

Alcohol intake, reproductive hormones, and menstrual cycle function: a prospective cohort study^{1,2}

Karen C Schliep,^{3,7} Shvetha M Zarek,^{3,4} Enrique F Schisterman,³ Jean Wactawski-Wende,⁵ Maurizio Trevisan,⁶ Lindsey A Sjaarda,³ Neil J Perkins,³ and Sunni L Mumford^{3*}

³Epidemiology Branch, Division of Intramural Population Health Research and ⁴Program of Reproductive and Adult Endocrinology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Department of Health and Human Services, Bethesda, MD; ⁵Department of Epidemiology and Environmental Health, University at Buffalo, Buffalo, NY; and ⁶Sophie Davis School of Biomedical Education, City College of New York, New York, NY

ABSTRACT

Background: Although habitual low-to-moderate alcohol intake has been linked with reduced all-cause mortality and morbidity, the effect of recent alcohol intake on female reproductive function has not been clearly established.

Objective: We assessed the relation between acute alcohol consumption, reproductive hormones, and markers of menstrual cycle dysfunction including sporadic anovulation, irregular cycle length, luteal phase deficiency, long menses, and heavy blood loss.

Design: A total of 259 healthy, premenopausal women from Western New York were followed for ≤ 2 menstrual cycles (2005–2007) and provided fasting blood specimens during ≤ 8 visits/cycle and four 24-h dietary recalls/cycle. Linear mixed models were used to estimate associations between previous day's alcohol intake and hormone concentrations, whereas Poisson regression was used to assess RR of cycle-average alcohol intake and menstrual cycle function.

Results: For every alcoholic drink consumed, the geometric mean total and free estradiol, total and free testosterone, and luteinizing hormone were higher by 5.26% (95% CI: 1.27%, 9.41%), 5.82% (95% CI: 1.81%, 9.99%), 1.56% (95% CI: 0.23%, 2.90%), 1.42% (95% CI: 0.02%, 2.84%), and 6.18% (95% CI: 2.02%, 10.52%), respectively, after adjustment for age, race, percentage of body fat, perceived stress, pain-medication use, sexual activity, caffeine, and sleep. Binge compared with nonbinge drinking (defined as reporting ≥ 4 compared with < 4 drinks/d, respectively) was associated with 64.35% (95% CI: 18.09%, 128.71%) and 63.53% (95% CI: 17.41%, 127.73%) higher total and free estradiol. No statistically significant associations were shown between cycle-average alcohol intake and menstrual cycle function.

Conclusion: Although recent moderate alcohol intake does not appear to have adverse short-term effects on menstrual cycle function, including sporadic anovulation, potential protective and deleterious long-term effects of alterations in reproductive hormones on other chronic diseases warrant additional investigation. *Am J Clin Nutr* 2015;102:933–42.

Keywords: alcohol, estradiol, menstrual cycle, ovulation, progesterone

INTRODUCTION

Alcohol intake is common in US women of reproductive age with over one-half of women aged 18–44 y consuming alcohol

(1). Although epidemiologic evidence has indicated that habitual moderate alcohol intake (1–2 drinks/d) is associated with reduced all-cause mortality and morbidity (2, 3), the effect of alcohol intake on female reproductive function has not been clearly established (4–19). It is challenging to study the effects of alcohol on female reproductive function because of the complex time-dependent hormonal feedback loops that regulate the menstrual cycle and women's fertility coupled with alcohol's relatively rapid metabolism (20), which clear the body of alcohol in 12–24 h (21). Because of potential sensitivity of menstrual cycle functions to critical windows of exposure, studies are needed that take into account recent alcohol intake relative to the outcome being assessed, such as ovulation, conception, and implantation.

Although there have been large observational studies that evaluated chronic alcohol exposure and menstrual cycle function (15, 16, 19), studies that evaluated acute alcohol intake and menstrual cycle function have been limited to experimental studies that assessed alcohol-mediated changes in reproductive hormone concentrations in nonpregnant women (22–25). The majority of these controlled studies showed relevant positive associations between acute, high alcohol (≥ 2 drinks/d) administration and elevated estradiol and testosterone concentrations, which are believed to be the result of potentially several mechanistic pathways including changes in hepatic redox states (22–25), the stimulation of the adrenal gland (26, 27), and the aromatization of testosterone to estradiol (28). Although these studies have helped to inform mechanistic pathways, they have limited generalizability because of small sample sizes, high alcohol exposures, and experimental conditions. In addition, most of these studies were limited to a single

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²Supplemental Tables 1–3 are available from the “Supplemental data” link in the online posting of the article and from the same link in the online table of contents at <http://ajcn.nutrition.org>.

⁷Present address: Division of Public Health, Department of Family and Preventive Medicine, University of Utah, Salt Lake City, UT.

*To whom correspondence should be addressed. E-mail: mumfords@mail.nih.gov.

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day's hormone assessment (24, 25), and none of the studies evaluated the effects of alcohol intake on clinical markers of menstrual cycle dysfunction such as ovulatory disorders, irregular cycle length, or abnormal bleeding patterns (22–25).

Because of the lack of population-based research in women in their natural settings, we sought to prospectively investigate the relation between acute alcohol consumption, reproductive hormones, and markers of menstrual cycle dysfunction in a healthy cohort of reproductive aged women with the use of old-standard assessments of alcohol quantity and type and biomarkers of reproductive function.

METHODS

Study population

Women aged 18–44 y who were regularly menstruating and from the Western New York region ($n = 259$) enrolled in the BioCycle Study (2005–2007) and were followed for ≤ 2 complete menstrual cycles (250 subjects completed 2 cycles; 9 subjects completed one cycle) (29). The study size was based on the power to detect differences in oxidative stress with endogenous reproductive hormone concentrations and antioxidants, which was the primary study outcome (29). To be eligible to participate, women could not have used oral contraceptives during the preceding 3 mo, been currently pregnant, or be actively trying to conceive during the next 3 mo. Only women with self-reported BMI (in kg/m^2) between 18 and 35 at screening, a cycle length between 21 and 35 d for each menstrual cycle for the past 6 mo, and no history of gynecologic problems (including endocrinologic disorders or known infertility) or chronic disease were included. The University at Buffalo Health Sciences Institutional Review Board approved the study and served as the institutional review board designated by the NIH for this study under a reliance agreement. All participants provided written informed consent.

Of 449 women who were screened, 318 women met the eligibility criteria, of whom 276 women enrolled. Seventeen women (6%) withdrew before completing the study. Women who withdrew were not markedly different from women who completed the study although a greater percentage of Asians (19%) than of blacks (6%) or whites (3%) withdrew. Reasons for withdrawal included scheduling conflicts ($n = 8$), loss to follow-up ($n = 4$), inability to tolerate blood draws ($n = 3$), inability to participate because of an illness ($n = 1$), or a loss of interest ($n = 1$) (29).

Hormone assessment

Women provided fasting serum samples at ≤ 8 clinic visits/cycle, with visit timing assisted with the use of fertility monitors (Clearblue Easy Fertility Monitor; Inverness Medical) to correspond to menstruation, midfollicular, late follicular, luteinizing hormone (LH)⁸ and follicle-stimulating hormone (FSH) surge, ovulation, and early luteal, midluteal, and late-luteal phases (30). A total of 94% of the participants completed 7–8 clinic visits/cycle. Total estradiol, progesterone, LH, FSH, and sex hormone binding globulin (SHBG) were measured with the use of

a solid-phase competitive chemiluminescent enzymatic immunoassay on the DPC IMMULITE 2000 analyzer (Siemens Medical Solutions Diagnostics) at Kaleida Laboratories.

