with ice packs (41). Saliva samples were collected via passive drool into a polypropylene tube (42). Participants were asked not to eat, drink, or chew gum 1 hour before collection. Like serum, saliva samples were stored at −80°C

until analysis; no sample was subjected to more than two freeze-thaw cycles. Saliva was used for CRP assay.

Survey data. In the first lab session, participants completed demographics questionnaires and reported on their sexual history including typical frequency of partnered sexual activity. At home, participants completed an online survey after each partnered sexual event. From these reports, frequency of intercourse events was tallied by phase, that is, within 7–10 days of the menses, follicular, ovulation, and luteal phase samples. No sexual event was tallied under multiple phases; activity reported on the cusp of phases was counted in the later phase. These data were used for analyses within the sexually active group only (see below).

Cytokine and CRP Assay

Cytokines. Unstimulated cytokine concentrations in serum were determined by ELISA using procedures recommended by kit manufacturers (Cytoset kits from Invitrogen Corporation). Intra-assay and interassay coefficients of variation were 2.1%–11.4% and 9.2%–18.5%, respectively. The lower limit of detection for assays was as follows: IFN-γ, 3.9 pg/mL; TNF-α, 1.7 pg/mL; IL-6, 2.0 pg/mL; IL-4, 2.0 pg/mL. As is typical for unstimulated samples in young, healthy individuals (43), there was a relatively high rate of undetectably low cytokine values: IFN-γ, 46%; TNF-α, 82%; IL-6, 67%; IL-4, 56%. Given that the rate of nondetection was high, and potentially related to the phenomenon of interest, we separately modeled predictors of whether or not the cytokine was detected (i.e., missingness analysis [44, 45]) and predictors of absolute values. This provided two types of information: first, the predictors of processes by which cytokines become stimulated (that is, detection vs. being too low to detect); and second, the predictors of processes that moderate or amplify cytokine response (that is, absolute levels of cytokine concentration).

CRP. CRP was determined from saliva. We were able to sample saliva at more time points than serum, giving us greater time resolution for our index of total inflammation. There is a strong association between serum and salivary CRP across multiple assay techniques (46–49). Salivary CRP reliably distinguishes healthy individuals from patients with inflammation conditions (50, 51) and between high and low serum CRP (52). Salivary CRP was determined by ELISA using procedures recommended by kit manufacturers (Salimetrics LLC). Intra-assay and interassay coefficients of variation were low (0.02%–14.9% and 13%–15%, respectively); the lower detection limit was 10 pg/mL. One CRP value was > 5 SD from the mean and excluded as an outlier.

RESULTS

Participant Characteristics

The majority of participants were white (68%), with 16% Asian and 16% mixed race or other. Participants were predominantly heterosexual (97%), with an average age of 23.57 (SD = 5.61) and an average of 15.74 years of education. The average body mass index and percent body fat were 23.61 (SD = 3.94) and 27.32% (SD = 7.78%), respectively. As both age and body composition are key predictors of inflammation generally (53) and CRP and proinflammatory cytokine expression specifically (54), we included age and percent body fat as covariates in the analyses below. However, groups did not differ on percent body fat [sexually active mean (M) = 27.64%, SD = 5.66; abstinent M = 26.02%, SD = 8.67, $t(30)$ = 0.61, P=.546] or age [sexually active M = 24.96, SD = 7.22; abstinent active M = 22.16, SD = 2.92, $t(30)$ = 1.47, P=.151]. Similarly, sexually active and abstinent participants were not significantly different in terms of race/ethnicity or sexual orientation (all P>.1).

Sexually active women were all in a relationship, while only three abstinent women reported a (nonsexual) dating partner; this difference was significant, $\chi^2(30) = 27.47$, P<.001. As relationship status was colinear with sexual activity status, we could not control for this difference between groups; this limitation is further considered in the Discussion. There was no significant difference in frequency of masturbation between abstinent (M = 1.65 times/week, SD = 1.57) and sexually active women (M = 1.80 times/week, SD = 2.43), $F(1,28) = 0.03$, P=.856.

Mean frequency of intercourse events reported on sexual event diaries was 6.67 times/cycle (range, 1–18). The effect of menstrual cycle phase on frequency of sexual events was nonsignificant [$F(1,3) = 0.08$, P=.96], indicating that women did not report more sexual events around ovulation. During recruitment, we screened out women who reported trying to conceive; however, sexual event diaries revealed that women who had reported regular condom use during screening did not always use condoms for every act of intercourse. Six participants reported using condoms on every sexual event diary that included intercourse, five reported no condom use, and four reported condom use on some but not all intercourse events. The effect of menstrual cycle phase on condom use was nonsignificant [$\chi^2(6) = 7.01$, P=.320], indicating women were not selectively using condoms only around ovulation. The present sample was underpowered to detect differences between condom users and nonusers. However, given the potential for exposure to ejaculate as one of the mechanisms by which women's immune system may respond to sexual activity, we conducted exploratory analyses of condom use as a predictor of change in inflammatory markers across the menstrual cycle; these analyses are presented in Supplemental Digital Content 1.

