Supplementary 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/non-detection followed by linear mixed models of continuous cytokine concentrations, and C-reactive protein 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 Table A-1. For TNF, IFN, and IL4, there were no significant effects of condom use nor 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 ($F(1, 7.53) = 27.19$, $p = 0.001$; Figure A2-1a). Women who reported using condoms at every sexual event had a significant increase in serum IL6 from menses to ovulation (Mean difference = 315.27 pg/mL, *SE* = 44.43 pg/mL, $p < 0.001$). In contrast, there was no cycle-related change in IL6 among women who reported no condom use (Mean difference = 24.19, *SE* = 39.89, $p = 0.56$).

There was evidence of an interaction between condom use and time for CRP concentrations ($F(3, 34.99) = 3.404$, $p = 0.028$, Figure 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, $p = 0.001$) 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, $p = 0.017$).

***Discussion*:** It is important not to over-interpret these exploratory analyses. The sample sizes of each sub-group were very small (condom users, $N = 6$; non-condom 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 vs. non-use. It is possible that, due 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. Firstly, when a difference emerged between immune markers in condom users and non-users, it was typically the condom *users* who showed change across the menstrual cycle while non-users 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 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.

Secondly, there were differences in the patterns of inflammation markers as measured from the general circulation (serum IL6) vs. 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.

Table A-1. Results of detection and mixed model analyses.

	Detection			Absolute value		
	B	*SE* B	*p*	Effect estimate	*SE*	*p*
IFN-γ						
Intercept	3.135	2.431	0.197	626.502	190.792	0.010
Age	-0.008	0.033	0.798	-2.246	3.790	0.574
Body fat %	-0.129	0.086	0.134	-16.073	5.457	0.042
Time [a]	-1.800	2.492	0.470	1.169	1.405	0.461
Rate of condom use	-0.012	0.008	0.154	3.714	26.565	0.894
Time x condom use[b]	0.113	0.095	0.235	-0.332	0.403	0.443
TNF-α						
Intercept	-0.784	2.213	0.723	1015.309	322.215	0.018
Age	-0.037	0.041	0.370	4.194	3.310	0.258
Body fat %	0.080	0.065	0.215	-40.110	15.541	0.039
Time [a]	0.016	0.049	0.741	3.523	0.917	0.004
Rate of condom use	-0.003	0.007	0.657	11.639	56.774	0.848
Time x condom use [b]	0.000	0.001	0.290	0.796	0.986	0.465
IL-6						
Intercept	2.355	1.826	0.197	111.438	471.775	0.818
Age	-0.022	0.035	0.535	37.788	8.698	0.004
Body fat %	-0.080	0.046	0.082	-27.052	14.472	0.095
Time [a]	0.580	0.388	0.135	2.861	3.036	0.385
Rate of condom use	-0.004	0.009	0.681	24.188	39.887	0.562
Time x condom use [b]	0.002	0.008	0.835	-3.395	0.651	0.001
IL-4						
Intercept	626.502	190.792	0.010	94.429	558.213	0.869
Age	-2.246	3.790	0.574	30.108	11.214	0.027
Body fat %	-16.073	5.457	0.042	-19.251	17.948	0.309
Time [a]	3.714	26.565	0.894	1.642	2.403	0.513
Rate of condom use	1.169	1.405	0.461	53.541	52.429	0.337
Time x condom use [b]	-0.332	0.403	0.443	-0.829	0.843	0.352
C-reactive protein [c]						
Intercept				11.258	2.053	0.000
Age				0.025	0.047	0.607
Body fat %				-0.109	0.058	0.075
Time = menses [d]				0.062	0.298	0.835
Time = follicular				0.030	0.259	0.907
Time = ovulation				-0.070	0.199	0.727
Rate of condom use				0.006	0.009	0.509
Time = menses x condom use [e]				-0.003	0.006	0.591
Time = follicular x condom use				-0.010	0.005	0.053
Time = ovulation x condom use				-0.005	0.004	0.215

[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 C-reactive protein 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

Figure Caption for Supplementary Materials.

Figure A-1a. Changes in IL-6 concentrations across the menstrual cycle in condom users vs. non-users.