Total testosterone was measured with the use of liquid chromatography–tandem mass spectrometry with the use of the Shimadzu Prominence Liquid Chromatogram (Shimadzu Scientific Instruments Inc.) with an ABSciex 5500 tandem mass spectrometer (AB SCIEX) at the Advanced Research and Diagnostics Laboratory. The calculation of free (i.e., bioavailable) estradiol and testosterone was performed via standardized methods (31, 32). Across the study period, CVs for these tests were as follows: $<14\%$ for progesterone; $<10\%$ for estradiol, testosterone, and SHBG; and $<5\%$ for LH and FSH.

Menstrual cycle–function assessment

Participants maintained a daily diary for 2 complete menstrual cycles in which they marked whether they experienced any menstrual bleeding (yes or no). Study personnel reviewed and recorded the diary data at each clinic visit.

Menses length was defined as the number of days of a bleeding episode that included ≥ 2 d of bleeding in a 3-d interval preceded by ≥ 2 bleed-free days (33). If participants reported any menstrual bleeding on a given day, they were instructed to complete a detailed follow-up menstrual flow questionnaire that consisted of pictograms that assessed the quantity, size, and observed amount of blood loss for each feminine product used as well as any extraneous blood loss that was not captured by sanitary protection (33). The total blood loss on each bleeding day was estimated with the use of an algorithm (34).

The prospectively collected cycle length (number of days between menstrual bleeding) was observed for ≤ 2 cycles. Day 1 of the cycle was defined as menstruating by 1600 on that day. The day of ovulation in ovulatory women was assigned on the basis of dates and concentrations of the LH peak with the use of the fertility monitor compared with the observed LH maximum value in serum and the first day of progesterone rise. The duration of the follicular phase was calculated as the number of days between the first day of menstrual bleeding and the day of ovulation; and the luteal length was calculated as the day after ovulation through the day before the onset of menses.

To complement previous research, we defined short cycles as ≤ 25 d, long cycles as ≥ 33 d, and normal cycles as 26–32 d (35). Luteal phase deficiency was defined as a luteal phase length <10 d as previously described (36). Long menses were defined as >7 d, and the total cycle blood flow was classified in tertiles as light (≤ 36.5 mL), medium (>36.5 and ≤ 72.5 mL), or heavy (>72.5 mL). We defined anovulation as any cycle with a peak progesterone concentration <5 ng/mL and no observed serum LH peak on the midluteal or late-luteal phase visits ($n = 42$ of 509 cycles; 8.3%) (37).

Alcohol assessment

We measured alcohol consumption during the 2 observed menstrual cycles with the use of the 24-h dietary recall (24HDR). Participants completed an interview-assisted 24HDR at the clinic after the collection of a fasting blood specimens during clinic visits that corresponding to menstruation, the midfollicular phase, the estimated day of ovulation, and the midluteal phase for a total of up

⁸ Abbreviations used: FSH, follicle-stimulating hormone; LH, luteinizing hormone; LPD, luteal phase deficiency; SHBG, sex hormone binding globulin; 24HDR, 24-h dietary recall.

to eight 24HDRs. Trained dietary interviewers, with the use of open-ended questions and visual amount-estimation tools (taking into account ice in beverages and the amount unconsumed), captured food and beverage quantities consumed. Alcohol (g/d) and beer, wine, distilled liquor, and spirits (i.e., cordial or liqueur) (all expressed in drinks/d) were calculated for each dietary assessment with the use of the Nutrition Data System for Research (version 2005; Nutrition Coordinating Center) which took into account the beverage type, brand (when necessary), and serving size in the calculation of total alcohol intake (38, 39).

Alcohol intake was also assessed via the daily diary whereby women recorded the number of alcoholic drinks they consumed each day (with one drink noted as equivalent to one can of beer, one glass of wine, or one shot of liquor). Women were to record none if no alcohol was consumed.

Covariate assessment

Age, race, depression (Center for Epidemiologic Studies Depression Scale), and reproductive history were obtained at baseline with the use of standard questionnaires (29). At the end of the follow-up period, the total percentage of body fat was measured with the use of dual energy X-ray absorptiometry (Hologic Discovery Elite, software version 12.4.1; Hologic). Total energy intake (kcal/d), fiber (g/d), and the alternate Mediterranean diet score (40) were calculated from the 24HDR. Daily minutes of vigorous exercise, cigarette use (total cigarettes smoked, noting zero if none), caffeinated coffee intake (total number of cups, noting zero if none), sexual activity (defined as vaginal intercourse), sleep duration (recorded as total numbers of hours and minutes of the previous night's sleep plus any nap time for the current day), perceived stress [not stressful (one), a little stressful (2), or very stressful (3)], and any pain-medication use (including aspirin, naproxen, ibuprofen, and acetaminophen) were captured via the daily diary.

Statistical analyses

Alcohol intake as reported in the 24HDR was allowed to vary across the cycle and was assessed both categorically [nonconsumer and 0–0.5 drinks/d, >0.5–1.0 drink/d, and >1.0 drink/d (14 g alcohol = 1 drink)] and continuously (drinks/d). In addition, we evaluated the effect of recent binge drinking by categorizing women as binge drinkers if they consumed ≥ 4 drinks/d on the day before the clinic visit as per their clinic-visit 24HDR. Although the 24HDR was considered our gold-standard method of the previous day's alcohol assessment, we also evaluated the previous day's daily diary record of alcohol intake and report of binge drinking in association with menstrual cycle function for comparison purposes. To capture the previous week's rather than the previous day's alcohol intake and binge-drinking episodes in relation to reproductive hormones, we further evaluated average alcoholic drink intake as recorded in the daily diary for the previous 7 d and the reporting of consumption of ≥ 4 alcoholic drinks/d in the daily diary on any of the 7 d before the clinic visit.

The variation in reported alcohol intake, both for the 24HDR and the daily diary, across the menstrual cycle was assessed with the use of linear mixed models to account for repeated measures within women (both across the cycle and between cycles). Pairwise comparisons were made between reported intake at menses,

the follicular phase, expected ovulation, and in the luteal phase of the menstrual cycle with the use of the Tukey's method to account for multiple comparisons.

Demographic, lifestyle, and dietary characteristics of participants were compared between categories of alcohol intake [nonconsumer and 0–0.5 drinks/d, >0.5–1.0 drink/d, and >1.0 drink/d (14 g alcohol = 1 drink)] averaged over the study period with the use of an ANOVA for normally distributed continuous variables and the Wilcoxon-Mann-Whitney test for nonnormally distributed continuous variables. Chi-square or Fisher exact tests, when appropriate, were used for categorical variables. Geometric mean (95% CI) reproductive hormone concentrations across the 2 menstrual cycles by study-average alcohol intake were also calculated. We modeled the relation between time-varying categorical and continuous total alcohol and alcoholic beverage intakes (for both the 24HDR and daily diary reported values) and log hormone concentrations with the use of linear mixed models with random intercepts. Although we were able to assess the previous day's daily diary-reported alcohol intake for each clinic visit, for the 4 clinic visits without 24HDRs, we imputed the nearest 24HDR reported alcohol intake value (i.e., for late follicular and LH and FSH surge clinic visits, we used reported intake from ovulation clinic visit, and for early luteal and late luteal, we used reported alcohol intake from the midluteal phase visit). Results of the models are presented as the percentage of change in geometric mean hormone concentrations for each increase of an alcoholic drink per day or binge drinking relative to nonbinge drinking with the use of the following formula:

$$(\exp\beta - 1) \times 100\% \quad (1)$$

Covariates to include in our adjusted analyses were determined with the use of a review of the previous literature (12, 15, 16, 19, 35, 41) and statistical testing for confounding identification. Variables retained in the final multivariable models included age (continuous); race (white compared with other); the percentage of body fat (continuous); and time varying, the previous day's perceived stress (continuous), sexual activity (yes or no), caffeinated coffee intake (continuous), pain-medication use (yes or no), and sleep (continuous).