Change in Cytokines across Cycle

For each cytokine, we first conducted a generalized estimating equation model of detection, which characterized factors that predicted whether cytokine values were above or below the limit of detection. These analyses considered a binary outcome variable (detection vs. nondetection, coded as 0 and 1). We then conducted linear mixed models of cytokine values, which characterized factors that predicted the absolute levels of cytokines. These analyses considered a continuous outcome variable, with values below the limit of detection coded as missing. The number of data points for detection analyses were the same across models (namely,

64, corresponding to two time points in 32 participants); however, available data for continuous cytokine value analyses depended on how many values were detected (IL-6, 22 data points from 13 participants; IFN-γ, 35 data points from 22 participants; TNF-α, 12 data points from six participants; IL-4, 29 data points from 17 participants; CRP, 126 data points from 31 participants).

In both types of models (Table 1), we used time point (menses, ovulation) as a repeated-measures variable, group (sexually active vs. abstinent) and the interaction of time and group as predictors, and age and percent body fat as covariates. In both sets of models we specified an unstructured repeated-measures covariance. Estimates of effect sizes (ϕ and Cohen's local f^2) were calculated following standard recommendations (55, 56).

For models showing a significant effect of sexual activity status, we then conducted follow-up analyses to evaluate the effect of frequency of intercourse. Frequency of sexual intercourse was treated as a continuous variable. As all abstinent women would have an intercourse frequency of 0 (creating a heavily skewed distribution), we restricted frequency analyses to sexually active women only.

IL-6. There was a significant increase in the number of detectable IL-6 values from menses to ovulation [χ^2 (1) = 4.96, P=.03]. However, the main effect of time on continuous values of IL-6 was not significant: $F(1, 10.57) = 1.05$, P=.33.

There was a significant effect of group [χ^2 (1) = 5.63, P=.018] on detection such that sexually active women were significantly more likely to have detectable IL-6 values than

TABLE 1

Parameter estimates for analyses of detection and continuous levels of inflammation markers by cycle phase (time) and group (sexually active vs. abstinent).

	Detection[a]				Continuous values				
Variable	**B**	**SE (B)**	***P***	**φ**	**Effect estimate**	**SE**	***P***	**f^2**	**N_{data} (%)[h]**
IL-6									N = 22 (33.33)
Intercept	0.59	1.14	.60	0.04	963.65	501.88	.07	0.20	
Age	−0.03	0.03	.42	0.11	5.60	10.30	.60	0.02	
Body fat %	−0.01	0.02	.58	0.04	−24.12	13.71	.10	0.14	
Time[b]	0.59	0.29	.04	0.24	−321.41	230.57	.19	0.26	
Sexual activity group[c]	1.33	0.50	.01	0.35	−96.50	51.41	.09	0.14	
Time × Sexual activity group[d]	−0.33	0.38	.38	0.10	139.68	108.78	.23	0.14	
TNF-α									N = 12 (18.8)
Intercept	−0.14	1.67	.94	0.02	74.99	130.28	.57	0.16	
Age	−0.06	0.04	.15	0.20	7.07	3.42	.05	0.32	
Body fat %	0.07	0.04	.10	0.24	−3.47	3.02	.26	0.04	
Time[b]	0.10	0.11	.36	<0.01	−117.69	52.41	.04	0.21	
Sexual activity group[c]	1.49	0.75	.05	0.25	−14.93	18.57	.44	0.03	
Time × Sexual activity group[d]	−0.11	0.11	.35	0.02	45.15	25.74	.10	0.15	
IFN-γ									N = 35 (54.7)
Intercept	0.48	1.17	.68	0.06	74.99	130.28	.57	0.03	
Age	−0.03	0.03	.31	0.12	7.07	3.42	.05	0.21	
Body fat %	−0.02	0.03	.43	0.12	−3.47	3.02	.26	0.07	
Time[b]	0.97	0.39	.01	0.33	−117.69	52.41	.04	0.25	
Sexual activity group[c]	0.53	0.51	.29	0.12	−14.93	18.57	.44	0.04	
Time Sexual activity group[d]	−0.52	0.46	.26	0.16	45.15	25.74	.10	0.16	
IL-4									N = 29 (45.3)
Intercept	0.18	1.26	.89	0.01	−161.84	308.77	.61	0.01	
Age	0.00	0.04	.99	<0.01	31.35	8.54	.00	0.45	
Body fat %	−0.01	0.03	.65	0.05	−9.21	7.34	.22	0.07	
Time[b]	0.09	0.27	.73	<0.01	−222.50	119.40	.08	0.17	
Sexual activity group[c]	0.23	0.47	.62	0.06	18.93	29.81	.54	0.03	
Time × Sexual activity group[d]	0.37	0.36	.31	0.16	−40.74	46.75	.40	0.06	
CRP[e]									N = 126 (90)
Intercept					7.07	1.37	.00	0.40	
Age					0.01	0.02	.65	<0.01	
Body fat %					0.06	0.04	.22	0.05	
Time = menses[f]					0.25	0.14	.08	0.11	
Time = mid-late follicular					−0.06	0.11	.57	0.01	
Time = ovulation					0.04	0.17	.83	<0.01	
Sexual activity group					−0.56	0.58	.34	0.03	
Time = Menses × Sexual activity group[g]					−0.14	0.18	.44	0.02	
Time = Follicular × Sexual activity group					−0.01	0.14	.97	<0.01	
Time = Ovulation × Sexual activity group					−0.12	0.23	.61	0.01	