Figure A-1b. Changes in CRP concentrations across the menstrual cycle in condom users vs. non-users.

Figure A-1a.

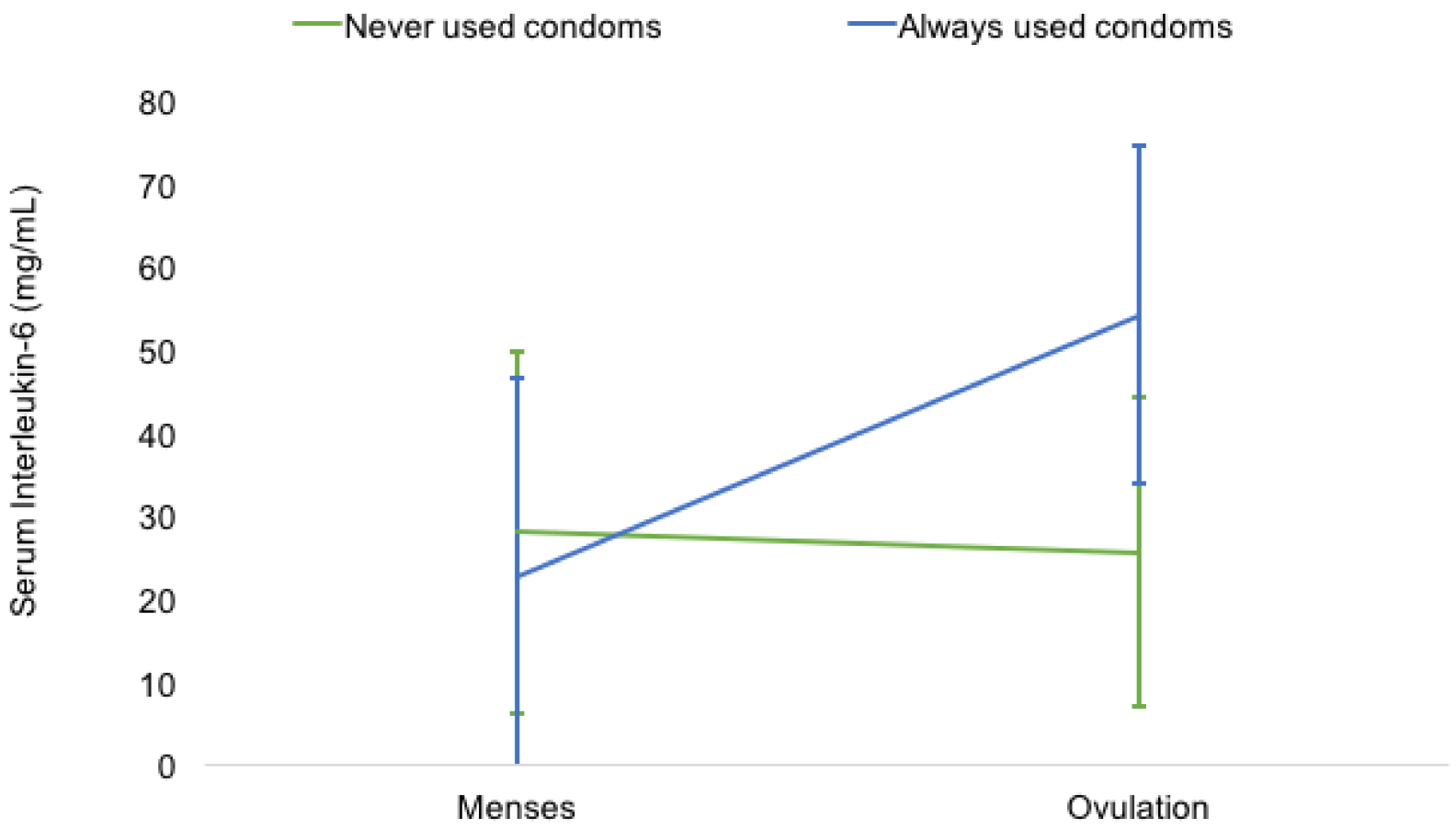
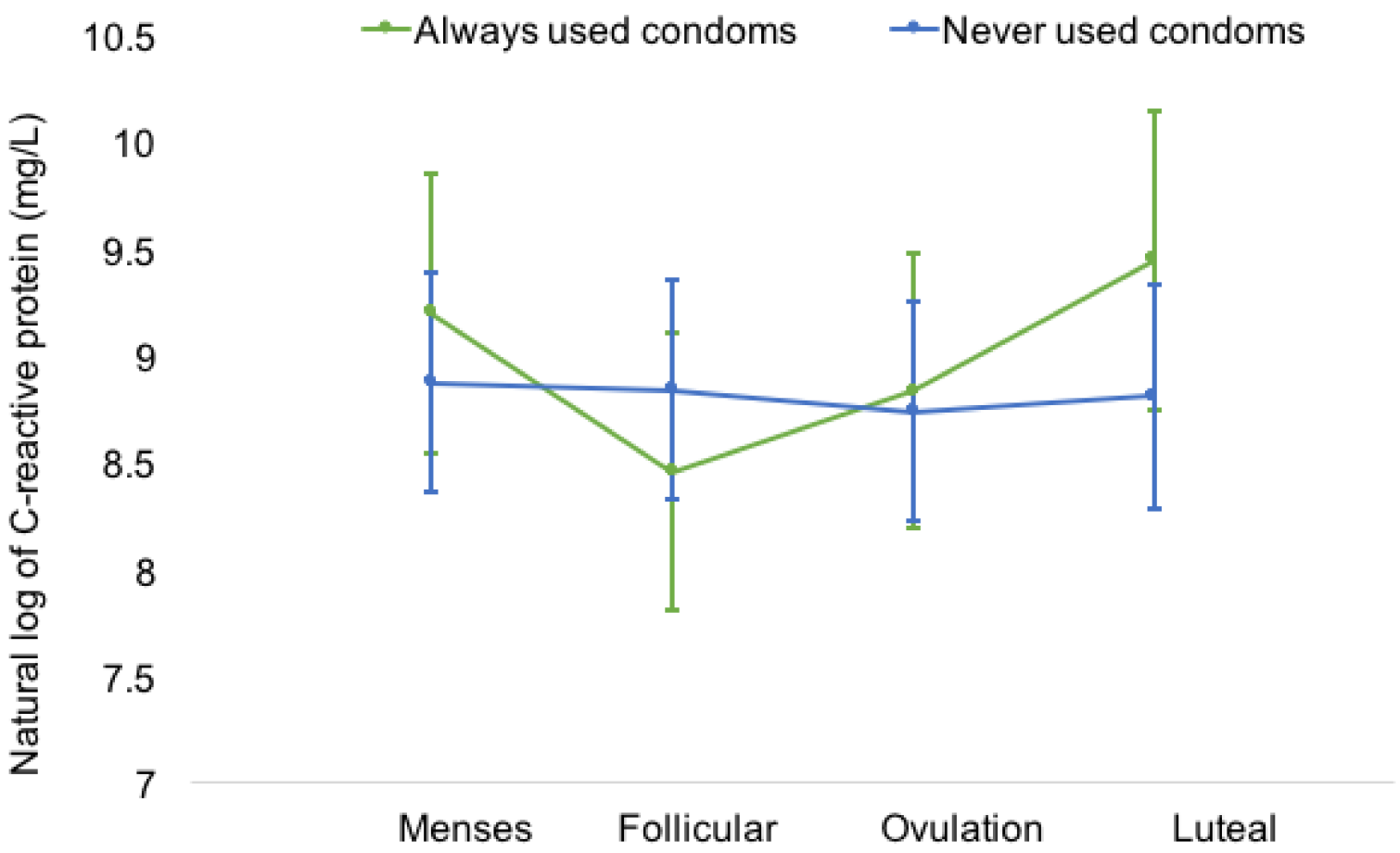

Never used condoms
Always used condoms
Serum Interleukin-6 (mg/mL)
80
70
60
50
40
30
20
10
0
Menses
Ovulation

Figure A-1b.


Always used condoms
Never used condoms
Natural log of C-reactive protein (mg/L)
10.5
10
9.5
9
8.5
8
7.5
7
Menses
Follicular
Ovulation
Luteal