Because estradiol, testosterone, progesterone, LH, and FSH concentrations change over the cycle in response to complex feedback mechanisms with other hormones, a traditional regression adjustment for other hormone concentrations across the cycle may be inadequate (42). Therefore, we also conducted analyses with the use of marginal structural models that adjusted for other reproductive hormones through a stabilized inverse probability of exposure weights to appropriately account for time-varying confounding by hormones affected by the previous alcohol concentrations (43). Weighted linear mixed-effects models with random intercepts were used to estimate the variables of marginal structural models. An effect modification was evaluated for age categories (<21, 21–29, 30–39, and >40 y), race (white, black, Asian, and other), and parity (nulliparous and parous) by fitting multiplicative interaction terms of these factors with alcohol intake in multivariate models.

Multivariable Poisson regression with robust error variance was used to assess RR for the association between alcohol intake and characteristics of menstrual cycle dysfunction. Alcohol intake and potential confounders were averaged across the cycle for this

analysis, with alcohol assessed continuously (drinks/d) (14 g alcohol = 1 drink), dichotomously (consumer compared with non-consumer), and categorically (nonconsumer and 0–0.5 drinks/d, >0.5–1.0 drink/d, and >1.0 drink/d). Characteristics of menstrual cycle dysfunction included sporadic anovulation, long or short cycles, luteal phase deficiency, long menses, and heavy menstruation. For the assessment of any dose-response relations, we calculated the *P*-trend value by taking the median for each alcohol category and analyzing alcoholic drinks per day as a continuous variable. Because of limited frequency of binge drinking reported in the 24HDR, we were not able to assess the effect of binge drinking in our adjusted menstrual cycle-variable models. Sensitivity analyses were conducted with the use of a more-stringent cutoffs of <3 ng/mL to define anovulation, <10 d of luteal length, and <10 ng luteal progesterone/mL to define luteal phase deficiency (LPD) and alternative cutoffs (on the basis of the distribution of our data) for long cycles (≥ 35 compared with 26–35 d, ≥ 37 compared with 26–37 d, and ≥ 39 compared with 26–39 d) to test the robustness of our findings related to anovulation, LPD, and long cycles (36, 37). All analyses were performed with SAS software (version 9.3; SAS Institute Inc.).

RESULTS

Two hundred twenty-six (87.3%) women reported the consumption of any alcohol over the study period with 27 reports of binge drinking by 11 women as per the 24HDR. In consumers, median intake was 0.1 drinks/d (IQR: 0.002–0.3 drinks/d). Participants were predominately consumers of wine (29%) followed by consumers of beer (20%), liquor (14%), and cordials (6%). Although there was an indication of higher alcohol intake around ovulation, we showed no significant differences in alcohol consumption over the menstrual cycle or over the study period as captured via the 24HDR (Figure 1) or daily diary (Figure 2). Higher alcohol consumption was positively associated with age, white race, nulliparity, vigorous exercise, sexual activity, the

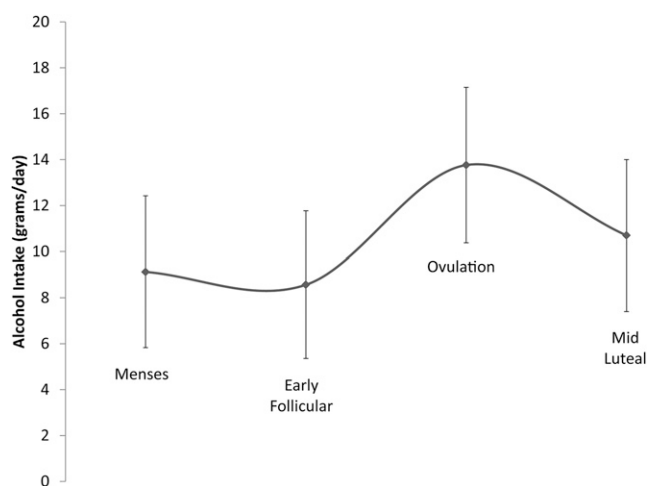


FIGURE 1 Mean (95% CI) variation in 24-h dietary recall-reported alcohol intake across the menstrual cycle. Comparisons were made via linear mixed models to account for repeated measures within women (both across the cycle and between cycles). Pairwise comparisons were made between reported intakes at menses, the follicular phase, expected ovulation, and in the luteal phase of the menstrual cycle with the use of Tukey's method to account for multiple comparisons. There were no significant differences in reported alcohol intake for the 24-h dietary recall (all $P > 0.05$).

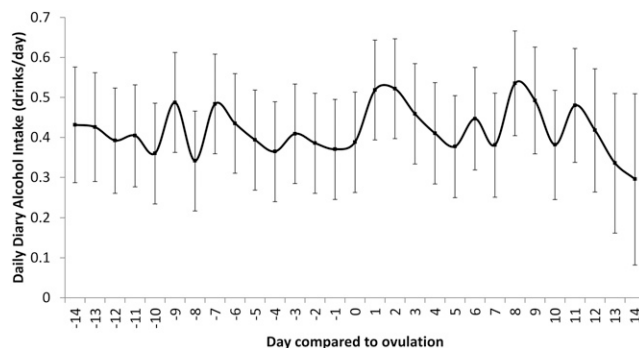


FIGURE 2 Mean (95% CI) variation in daily diary-reported alcoholic drink intake across the menstrual cycle. Comparisons were made via linear mixed models to account for repeated measures within women (both across the cycle and between cycles). Pairwise comparisons were made between reported intakes for days of the menstrual cycle with the use of Tukey's method to account for multiple comparisons. There were no significant differences in reported alcohol intake for the daily diary (all $P > 0.05$).

Mediterranean diet score, and total energy, fiber, and caffeinated coffee intake (Table 1).

Cycle geometric mean concentrations of reproductive hormones for the study population were 104.3 pg/mL (95% CI: 99.7, 109.1 pg/mL) for estradiol, 1.65 pg/mL (95% CI: 1.59, 1.72 pg/mL) for free estradiol, 28.5 ng/dL (95% CI: 27.3, 29.7 ng/dL) for testosterone, 0.18 ng/dL (95% CI: 0.18, 0.19 ng/dL) for free testosterone, 2.6 ng/mL (95% CI: 2.4, 2.9 ng/mL) for luteal progesterone, 6.1 mIU/mL (95% CI: 5.9, 6.3 mIU/mL) for FSH, 9.0 ng/mL (95% CI: 8.7, 9.4 ng/mL) for LH, and 43.6 nmol/L (95% CI: 41.2, 46.0 nmol/L) for SHBG (Table 2). Alcohol consumption, as per the 24HDR, was significantly associated with higher total and free estradiol after adjustment for age, race, the percentage of body fat, perceived stress, sexual activity, caffeine, pain-medication use, and sleep (Table 3). For each increased drink per day, the average log total and free estradiol was higher by 5.26% (95% CI: 1.27%, 9.41%) and 5.82% (95% CI: 1.81%, 9.99%), respectively; and the average log total and free testosterone was higher by 1.56% (95% CI: 0.23%, 2.90%) and 1.42% (95% CI: 0.02%, 2.84%), respectively. Women who engaged in binge drinking had, on average, 64.35% (95% CI: 18.09%, 128.71%) and 63.53% (95% CI: 17.41%, 127.83%) higher concentrations of log total and free estradiol, respectively, compared with those of nonbinge drinkers. Similar positive associations were shown with total alcohol intake and LH with concentrations higher by 6.18% (95% CI: 2.02%, 10.52%) for each 1-drink/d increase. No significant associations were seen between alcohol and luteal progesterone, FSH, or SHBG over the cycle. A categorical assessment revealed that >1-drink/d average intake was associated with significantly elevated free estradiol (11.26%, 95% CI: 0.21%, 23.53%), testosterone (3.61%, 95% CI: -0.19%, 7.55%), and LH (15.93%, 95% CI: 3.10%, 30.36%) concentrations compared with those with no intake; but intakes >0–0.5 and >0.5–1 drink/d were not associated with hormones (Supplemental Table 1). Wine and liquor appeared to drive the association between alcohol intake and free estradiol with log concentrations higher by 8.33% (95% CI: 1.01%, 15.03%) and 9.68% (95% CI: 2.56%, 17.28%), respectively, for each increased drink per day. No significant associations were shown with intakes of beer or spirits (data not shown). Estimates did not appreciably alter after additional adjustment for nulliparity, depression score, smoking, vigorous exercise other dietary factors including total