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abstinent women. Similarly, there was a significant effect of group on continuous IL-6 values [$F(2, 12.81) = 4.47$, $P=.034$], with sexually active women showing significantly higher IL-6 levels than abstinent women.

Follow-up analyses within the sexually active women revealed a significant interaction only between time and frequency of intercourse in predicting continuous IL-6 values [$F(1, 6.80) = 167.13$, $P<.001$]. Higher frequency of intercourse was associated with greater decreases in IL6 from menses to ovulation (see Fig. 1).

IFN-γ. There was a significant increase in the number of detectable IFN-γ values from menses to ovulation [χ^2 (1) = 9.50, $P=.002$]. However, the effect of time on IFN-γ values was not significant: $F(1, 14.27) = 0.002$, $P=.967$.

Sexual activity group did not predict detection of IFN-γ [χ^2 (1) = 9.50, $P=.29$]. It did predict continuous values of IFN-γ [$F(2, 19.23) = 4.82$, $P=.02$], with abstinent women showing significantly lower levels of IFN-γ than sexually active women.

Among the sexually active women only, follow-up analyses revealed a significant interaction between time and frequency of intercourse in predicting continuous levels of IFN-γ [$F(1, 1.89) = 29.99$, $P=.036$]. As with IL-6, higher frequency of intercourse was associated with greater decreases in IFN-γ from menses to ovulation (Fig. 1).

TNF-α. There was no significant change over time in either detection [χ^2 (1) = 0.72, $P=.40$] or continuous values of TNF-α [$F(1, 5.67) = 1.35$, $P=.29$]. The effect of sexual activity group on detection of TNF-α was marginally significant, χ^2 (1) = 3.79, $P=.052$, with sexually active women more likely to have detectable values than abstinent women. There was no significant effect of group on continuous TNF-α values: $F(2, 7.61) = 1.65$, $P=.253$.

IL-4. Finally, there was no significant main effect of time in detection of IL-4 [χ^2 (1) = 2.32, $P=.128$] or continuous values of IL-4 [$F(1,12.39) = 0.06$, $P=.804$].

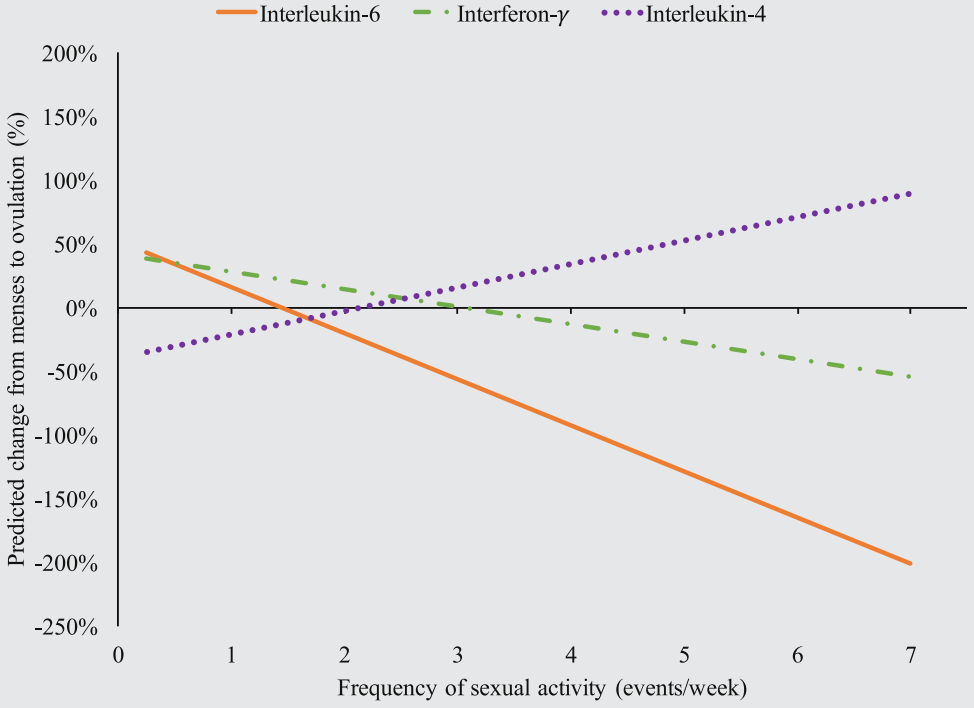
Sexual activity group did not significantly predict detection of IL-4 [χ^2 (1) = 0.95, $P=.330$]. The group did significantly predict continuous values of IL-4 [$F(2, 17.25) = 9.65$, $P=.002$], however, with sexually active women showing higher levels of IL-4 than abstinent women.