TABLE 1Demographic, lifestyle, and dietary characteristics of participants by average alcohol intake across the study period¹

	Alcohol intake, drinks/d					<i>P</i>
	Total	None	>0–0.5	>0.5–1.0	>1.0	
Participants, <i>n</i> (%)	259	33 (13)	194 (75)	18 (7)	14 (5)	—
Age, <i>y</i> , <i>n</i> (%)						0.01
<21	73 (28)	14 (42)	55 (28)	3 (17)	1 (7)	
21–29	102 (39)	13 (39)	81 (42)	5 (28)	3 (21)	
30–39	51 (20)	6 (18)	34 (18)	6 (33)	5 (36)	
>40	33 (13)	0 (0)	24 (12)	4 (22)	5 (36)	
Race, <i>n</i> (%)						0.09
White	154 (60)	14 (42)	114 (59)	13 (72)	13 (93)	
Black	51 (20)	11 (33)	37 (19)	2 (11)	1 (7)	
Asian	37 (14)	4 (12)	31 (16)	2 (11)	0 (0)	
Other	17 (7)	4 (12)	12 (6)	1 (6)	0 (0)	
Body fat, %	29.5 ± 5.9 ²	30.6 ± 5.1	29.2 ± 6.0	29.6 ± 7.1	30.5 ± 4.6	0.57
Nulliparous, <i>n</i> (%)	187 (74)	29 (88)	143 (76)	9 (50)	6 (43)	0.001
Depression score	5 (1–9) ³	5 (2–10)	5 (1–9)	3 (2–8)	1 (0–3)	0.07
Cigarette use, <i>n</i> (%)	42 (16)	4 (12)	28 (14)	5 (28)	5 (36)	0.09
Sleep, h	7.3 ± 1.0	7.2 ± 0.9	7.3 ± 1.0	6.8 ± 0.5	7.1 ± 0.7	0.18
Vigorous exercise, min	9.2 (1.9–20.2)	7.3 (0.4–22.8) ^{a,b}	8.9 (1.8–20.1) ^a	9.1 (3.8–14.1) ^a	21.4 (9.8–32.9) ^b	0.03
Pain-medication use, <i>n</i> (%)	175 (68)	22 (67)	127 (66)	14 (78)	12 (86)	0.34
Sexual activity, <i>n</i> (%)						0.003
Never	59 (23)	8 (24)	50 (26)	1 (6)	0 (0)	
Not current	64 (25)	15 (45)	41 (22)	4 (22)	4 (29)	
<1 time/wk	71 (28)	9 (27)	53 (27)	6 (33)	3 (21)	
≥1 time/wk	64 (25)	1 (3)	49 (25)	7 (39)	7 (50)	
Total energy, kcal	1613.3 ± 367.3	1389.2 ± 258.9 ^a	1623.4 ± 375.8 ^b	1849.1 ± 325.1 ^b	1699.1 ± 229.3 ^b	<0.001
Fiber, g/d	13.6 ± 5.6	10.9 ± 3.0 ^a	14.0 ± 6.0 ^b	14.7 ± 4.4 ^{a,b}	14.3 ± 3.9 ^{a,b}	0.02
Mediterranean diet score	2.9 ± 0.9	2.4 ± 0.8 ^a	2.9 ± 1.0 ^b	3.2 ± 0.7 ^b	2.9 ± 0.8 ^{a,b}	0.02
Caffeinated coffee, cups/d	0.3 (0.0–1.1)	0.1 (0.0–0.6) ^a	0.3 (0.0–1.0) ^a	1.4 (0.9–2.4) ^b	2.0 (0.5–2.6) ^b	0.002

¹One drink = 14 g alcohol. Descriptive statistics were compared between categories of alcohol intake averaged over the study period with the use of an ANOVA for normally distributed continuous variables and the Wilcoxon-Mann-Whitney test for nonnormally distributed continuous variables. Chi-square or Fisher's exact tests, when appropriate, were used for categorical variables. Means that do not share a common superscript letter are significantly different at $P < 0.05$ on the basis of Tukey's test. There were no missing values for age; race; percentage of body fat; cigarette and pain medication use; average alcohol, caffeinated coffee, fiber, and energy intakes; vigorous exercise; and Mediterranean diet score. There were 11 missing values for the depression score, 4 missing values for parity, one missing value for average sleep, and one missing value for sexual activity.

²Mean ± SD (all such values).

³Median; IQR in parentheses (all such values).

energy or fiber intake, and the Mediterranean diet score. Accounting for time-varying confounding by hormones with the use of marginal structural models also yielded similar results. No significant interactions between alcohol and age, race, or parity were identified (all $P > 0.05$).

The previous day's daily diary alcohol intake and report of binge drinking resulted in similar but attenuated estimates with only total and free estradiol appearing to be significantly elevated with increased alcohol intake after adjustment for age, race, the percentage of body fat, perceived stress, sexual activity, caffeine, pain-medication use, and sleep (**Supplemental Table 2**). Null associations were shown when we assessed the previous week's average daily diary alcohol intake or report of binge drinking on any of the previous 7 d.

Overall, after adjustment for confounding factors, there was little association shown between alcohol consumption and various markers of menstrual cycle dysfunction. However, there was a slight indication, albeit nonsignificant, of lower adjusted risk of sporadic anovulation and elevated risk of heavy menses with higher alcohol intake (**Table 4**). The frequency of anovulation was 9.9% in nonconsumers, 9.0% in women who consumed 0–0.5 drinks/d, 2.4% in women who consumed >0.5–1.0 drink/d,

and 0.0% in women who consumed >1.0 drink/d (adjusted P -trend value = 0.18). In addition, women who consumed, on average, >1.0 drink/d had 1.49 (95% CI: 0.97, 2.29) higher risk of heavy menses than did nonconsumers after adjustment for confounding factors. Sensitivity analyses that looked at alcohol intake and the alternative definitions of anovulation (<3 ng/mL) or LPD (<10 d luteal length and <10 ng luteal progesterone/mL) resulted in similar findings compared with those when defining anovulation as all luteal progesterone measurements <5 ng/mL or LPD as a luteal phase duration <10 d (data not shown). Sensitivity analyses that looked at alcohol intake and alternative cutoffs for long cycles (≥35 compared with 26–35 d, ≥37 compared with 26–37 d, and ≥39 compared with 26–39 d) showed that women with longer cycles tended to be more likely to be nonconsumers compared with consumers of alcohol, but no findings were significant (**Supplemental Table 3**).