Follow-up analyses among sexually active women revealed a significant interaction only between time and frequency of intercourse: $F(1, 6.90) = 43.40$, $P<.001$. Higher frequency of intercourse was associated with greater increases in IL-4 from menses to ovulation (Fig. 1).

CRP

As there were few CRP values below the limit of detection, we did not conduct detection analyses for CRP. As there were four saliva samples per cycle (at menses, mid-late follicular, ovulation, and luteal phases), each woman had four CRP values. This meant that the effect of time could potentially be nonlinear;

FIGURE 1



Interaction between menstrual cycle phase and frequency of intercourse in predicting IL-6, IL-4, and IFN-γ. Predicted change controls for individual differences in age and body; positive % change indicates increase from menses to ovulation, while negative % change indicates decrease from menses to ovulation.

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indeed, given the above-cited research showing a curvilinear pattern of CRP across the menstrual cycle, we expected a nonlinear effect of time. To better model potentially nonlinear effects over time, we conducted a repeated-measures multivariate analysis of covariance (MANCOVA), with time and sexual activity group as predictors, controlling for age and body fat percent. The outcome variable, salivary CRP levels, was natural-log transformed to reduce nonnormality.

There was a significant interaction of the quadratic (i.e., curvilinear) effect of time and sexual activity group: $F(1, 26) = 8.41$, $P = .008$ (Fig. 2). Abstinent women had generally lower CRP values than did sexually active women. Also, there was little change in CRP across the menstrual cycle in abstinent women. In sexually active women, however, there was significant variability over time, with higher levels at menses and in the luteal phase and a nadir at midcycle. Finally, follow-up analyses among sexually active women only revealed no significant interaction between time and frequency of intercourse [$F(1, 12) = 3.38$, $P = .096$]. In other words, women who engaged in intercourse once a week had similar patterns of change in CRP as women who engaged in intercourse every day.

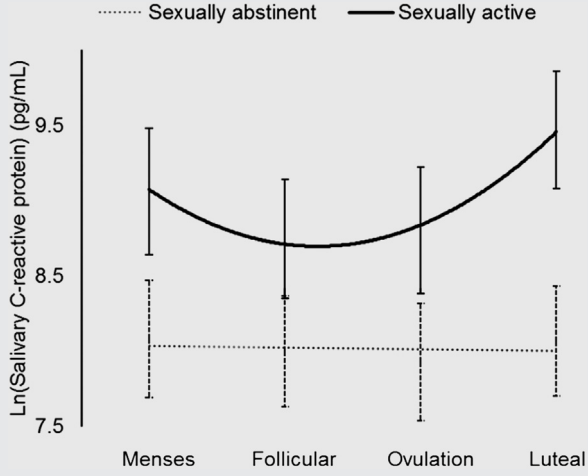
DISCUSSION

This was the first study to examine differences in menstrual cycle–related change in markers of inflammation between abstinent and sexually active women. In general, abstinent women had significantly lower levels of inflammation markers than did sexually active women. Among women who were sexually active, there was a significant interaction between cycle phase and frequency of intercourse, with higher frequency of intercourse associated with greater cycle-related change. That is, higher sexual frequency was associated with greater decreases in IL-6 and IFN-γ (proinflammatory cytokines) from menses to ovulation. Similarly, higher sexual frequency was associated with greater decreases in IL-4 (an anti-inflammatory cytokine). Finally, there was a significant group-wise difference in cycle-related change in CRP (a marker of total inflammation). Abstinent women had little change in CRP over time, but sexually active women showed a curvilinear pattern, with high CRP at early and late cycle and lowest CRP at ovulation. Taken together, these findings suggest that sexual activity may contribute to a complex interaction between pro- and anti-inflammatory cytokines, which ultimately direct the midcycle decline in inflammation in sexually active women.

Among sexually active women, there was an interaction between reproductive phase and frequency of sexual activity across four of the five measured markers of inflammation. The higher the frequency of intercourse, the greater the midcycle decline in proinflammatory markers (and midcycle increase in anti-inflammatory markers). Prior research in healthy women has shown that sexual activity is associated with lower levels of IgA (16, 19), IgE-mediated allergic responses (57), and Th1-dominant cytokine profiles (20). One common theme throughout these seemingly diverse immune factors is their potential detrimental effect on reproduction, via disruption of conception (as IgA may disrupt sperm transport and viability [58]), interference with establishment of the placenta (as when IgE is diverted from supporting the placenta towards allergic reactions [59]), or early termination of the pregnancy (as is the case for Th1 cells [60] particularly in the context of low Th2 cells [61]). Similarly, the presence of proinflammatory cytokines is associated with spontaneous abortion (62), even in the absence of overt infection or wound (63, 64). Thus, suppressing these proinflammatory cytokines could improve the chances of sexual activity leading to offspring. Intriguingly, the down-regulation of inflammation markers appeared only when they were necessary—that is, when a woman approached the fertile phase of the menstrual cycle and when she was sufficiently sexually active that conception could be likely. This timing likely reflects the immune system's ongoing evaluation of the balance between defensive and reproductive priorities: favoring defense during nonfertile times but favoring reproduction during fertile times.