DISCUSSION

To our knowledge, this is the first prospective study to evaluate the short-term relation between alcohol intake and menstrual cycle

TABLE 2
Reproductive hormone concentrations across the study period by average alcohol intake¹

	Alcohol intake, drinks/d					<i>P</i>
	Total	None	>0–0.5	>0.5–1.0	>1.0	
Participants, <i>n</i> (%)	259	33 (13)	194 (75)	18 (7)	14 (5)	—
Total estradiol, pg/mL	104.3 (99.7, 109.1) ²	97.7 (86.1, 110.8)	104.6 (99.3, 110.2)	109.2 (92, 129.5)	110.3 (90.9, 133.8)	0.65
Free estradiol, pg/mL	1.65 (1.59, 1.72)	1.60 (1.43, 1.8)	1.65 (1.58, 1.73)	1.68 (1.44, 1.96)	1.71 (1.44, 2.04)	0.93
Total testosterone, ng/dL	28.5 (27.3, 29.7)	27.9 (24.8, 31.5)	29.1 (27.7, 30.6)	25.1 (21.3, 29.5)	26.5 (22.1, 31.8)	0.28
Free testosterone, ng/dL	0.18 (0.18, 0.19)	0.19 (0.17, 0.21)	0.19 (0.18, 0.19)	0.16 (0.14, 0.19)	0.17 (0.14, 0.2)	0.22
Luteal progesterone, ng/mL	2.6 (2.4, 2.9)	2.3 (1.8, 3)	2.6 (2.3, 2.8)	3.6 (2.6, 5.1)	2.9 (2.0, 4.3)	0.18
FSH, mIU/mL	6.1 (5.9, 6.3)	5.8 (5.3, 6.5)	6.0 (5.8, 6.3)	6.5 (5.7, 7.5)	7.8 (6.7, 9.1)	0.01
LH, ng/mL	9.0 (8.7, 9.4)	9.4 (8.4, 10.6)	8.7 (8.3, 9.2)	10.3 (8.9, 12.0)	10.8 (9.1, 12.8)	0.02
SHBG, nmol/L	43.5 (41.2, 46.0)	39.1 (33.4, 45.8)	44.0 (41.2, 46.9)	44.7 (36.1, 55.3)	47.0 (36.9, 59.8)	0.50

¹One drink = 14 g alcohol. Log-transformed reproductive hormone concentrations were compared between categories of alcohol intake averaged over the study period with the use of an ANOVA. A total of 94% of 259 participants completed 7–8 clinic visits/menstrual cycle resulting in 3903 serum samples. FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone binding globulin.

²Geometric mean; 95% CI in parentheses (all such values).

function in healthy eumenorrheic women. We showed that the previous day's alcohol intake, notably of wine and beer, was significantly associated with elevated total and free estradiol, testosterone, and LH, and binge drinking magnified these effects. Although significant alterations in hormones were shown, we observed no significant elevated risk of menstrual cycle dysfunction. These results suggest that recent alcohol intake at moderate concentrations [mean: 9.3 g/d (0.67 drinks/d) in consumers] within healthy women with no known reproductive disorders is unlikely to be associated with menstrual cycle-related infertility.

Our findings of a positive association between acute alcohol intake and estradiol and testosterone concentrations are consistent with the majority of experimental studies in premenopausal women (23–25, 44). Results from epidemiologic studies that investigated

the association of chronic alcohol intake, which was primarily assessed via a food-frequency questionnaire, and steroid hormones have been less consistent (26, 45–48) but, in general, showed significant positive associations only at high intakes in large samples (46). Although our finding of stronger associations for wine and beer compared with for liquor and spirits is supported by previous research (35), experimental evidence that has teased apart the effects of ethanol as opposed to the liquid “food matrix” by which ethanol is delivered (e.g., beer and wine rich in vitamins, minerals, and phytochemicals) has not been conclusively shown (20).

To our knowledge, our finding of elevated LH concentrations with increased acute alcohol intake is novel. Only one other epidemiologic study has looked at the relation between alcohol and LH and showed no association with habitual intake (48).

TABLE 3
Adjusted percentage of differences in average hormone concentrations by alcohol intake (*n* = 509 cycles in 259 women)¹

	24-h dietary recall	
	Alcohol, ² drinks/d	Alcohol, ³ binge vs. nonbinge
Total estradiol, pg/mL	5.26 (1.27, 9.41) ⁴	64.35 (18.09, 128.71) ⁴
Free estradiol, pg/mL	5.82 (1.81, 9.99) ⁴	63.53 (17.41, 127.73) ⁴
Total testosterone, ng/dL	1.56 (0.23, 2.90) ⁴	8.96 (–2.42, 21.66)
Free testosterone, ng/dL	1.42 (0.02, 2.84) ⁴	8.39 (–3.54, 21.79)
Luteal progesterone, ng/mL	–3.35 (–12.58, 6.84)	17.63 (–52.34, 190.32)
FSH, mIU/mL	2.03 (–0.88, 5.02)	3.80 (–19.05, 33.11)
LH, ng/mL	6.18 (2.02, 10.52) ⁴	19.71 (–14.83, 68.25)
SHBG, nmol/L	0.00 (–1.00, 1.01)	0.69 (–7.74, 9.89)

¹All values are percentages of geometric mean differences; 95% CIs in parentheses. One drink = 14 g alcohol. Analyses were performed with the use of linear mixed effects models on the log scale of hormones and adjusted for age (continuous), race (white compared with other), percentage of body fat (continuous), and time-varying perceived stress (continuous), sexual activity (continuous), caffeinated coffee intake (continuous), pain-medication use (yes or no), and sleep (continuous). A total of 94% of 259 participants completed 7–8 clinic visits/menstrual cycle resulting in 3903 serum samples. FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone binding globulin.

²Intake was assessed at 4 times in each cycle (i.e., menses, midfollicular, ovulation, and midluteal clinic visits) via a 24-h dietary recall.

³Binge vs. nonbinge drinking was defined as ≥ 4 compared with < 4 drinks, respectively, on the day preceding clinic visit. There were 27 reports (0.7%) in 11 women (4.2%) of binge drinking per the 24-h dietary recall.

⁴Significant at $P < 0.05$. Results of the models are presented as the percentage of change in geometric mean hormone concentrations for each increase of an alcoholic drink per day or binge drinking relative to nonbinge drinking with the use of the following formula: $(\exp \beta - 1) \times 100\%$.

TABLE 4
Menstrual cycle variables according to alcohol intake ($n = 509$ cycles in 259 women)¹