Relative to sexually active women, abstinent women were less likely to have detectable levels of inflammation markers and lower levels of inflammation markers overall. It should be reiterated that all of these participants were healthy and thus the relatively higher levels of inflammation markers in sexually active women were not reflective of pathology or of significant injury or infection. Cytokines are signals; thus, the natural variation observed in these healthy women likely reflects differences in immune signaling and communication with other systems. Indeed, sexually active women had higher levels of both pro- and anti-inflammatory cytokines, further suggesting an increased need for immune system communication about inflammation rather than an increased need for inflammation per se. This principle is further echoed in the differential patterns of change across the menstrual cycle in sexually active versus abstinent women: while sexually active women had

FIGURE 2



Interaction between menstrual cycle phase and frequency of intercourse in predicting CRP. CRP values are estimated marginal means from analysis.

Lorenz. Sexual activity and inflammation. Fertil Steril 2016.

significant variation according to reproductive phase (fertile vs. nonfertile), abstinent women had less change. Taking all of these findings together, there is support for the hypothesis that in healthy women, the immune system uses information about sexual activity status to coordinate trade-offs with reproduction.

Females must commit considerable time and energy to reproduction, and there is risk associated with pregnancy and childbirth. In response, the female reproductive system has evolved to be highly attuned to environmental factors related to the current availability of physiologic and psychosocial resources. The propensity to balance trade-offs between reproduction and somatic growth and maintenance (including immune defense) across the lifespan has been noted in both humans and nonhumans (11, 65–69). Humans are notable for their relatively high level of investment of time and resources for each offspring and the diversity of social cues that appear to influence trade-offs in reproductive-somatic investment (11). It is possible that frequency of sexual activity is one such cue, eliciting the aspects of the immune response that promote reproduction by signaling that conditions are favorable. For example, more frequent sexual activity may indicate relationship stability and satisfaction (70), health of the woman and her partner (71), or even eagerness for reproduction (72), all of which could benefit offspring.

Of course, the selection pressure to develop different inflammation profiles in sexually active versus abstinent women would only be present if both states occurred in our evolutionary past. Indeed, there is evidence that hunter-gatherer societies may have used sexual abstinence as a means of population control (73–75). Similarly, some early societies systematically regulated the sexual availability of females after their husband's death or before marriage (76, 77). Still other societies (predominantly in African and India [73, 78–80]) practiced (and continue to practice) "terminal abstinence" in which a premenopausal woman commits to permanent sexual abstinence to balance family dynamics (e.g., to redirect her caretaking energies to grandchildren rather than new children of her own). Finally, scholars have suggested that sexual abstinence was particularly common among societies in which sexually transmitted diseases were prominent (81). In sum, while widespread abstinence among healthy reproductive-age females was probably rare, it likely did occur sufficiently often to have an effect on immunity.

Much more work is needed to elucidate the mechanisms by which sexual activity influences immune response. Given significant differences between sexually active and inactive women in E_2 and P_4 across the cycle (82), it is possible that endocrine factors play a role. Both E_2 and P_4 have immune effects, with the former thought to be generally anti-inflammatory (with notable exceptions [83]) and the latter thought to be generally proinflammatory, particularly within the female reproductive tract (84). Insofar as sexual activity changes E_2/P_4 profiles across the menstrual cycle, there should be associated changes in immune response (20), including inflammation. Another possible endocrine mediator is oxytocin, which is released during sexual activity (85–87). Oxytocin has a broad effect on inflammation, suppressing the inflammatory action of proinflammatory cytokines (88) and possibly increasing production of IL-4 (87). And of course, there are many other potential mechanisms, such as such as immune adaptation to repeated contact with a partner's microbiome (89), immune redistribution to combat sexually transmitted infections or other immune challenges associated with repeated contact with the intimate partner, activation of the autonomic nervous system during sexual arousal (90), or input from the central nervous system during sexual stimulation. It is very likely, given the complexity of the associations noted here, that no one of these mechanisms will fully explain the impact of sexual activity on immune function.

Limitations

This was an exploratory study in a small sample of healthy, regularly cycling women. Most notably, our continuous variable analyses were limited by high rates of nondetects in several of the cytokines measured. It is also possible that missingness influenced effect estimates for our continuous value models: simply put, missing data cannot contribute as much to effect estimates as nonmissing data. If an effect were significant in both detection and continuous value analyses, this would warrant suspicion as it would indicate that the significant effect in the continuous value analysis may be driven by nondetects. This condition applied in one case—the main effect of time on IFN-γ—and these findings should be thus treated with particular caution.

We were limited in the number of time points and cycles over which we could sample inflammation. Some work measuring cycle-related shifts in inflammation have found substantial within-woman variation across multiple cycles (4, 13, 91), which further supports the need to replicate these effects in larger samples over longer time periods. Our sample was relatively homogenous in terms of age, race/ethnicity, and parity; all three factors have strong effects on inflammation patterns (91–94). Finally, in the present study we excluded women taking hormonal medications and thus the generalizability to women using hormonal contraceptives (approximately 27.6% of women in the United States [95]) is unknown. More research extending these findings into the population of women taking hormonal medications is needed to shed light on both the generalizability and the possible endocrine mechanisms underlying the observed effects.

Future Directions

While the greatest difference between our sexually active and abstinent groups was the level of sexual activity, there may be other factors that covaried with sexual activity status; most notably, while all of the sexually active women were partnered, only a few of the abstinent women were partnered. Research on mechanisms is needed to evaluate whether and how sexual activity (vs. other factors such as partnership) influences women's immune patterns. For example, relative to women who do not live with an intimate partner, women who do live with an intimate partner tend to eat a more anti-inflammatory diet with more vegetables and less meat

(96). While partnership is unlikely to fully explain our findings regarding sexual frequency, it is possible that partnership moderates the effect of sexual activity on inflammation. Further study of the effect of sexual activity in women in different kinds of relationships, and/or who have sex with multiple partners, is warranted. It would be valuable to examine the effect of sexual activity within the same woman over time—for example, in an experimental manipulation of sexual activity from cycle to cycle. It would also be valuable to know the timing and duration of the effect—that is, how long after sexual activity do these immune effects peak? Finally, life history theory suggests that we may expect different immune effects among nulliparous and multiparous women and between younger and older (but still premenopausal) women. These groups would be subject to different evolutionary selection pressures and thus potentially different thresholds for the trade-offs between reproduction and somatic maintenance (66, 97).

Clinical Implications

Our findings are consistent with prior literature documenting similar patterns in markers of inflammation (4, 5, 7, 8). Such variability in inflammation in healthy women has important clinical implications, as markers of inflammation are often used in prognosis of risk for cardiovascular disease (CVD) and other inflammation-related conditions (98). In our sample, women had the lowest levels of inflammation markers at midcycle, particularly if they were sexually active. In other words, there is a significant chance a sexually active woman will be differently classified (and possibly misclassified) if she presents at midcycle (around ovulation) versus other points in her cycle. These findings suggest that in sexually active women of reproductive age, there is limited prognostic value of a single measurement of CRP (such as is typically conducted in epidemiologic studies). Clinicians and researchers using biomarkers of CVD risk may find it useful to take multiple measurements across the cycle or, if this is not feasible, schedule measurements at midcycle (if possible, around the time ovulation is detected) when CRP can be expected to be at a minimum for both sexually active and sexually abstinent women.

Given the prognostic value of CRP and other inflammation markers, much research has investigated sources of intraindividual variations in the measurement of these markers (99). Sometimes overlooked in the search for stability across measurements, however, is the potential prognostic value of variability. Although some researchers have suggested that extreme variability of CRP is indicative of pathology (100), moderate variation in response to cues from the social or physical environment—such as reproductive activity—may represent adaptability and as such the potential for good health. Further research is needed to examine whether cycle-related changes in biomarkers of inflammation, corresponding to responsivity of the immune system to environmental demands, are associated with other markers of health.

Conclusions

We observed a significant midcycle decrease in inflammatory markers in sexually active but not in abstinent women. In addition, frequency of partnered genital sexual activity moderated this effect such that women reporting more frequent sexual activity demonstrated greater midcycle decreases in inflammation markers than women reporting less frequent sexual activity. The present study was limited in sample size and restricted to young, primarily white women, regularly cycling and not taking hormonal medications; thus, further research is needed to replicate and extend these findings to other populations. While several studies have previously documented menstrual cycle–related variations in inflammation, this is the first to examine the effect of sexual activity on these patterns. Our findings call for critical evaluation of inflammation biomarkers in clinical care of reproductive-age women and offer a new avenue for research on the intersection of immune health, reproduction, and sexual behaviors in women.

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SUPPLEMENTAL ELECTRONIC MATERIAL 1. CONDOM USE ANALYSES

METHODS

In the analyses below, we considered the rate of condom use during sexual activity as a predictor of menstrual variations in inflammatory markers among sexually active women only. Using the sexual event diaries, we characterized the rate of condom use as a continuous variable ranging from 0% (condom use during intercourse reported on no sexual event diary) to 100% (condom use during intercourse reported on all sexual event diaries). The average condom use rate was 44% (SD = 46%).

As with the main analyses, we conducted generalized estimating equation models of detection/nondetection followed by linear mixed models of continuous cytokine concentrations and CRP concentration. In all models, we used time point (menses, ovulation) as the repeated-measures variable, rate of condom use and the interaction of time and condom use as predictors, and age and percent body fat as covariates.