	n (%) ²	Unadjusted RR	P	Adjusted RR	P
Anovulation					
Continuous, drinks/d	—	0.36 (0.15, 0.88)	0.02	0.61 (0.23, 1.62)	0.32
Nonconsumer	15/151 (9.9)	1.00	0.57	1.00	0.98
Consumer	27/358 (7.5)	0.84 (0.46, 1.55)		0.99 (0.54, 1.83)	
Nonconsumer	15/151 (9.9)	1.00	0.02	1.00	0.18
0–0.5 drinks/d	26/290 (9.0)	0.96 (0.52, 1.74)		1.07 (0.58, 1.95)	
>0.5–1 drink/d	1/42 (2.4)	0.30 (0.06, 1.43)		0.44 (0.07, 2.89)	
>1 drink/d	0/26 (0.0)	—		—	
Short cycles					
Continuous, drinks/d	—	0.85 (0.55, 1.31)	0.45	0.66 (0.41, 1.08)	0.10
Nonconsumer	22/117 (18.8)	1.00	0.83	1.00	0.61
Consumer	59/293 (20.1)	1.05 (0.67, 1.63)		0.89 (0.56, 1.41)	
Nonconsumer	22/117 (18.8)	1.00	0.55	1.00	0.16
0–0.5 drinks/d	51/236 (21.6)	1.11 (0.71, 1.72)		0.96 (0.61, 1.51)	
>0.5–1 drink/d	3/34 (8.3)	0.48 (0.18, 1.27)		0.35 (0.11, 1.07)	
>1 drink/d	5/23 (21.7)	1.16 (0.51, 2.59)		0.81 (0.39, 1.66)	
Long cycles					
Continuous, drinks/d	—	0.76 (0.44, 1.30)	0.32	0.95 (0.56, 1.64)	0.87
Nonconsumer	21/116 (18.1)	1.0	0.59	1.00	0.98
Consumer	45/279 (16.1)	0.89 (0.58, 1.37)		0.99 (0.64, 1.55)	
Nonconsumer	21/116 (18.1)	1.00	0.58	1.00	0.79
>0–0.5 drinks/d	37/222 (16.7)	0.90 (0.57, 1.41)		0.97 (0.62, 1.53)	
>0.5–1 drink/d	6/37 (16.2)	1.01 (0.48, 2.11)		1.39 (0.62, 3.11)	
>1 drink/d	2/20 (10.0)	0.60 (0.17, 2.14)		0.84 (0.22, 3.31)	
Luteal phase deficiency					
Continuous, drinks/d	—	0.64 (0.32, 1.31)	0.23	0.76 (0.36, 1.59)	0.46
Nonconsumer	9/132 (6.8)	1.00	0.52	1.00	0.41
Consumer	32/331 (9.7)	1.22 (0.64, 2.34)		1.34 (0.67, 2.67)	
Nonconsumer	9/132 (6.8)	1.00	0.22	1.00	0.48
>0–0.5 drinks/d	27/266 (10.2)	1.31 (0.69, 2.47)		1.39 (0.69, 2.80)	
>0.5–1 drink/d	4/40 (10.0)	0.89 (0.26, 3.09)		1.23 (0.40, 3.72)	
>1 drink/d	1/26 (2.4)	0.56 (0.16, 2.01)		0.67 (0.12, 3.67)	
Long menses					
Continuous, drinks/d	—	1.03 (0.93, 1.14)	0.55	1.02 (0.91, 1.14)	0.77
Nonconsumer	9/132 (6.8)	1.00	0.68	1.00	0.72
Consumer	24/314 (7.6)	1.14 (0.61, 2.13)		0.88 (0.44, 1.76)	
Nonconsumer	9/132 (6.8)	1.00	0.19	1.00	0.79
>0–0.5 drinks/d	16/250 (6.4)	1.02 (0.52, 2.01)		0.87 (0.43, 1.80)	
>0.5–1 drink/d	5/39 (12.8)	1.69 (0.67, 4.25)		1.12 (0.43, 2.91)	
>1 drink/d	3/25 (12.0)	1.58 (0.50, 5.0)		0.66 (0.18, 2.49)	
Heavy menses³					
Continuous, drinks/d	—	1.33 (1.11, 1.58)	0.001	1.19 (0.97, 1.46)	0.09
Nonconsumer	38/130 (28.2)	1.00	0.35	1.00	0.59
Consumer	116/331 (35.1)	1.15 (0.86, 1.53)		1.08 (0.81, 1.45)	
Nonconsumer	38/130 (28.2)	1.00	0.01	1.00	0.21
>0–0.5 drinks/d	89/265 (33.6)	1.11 (0.83, 1.49)		1.08 (0.80, 1.45)	
>0.5–1 drink/d	12/41 (29.3)	1.04 (0.66, 1.66)		0.91 (0.57, 1.48)	
>1 drink/d	15/25 (60.0)	1.88 (1.27, 2.80)		1.49 (0.97, 2.29)	

¹Intake was assessed at 4 times each cycle (i.e., menses, midfollicular, ovulation, and midluteal clinic visits) via a 24-h dietary recall. One drink = 14 g alcohol. Analyses were performed with the use of multivariate RR estimation by Poisson regression with adjusted models controlled for age (continuous), race (Caucasian compared with other), percentage of body fat (continuous), pain-medication use (yes or no), cycle-average perceived stress (continuous), sexual activity (continuous), caffeinated coffee (continuous), and sleep (continuous). P -trend was calculated for 4-level categorical variables (nonconsumer, >0–0.5 drinks/d, >0.5–1.0 drink/d, and >1.0 drink/d) by taking the median of each category and running as a continuous variable. Anovulation was defined as peak progesterone concentrations ≤ 5 ng/mL and no observed serum luteinizing hormone peak on the midluteal or late-luteal phase visit; there were no missing values. Short cycles were defined as ≤ 25 d compared with >25 d in length. For analyses, risk of short cycles (≤ 25 d in length) was compared with reference of having a normal length cycle (26–32 d in length). Menstrual cycle length available for 476 cycles. Long cycles were defined as ≥ 33 d compared with <33 days in length. For analyses, risk of long cycles (≥ 33 d in length) was compared with reference of having a normal length cycle (26 to 32 d in length). Menstrual cycle length was available for 476 cycles. Luteal phase deficiency was defined as the luteal phase length less 10 d. Luteal phase deficiency was available for 463 cycles. Long menses was defined as >7 d compared with less. Menses duration was available for 499 cycles.

²The numerator is the number of anovulatory, short, long, luteal phase defect, long menses, and heavy menses cycles, and the denominator is the number of cycles for the relevant alcohol category. Alcohol was assessed continuously, dichotomously (nonconsumer and consumer), and categorically (nonconsumer, >0–0.5 drinks/d, >0.5–1 drink/d, >1 drink/d).

³Total cycle blood flow was classified in tertiles as light (≤ 36.5 mL), medium (>36.5 and ≤ 72.5 mL), or heavy (>72.5 mL) and evaluated as risk of heavy compared with medium or light menses. Blood loss was available for 461 cycles.

Differences between studies included 1) our assessment of acute alcohol exposure via a 24HDR compared with chronic exposure via a food-frequency questionnaire (48) and 2) their reliance on a single follicular serum sample between days 1 and 5 of the menstrual cycle when LH variability was low compared with our assessment of ≤ 8 serum measurements that were timed with fertility monitors, including the expected LH peak just before ovulation. Because of the requisite LH surge needed to trigger ovulation, our finding may help explain the association reported between alcohol intake, specifically wine intake, and a decreased waiting time to pregnancy in the Danish National Birth Cohort (35), although a better understanding of the mechanism for alcohol's effects on LH is needed because of the limited experimental research (23).

We showed no significant associations between the previous day's or the previous week's alcohol intake and FSH or SHBG, which corroborated findings from the majority of previous studies that assessed chronic exposure in premenopausal women (26, 47, 48). Although results between studies are not directly comparable because of our assessment of acute compared with chronic exposure, alcohol-consumption distributions were similar to those in the current study, with an average of < 1 drink/d in all 3 studies. Our null finding for any effect of alcohol on luteal progesterone is supported by the only other previous epidemiologic study to be conducted in premenopausal women (49).

To our knowledge, this is the first study to use both the 24HDR and a daily diary to assess the short-term relation between alcohol consumption and menstrual cycle function in healthy eumenorrheic women. Results were similar but significantly attenuated for the daily diary than for the 24HDR. The 24HDR, which we considered the gold standard, was administered at the clinic visit by trained staff with the use of a multipass approach that captured precise alcohol quantities and types. In contrast, the alcohol assessment via the daily diary in our study, which was comparable to that in other reproductive health studies (50, 51), simply asked participants to report the number of alcoholic drinks, which may have been prone to rounding and consequent misclassification. Indeed, we showed only a fair agreement between the 24HDR and daily diary for capturing alcoholic drink intake for the same day (weighted $\kappa = 0.23$). Despite the daily diary's limitations, our ability to compare the previous day's alcohol consumption and the previous week's alcohol consumption is an improvement over previous studies and supports the hypothesis that alcohol's effects on reproductive hormones are acute.