RESULTS

Results are presented in Supplemental Table 1. For TNF, IFN, and IL-4, there were no significant effects of condom use or interactions between condom use and time. For IL-6, there was no effect of condom use on likelihood of detection, but there was evidence of a significant interaction between condom use and time [$F(1, 7.53) = 27.19$, $P=.001$; Supplemental Fig. 1]. Women who reported using condoms at every sexual event had a significant increase in serum IL-6 from menses to ovulation (mean difference = 315.27 pg/mL, SE = 44.43 pg/mL, $P<.001$). In contrast, there was no cycle-related change in IL-6 among women who reported no condom use (mean difference = 24.19, SE = 39.89, $P=.56$).

There was evidence of an interaction between condom use and time for CRP concentrations [$F(3, 34.99) = 3.404$, $P=.028$, Fig. 12–1b]. Again, women who reported no condom use showed no significant variation in CRP across the cycle. In contrast women who reported condoms at every sexual event had a U-shaped curve in CRP across the cycle, with a significant decrease in salivary CRP from menses to the follicular phase (mean difference = −2.10 pg/mL, SE = 1.22 pg/mL, $P=.001$), which was sustained at ovulation, followed by a significant increase in CRP during the luteal phase (mean difference = 2.70 mg/L, SE = 1.49 mg/L, $P=.017$).

DISCUSSION

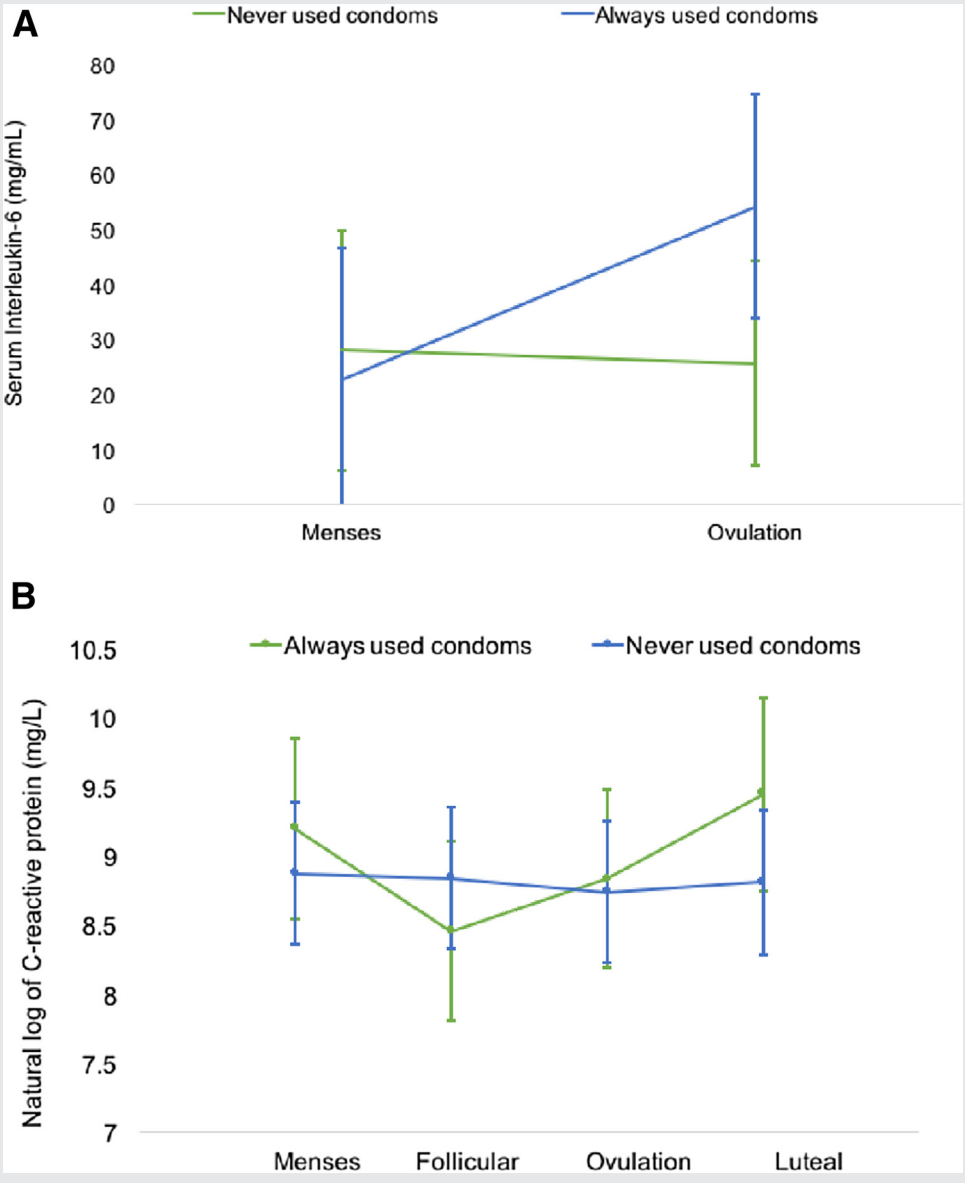
It is important not to overinterpret these exploratory analyses. The sample sizes of each subgroup were very small (condom users, n = 6; noncondom users, n = 5; inconsistent condom users, n = 4), and condom use was not randomly assigned, making it impossible to control for the diversity of factors that lead to condom use versus nonuse. It is possible that, owing to the small subsamples, we did not detect true differences between groups. Post hoc power analyses suggest that we would have missed any effect of $d < .6$, which includes moderate or small effects that still may be of clinical or theoretic interest.