Although no significant differences were noted between cycle-average alcohol intake and abnormal menstrual cycle function, we showed some indication for lower risk of anovulation and elevated risk of heavy menses, the latter of which has been corroborated by previous research (16). Hahn et al. (16) conducted a cross-sectional study of 2613 women who were taking part in the Danish Web-based Pregnancy Planning Study and reported that heavy alcohol consumption (≥ 14 drinks/wk) over the previous month was associated with a 1.48-higher (95% CI: 0.80, 2.72-higher) prevalence ratio of heavy menstruation than in women who abstained from alcohol after adjustment for age, BMI, physical activity, smoking, caffeine consumption, and the last method of contraception. Although a direct comparison between the study of Hahn et al. (16) and our study is difficult because our study prospectively captured alcohol intake via 24HDRs and menstrual cycle dysfunction via serum hormone biomarkers, daily diaries, and validated menses questionnaires, whereas the previous study was cross-sectional (16) in

design and relied on self-reported menstrual history from a baseline questionnaire (16), it is reassuring to find a similar higher prevalence estimate and precision of 48% (95% CI: -20% , 272%) compared with our risk of 49% (95% CI: -3% , 227%). Although moderate alcohol consumption does not appear to be associated with menstrual cycle dysfunction, additional research into the potential adverse effects of binge drinking on oocyte quality and early embryogenesis is warranted because of recent evidence in primates that showed negative effects of binge drinking before ovulation on pregnancy (52).

There were many strengths of the current investigation including multiple measures of hormone assessment over 2 menstrual cycles (with the use of standardized methods to time the cycle phase) and multiple measures of not only alcohol intake but also of important lifestyle factors. Limitations of our study included the small number of moderate and high alcohol consumers in our cohort, which weakened our power to detect the magnitude of association between high, acute alcohol consumption and higher estradiol and LH concentrations and the potential for an adverse menstrual cycle function in binge drinkers. However, alcohol intakes in the current study are comparable with those seen in other healthy, American female populations, thereby increasing the generalizability of our results (26, 47, 48). Finally, because of recent research that indicated that common polymorphisms in the *N*-acetyltransferase-2 gene, which codes for the *N*-acetyltransferase-2 enzyme that is responsible for alcohol metabolism, may modify the effects of alcohol on fecundability (51), future studies looking at the association between alcohol and menstrual cycle function should consider further capturing genetic and metabolic information.

In conclusion, we observed a modest, significant positive association between acute alcohol intake and total and free estradiol, testosterone, and LH; no significant adverse effect on short-term ovulatory function; and some indication of decreased risk of anovulation. Although these findings do not support a recommendation of moderate alcohol intake to decrease sporadic anovulation risk, it is reassuring evidence for this study population that low-to-moderate alcohol intake is not associated with ovulatory dysfunction, which is one cause of infertility. Additional studies are warranted to assess the longer-term effects of moderate alcohol intake on reproductive function and fertility (53, 54).

The authors' responsibilities were as follows—KCS: statistical analyses and primary responsibility for the final content of the manuscript; KCS, EFS, and SLM: project conception, development of overall research plan, and study oversight; KCS and SLM: writing of the manuscript; EFS, JW-W, and SLM: study concept and design; and all authors: interpretation of the data and critical revision of the manuscript for important intellectual content. None of the authors reported a conflict of interest related to the study.

REFERENCES

1. Center for Disease Control (CDC). Alcohol use and binge drinking among women of childbearing age—United States, 2006–2010. *MMWR Morb Mortal Wkly Rep* 2012;61:534–8.
2. Rees K, Hartley L, Flowers N, Clarke A, Hooper L, Thorogood M, Stranges S. 'Mediterranean' dietary pattern for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev* 2013;8:CD009825.
3. Goldberg JJ, Mosca L, Piano MR, Fisher EA, Nutrition Committee, Council on Epidemiology and Prevention, and Council on Cardiovascular Nursing of the American Heart Association. *AHA Science Advisory: wine and your heart: a science advisory for healthcare professionals from the Nutrition Committee, Council on Epidemiology and Prevention, and Council on Cardiovascular Nursing of the American Heart Association. Circulation* 2001;103:472–5.

4. Eggert J, Theobald H, Engfeldt P. Effects of alcohol consumption on female fertility during an 18-year period. *Fertil Steril* 2004;81:379–83.
5. Hassan MA, Killick SR. Negative lifestyle is associated with a significant reduction in fecundity. *Fertil Steril* 2004;81:384–92.
6. Tolstrup JS, Kjaer SK, Holst C, Sharif H, Munk C, Osler M, Schmidt L, Andersen AM, Grønbaek M. Alcohol use as predictor for infertility in a representative population of Danish women. *Acta Obstet Gynecol Scand* 2003;82:744–9.
7. Grodstein F, Goldman MB, Cramer DW. Infertility in women and moderate alcohol use. *Am J Public Health* 1994;84:1429–32.
8. Olsen J, Bolumar F, Boldsen J, Bisanti L. Does moderate alcohol intake reduce fecundability? A European multicenter study on infertility and subfecundity. *European Study Group on Infertility and Subfecundity. Alcohol Clin Exp Res* 1997;21:206–12.
9. Juhl M, Nyboe Andersen AM, Grønbaek M, Olsen J. Moderate alcohol consumption and waiting time to pregnancy. *Hum Reprod* 2001;16:2705–9.
10. Florack EI, Zielhuis GA, Rolland R. Cigarette smoking, alcohol consumption, and caffeine intake and fecundability. *Prev Med* 1994;23:175–80.
11. Curtis KM, Savitz DA, Arbuckle TE. Effects of cigarette smoking, caffeine consumption, and alcohol intake on fecundability. *Am J Epidemiol* 1997;146:32–41.
12. Parazzini F, Chatenoud L, Di Cintio E, La Vecchia C, Benzi G, Fedele L. Alcohol consumption is not related to fertility in Italian women. *BMJ* 1999;318:397.
13. Chavarro JE, Rich-Edwards JW, Rosner BA, Willett WC. Caffeinated and alcoholic beverage intake in relation to ovulatory disorder infertility. *Epidemiology* 2009;20:374–81.
14. Mutsaerts MA, Groen H, Huiting HG, Kuchenbecker WK, Sauer PJ, Land JA, Stolk RP, Hoek A. The influence of maternal and paternal factors on time to pregnancy—a Dutch population-based birth-cohort study: the GECKO Drenthe study. *Hum Reprod* 2012;27:583–93.
15. Lyngsø J, Toft G, Hoyer BB, Guldbandsen K, Olsen J, Ramlaue-Hansen CH. Moderate alcohol intake and menstrual cycle characteristics. *Hum Reprod* 2014;29:351–8.
16. Hahn KA, Wise LA, Riis AH, Mikkelsen EM, Rothman KJ, Banholzer K, Hatch EE. Correlates of menstrual cycle characteristics among nulliparous Danish women. *Clin Epidemiol* 2013;5:311–9.
17. Wilsnack SC, Klassen AD, Wilsnack RW. Drinking and reproductive dysfunction among women in a 1981 national survey. *Alcohol Clin Exp Res* 1984;8:451–8.
18. Kritz-Silverstein D, Wingard DL, Garland FC. The association of behavior and lifestyle factors with menstrual symptoms. *J Womens Health Gend Based Med* 1999;8:1185–93.
19. Cooper GS, Sandler DP, Whelan EA, Smith KR. Association of physical and behavioral characteristics with menstrual cycle patterns in women age 29–31 years. *Epidemiology* 1996;7:624–8.
20. Ferreira MP, Willoughby D. Alcohol consumption: the good, the bad, and the indifferent. *Appl Physiol Nutr Metab* 2008;33:12–20.
21. Substance Abuse and Mental Health Services Administration (US). Urine collection and testing procedures and alternative methods for monitoring drug use. *Treatment Improvement Protocol (TIP) Series. Vol. 47. Rockville (MD): Center for Substance Abuse Treatment. 2006.*
22. Mendelson JH, Mello NK, Cristofaro P, Ellingboe J, Skupny A, Palmieri SL, Benedikt R, Schiff I. Alcohol effects on naloxone-stimulated luteinizing hormone, prolactin and estradiol in women. *J Stud Alcohol* 1987;48:287–94.
23. Sarkola T, Mäkisalo H, Fukunaga T, Eriksson CJ. Acute effect of alcohol on estradiol, estrone, progesterone, prolactin, cortisol, and luteinizing hormone in premenopausal women. *Alcohol Clin Exp Res* 1999;23:976–82.
24. Vatsalya V, Issa JE, Hommer DW, Ramchandani VA. Pharmacodynamic effects of intravenous alcohol on hepatic and gonadal hormones: influence of age and sex. *Alcohol Clin Exp Res* 2012;36:207–13.
25. Reichman ME, Judd JT, Longcope C, Schatzkin A, Clevidence BA, Nair PP, Campbell WS, Taylor PR. Effects of alcohol consumption on plasma and urinary hormone concentrations in premenopausal women. *J Natl Cancer Inst* 1993;85:722–7.
26. Dorgan JF, Reichman ME, Judd JT, Brown C, Longcope C, Schatzkin A, Campbell WS, Franz C, Kahle L, Taylor PR. The relation of reported alcohol ingestion to plasma levels of estrogens and androgens in premenopausal women (Maryland, United States). *Cancer Causes Control* 1994;5:53–60.
27. Rivier C. Alcohol stimulates ACTH secretion in the rat: mechanisms of action and interactions with other stimuli. *Alcohol Clin Exp Res* 1996;20:240–54.
28. Gavalier JS, Van Thiel DH. The association between moderate alcoholic beverage consumption and serum estradiol and testosterone levels in normal postmenopausal women: relationship to the literature. *Alcohol Clin Exp Res* 1992;16:87–92.
29. Wactawski-Wende J, Schisterman EF, Hovey KM, Howards PP, Browne RW, Hediger M, Liu A, Trevisan M, BioCycle Study Group. BioCycle Study: design of the longitudinal study of the oxidative stress and hormone variation during the menstrual cycle. *Paediatr Perinat Epidemiol* 2009;23:171–84.
30. Howards PP, Schisterman EF, Wactawski-Wende J, Reschke JE, Frazer AA, Hovey KM. Timing clinic visits to phases of the menstrual cycle by using a fertility monitor: the BioCycle Study. *Am J Epidemiol* 2009;169:105–12.
31. Södergård R, Bäckström T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. *J Steroid Biochem* 1982;16:801–10.
32. Sartorius G, Ly LP, Sikaris K, McLachlan R, Handelsman DJ. Predictive accuracy and sources of variability in calculated free testosterone estimates. *Ann Clin Biochem* 2009;46:137–43.
33. Dasharathy SS, Mumford SL, Pollack AZ, Perkins NJ, Mattison DR, Wactawski-Wende J, Schisterman EF. Menstrual bleeding patterns among regularly menstruating women. *Am J Epidemiol* 2012;175:536–45.
34. Wyatt KM, Dimmock PW, Walker TJ, O'Brien PM. Determination of total menstrual blood loss. *Fertil Steril* 2001;76:125–31.
35. Juhl M, Olsen J, Andersen AM, Grønbaek M. Intake of wine, beer and spirits and waiting time to pregnancy. *Hum Reprod* 2003;18:1967–71.
36. Schliep KC, Mumford SL, Hammoud AO, Stanford JB, Kissell KA, Sjaarda LA, Perkins NJ, Ahrens KA, Wactawski-Wende J, Mendola P, et al. Luteal phase deficiency in regularly menstruating women: prevalence and overlap in identification based on clinical and biochemical diagnostic criteria. *J Clin Endocrinol Metab* 2014;99:E1007–14.
37. Lynch KE, Mumford SL, Schliep KC, Whitcomb BW, Zarek SM, Pollack AZ, Bertone-Johnson ER, Danaheer M, Wactawski-Wende J, Gaskins AJ, et al. Assessment of anovulation in eumenorrheic women: comparison of ovulation detection algorithms. *Fertil Steril* 2014;102:511–8.e2.
38. Schakel SF, Sievert YA, Buzzard IM. Sources of data for developing and maintaining a nutrient database. *J Am Diet Assoc* 1988;88:1268–71.
39. Nutrition Coordinating Center. University of Minnesota Nutrition Data System for Research [Internet]. University of Minnesota, Minneapolis, MN; 2005. [cited 2014 Oct 24]. Available from: <http://www.ncc.umn.edu/products/nds.html>.
40. Gaskins AJ, Rovner AJ, Mumford SL, Yeung E, Browne RW, Trevisan M, Perkins NJ, Wactawski-Wende J, Schisterman EF, BioCycle Study Group. Adherence to a Mediterranean diet and plasma concentrations of lipid peroxidation in premenopausal women. *Am J Clin Nutr* 2010;92:1461–7.
41. Battaglia C, Battaglia B, Mancini F, Nappi RE, Paradisi R, Venturoli S. Moderate alcohol intake, genital vascularization, and sexuality in young, healthy, eumenorrheic women. A pilot study. *J Sex Med* 2011;8:2334–43.
42. Robins JM, Hernán MA, Brumback B. Marginal structural models and causal inference in epidemiology. *Epidemiology* 2000;11:550–60.
43. Cole SR, Hernán MA. Constructing inverse probability weights for marginal structural models. *Am J Epidemiol* 2008;168:656–64.
44. Mendelson JH, Lukas SE, Mello NK, Amass L, Ellingboe J, Skupny A. Acute alcohol effects on plasma estradiol levels in women. *Psychopharmacology (Berl)* 1988;94:464–7.
45. Maskarinec G, Morimoto Y, Takata Y, Murphy SP, Stanczyk FZ. Alcohol and dietary fibre intakes affect circulating sex hormones among premenopausal women. *Public Health Nutr* 2006;9:875–81.
46. Rinaldi S, Peeters PH, Bezemer ID, Dossus L, Biessy C, Sacerdote C, Berrino F, Panico S, Palli D, Tumino R, et al. Relationship of alcohol intake and sex steroid concentrations in blood in pre- and post-menopausal women: the European Prospective Investigation into Cancer and Nutrition. *Cancer Causes Control* 2006;17:1033–43.

47. Kinney A, Kline J, Kelly A, Reuss ML, Levin B. Smoking, alcohol and caffeine in relation to ovarian age during the reproductive years. *Hum Reprod* 2007;22:1175–85.
48. Lucero J, Harlow BL, Barbieri RL, Sluss P, Cramer DW. Early follicular phase hormone levels in relation to patterns of alcohol, tobacco, and coffee use. *Fertil Steril* 2001;76:723–9.
49. Verkasalo PK, Thomas HV, Appleby PN, Davey GK, Key TJ. Circulating levels of sex hormones and their relation to risk factors for breast cancer: a cross-sectional study in 1092 pre- and postmenopausal women (United Kingdom). *Cancer Causes Control* 2001;12:47–59.
50. Buck Louis GM, Dmochowski J, Lynch C, Kostyniak P, McGuinness BM, Vena JE. Polychlorinated biphenyl serum concentrations, lifestyle and time-to-pregnancy. *Hum Reprod* 2009;24:451–8.
51. Taylor KC, Small CM, Dominguez CE, Murray LE, Tang W, Wilson MM, Bouzyk M, Marcus M. Alcohol, smoking, and caffeine in relation to fecundability, with effect modification by NAT2. *Ann Epidemiol* 2011;21:864–72.
52. VandeVoort CA, Grimsrud KN, Midic U, Mtango N, Latham KE. Transgenerational effects of binge drinking in a primate model: implications for human health. *Fertil Steril* 2015;103:560–9.
53. Hankinson SE, Eliassen AH. Circulating sex steroids and breast cancer risk in premenopausal women. *Horm Cancer* 2010;1:2–10.
54. Eliassen AH, Hankinson SE. Endogenous hormone levels and risk of breast, endometrial and ovarian cancers: prospective studies. *Adv Exp Med Biol* 2008;630:148–65.