Nevertheless, two interesting patterns emerged that may serve as a starting place for further study. First, when a difference emerged between immune markers in condom users and nonusers, it was typically the condom users who showed change across the menstrual cycle, while nonusers showed stability. This suggests that the influence of condom use on healthy women's immune function may not be limited to exposure to the partner's penis and (potentially) ejaculate; if this were so, we would expect any cycle-related immune variations to have occurred in the non–condom user group. Other factors, such as exposure to the condom itself, or relationship factors leading to condom use as the primary form of contraception, may play a role in immune variations across the menstrual cycle.

Second, there were differences in the patterns of inflammation markers as measured from the general circulation (serum IL-6) versus mucosa (salivary CRP), with the former increasing at ovulation and the latter decreasing. This suggests condom use may influence humoral and mucosal immunity via differential mechanisms, leading to ultimately different levels of inflammation for different sites. It is also possible that decreases in inflammation at one site (e.g., in saliva) correspond to increased recruitment of inflammation mediators to another site (e.g., in blood) rather than a suppression of inflammation per se.

Further study of menstrual variations of inflammation that experimentally manipulates condom use, using a more diverse and larger sample of participants, is warranted to determine the replicability of these results and elucidate the mechanisms behind these patterns.

SUPPLEMENTAL FIGURE 1



(**A**) Changes in IL-6 concentrations across the menstrual cycle in condom users versus nonusers. (**B**) Changes in CRP concentrations across the menstrual cycle in condom users versus nonusers.

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SUPPLEMENTAL TABLE 1

Results of detection and mixed model analyses.

	Detection			Absolute value		
Variable	**B**	***SE* B**	***P***	**Effect estimate**	***SE***	***P***
IFN-γ						
Intercept	3.135	2.431	.197	626.502	190.792	.010
Age	−0.008	0.033	.798	−2.246	3.790	.574
Body fat %	−0.129	0.086	.134	−16.073	5.457	.042
Time[a]	−1.800	2.492	.470	1.169	1.405	.461
Rate of condom use	−0.012	0.008	.154	3.714	26.565	.894
Time × Condom use[b]	0.113	0.095	.235	−0.332	0.403	.443
TNF-α						
Intercept	−0.784	2.213	.723	1,015.309	322.215	.018
Age	−0.037	0.041	.370	4.194	3.310	.258
Body fat %	0.080	0.065	.215	−40.110	15.541	.039
Time[a]	0.016	0.049	.741	3.523	0.917	.004
Rate of condom use	−0.003	0.007	.657	11.639	56.774	.848
Time × Condom use[b]	0.000	0.001	.290	0.796	0.986	.465
IL-6						
Intercept	2.355	1.826	.197	111.438	471.775	.818
Age	−0.022	0.035	.535	37.788	8.698	.004
Body fat %	−0.080	0.046	.082	−27.052	14.472	.095
Time[a]	0.580	0.388	.135	2.861	3.036	.385
Rate of Condom use	−0.004	0.009	.681	24.188	39.887	.562
Time × Condom use[b]	0.002	0.008	.835	−3.395	0.651	.001
IL-4						
Intercept	626.502	190.792	.010	94.429	558.213	.869
Age	−2.246	3.790	.574	30.108	11.214	.027
Body fat %	−16.073	5.457	.042	−19.251	17.948	.309
Time[a]	3.714	26.565	.894	1.642	2.403	.513
Rate of Condom use	1.169	1.405	.461	53.541	52.429	.337
Time × Condom use[b]	−0.332	0.403	.443	−0.829	0.843	.352
CRP[c]						
Intercept				11.258	2.053	.000
Age				0.025	0.047	.607
Body fat %				−0.109	0.058	.075
Time = Menses[d]				0.062	0.298	.835
Time = Follicular				0.030	0.259	.907
Time = Ovulation				−0.070	0.199	.727
Rate of condom use				0.006	0.009	.509
Time = Menses × Condom use[e]				−0.003	0.006	.591
Time = Follicular × Condom use				−0.010	0.005	.053
Time = Ovulation × Condom use				−0.005	0.004	.215

[a] Parameter estimate for T1 (menses); estimate for T2 (ovulation) is set to zero as parameter is redundant.
[b] Indicates parameter estimate for T1 (menses) * condom rate; estimate for T2 (ovulation) * condom rate is set to zero as the parameter is redundant.
[c] No detection analyses were conducted for CRP values.
[d] Estimate for T4 (luteal) is set to zero as the parameter is redundant.
[e] Estimate for T4 (luteal) * condom rate is set to zero as the parameter is redundant.

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