American Journal of Epidemiology

Vol. 189, No. 7
DOI: 10.1093/aje/kwz264
Advance Access publication:
December 17, 2019

Original Contribution

Estimated Number of Lifetime Ovulatory Years and Its Determinants in Relation to Levels of Circulating Inflammatory Biomarkers

Tianyi Huang*, Amy L. Shafrir, A. Heather Eliassen, Kathryn M. Rexrode, and Shelley S. Tworoger

* Correspondence to Dr. Tianyi Huang, Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, 181 Longwood Avenue, Room 353, Boston, MA 02115 (e-mail: tih541@mail.harvard.edu).

Initially submitted April 4, 2019; accepted for publication September 13, 2019.

Reproductive events, such as ovulation, trigger an inflammatory cascade. Few studies have examined their long-term influence on inflammatory profiles. We included 3,393 premenopausal and 3,915 postmenopausal women with intact ovaries/uterus from the Nurses' Health studies (Nurses' Health Study (1989–1990) and Nurses' Health Study II (1996–1999)) in an analysis of the association between lifetime ovulatory years (LOY) and levels of inflammatory biomarkers. We estimated LOY as age at menopause (age at blood collection for premenopausal women) minus age at menarche, subtracting years of oral contraceptive (OC) use and 1 year per pregnancy. After adjustment for other inflammation-related factors (e.g., body mass index, exercise, diet), every 5-year increase in LOY was associated with lower C-reactive protein (CRP) levels in both premenopausal (difference = −11.5%, 95% confidence interval: −15.0, −8.0; $P < 0.0001$) and postmenopausal (difference = −7.2%, 95% confidence interval: −10.0, −4.3; $P < 0.0001$) women. Older age at menopause ($P = 0.007$), earlier menarche ($P = 0.007$), and shorter duration of OC use ($P = 0.002$) were associated with lower CRP levels in postmenopausal women, whereas duration of OC use was positively associated with CRP levels in premenopausal women ($P < 0.0001$). LOY was modestly inversely associated with interleukin 6 in postmenopausal women ($P = 0.03$). Notably, the associations of CRP with LOY were similar in magnitude to associations with exercise and a healthy diet, though weaker than the association with body mass index. Although many reproductive events induce acute inflammation, increased LOY was associated with lower chronic systemic inflammation even after menopause.

age at menopause; C-reactive protein; inflammation; lifetime ovulatory years; menopause; oral contraceptives; ovulation; reproductive factors

Abbreviations: BWH, Brigham and Women's Hospital; CI, confidence interval; CRP, C-reactive protein; IL-6, interleukin 6; LOY, lifetime ovulatory years; NHS, Nurses' Health Study; NHSII, Nurses' Health Study II; OC, oral contraceptive; SD, standard deviation; sTNFR2, soluble tumor necrosis factor α receptor 2.

Editor's note: *Invited commentaries on this article appear on pages 671 and 674, and the authors' response appears on page 677.*

A longer reproductive life span, usually measured by the number of years between menarche and menopause, has been associated with lower risks of diabetes, cardiovascular disease, and death in women (1–4). It is often postulated that longer exposure to endogenous estrogen during reproductive years may confer potential health benefits. Interestingly, reproductive factors that disrupt hormonal profiles and reduce the number of ovulatory cycles, such as longer duration of oral contraceptive (OC) use and more pregnancies, have also been associated with increased risk of cardiometabolic disease (5–10). Conversely, longer estimated lifetime ovulatory years (LOY), a measure which considers duration of OC use and pregnancies in addition to ages at menarche and menopause, has been consistently associated with higher risk of ovarian and endometrial cancers (11–14).

Despite the predominant focus on hormonal mechanisms underlying the associations of reproductive life span and LOY with chronic disease risk, the potential role of inflammation has been understudied. Reproductive events such as

ovulation and menstruation trigger the acute inflammatory cascade because of repeated damage to/repair of ovarian surface epithelium and endometrium (15, 16). Exposure to endogenous or exogenous estrogen may also modulate inflammation, although differential inflammatory effects of estrogen have been observed, possibly through action on different subtypes of estrogen receptors (17–19). While many studies have supported a protective effect of progesterone on inflammatory responses (20, 21), some studies have suggested that progesterone exposure may activate genes involved in cytokine production and induce inflammation under certain circumstances (22, 23). In addition, the evolving formulations of OCs in the past several decades, characterized by substantial reductions in the dose of estrogen/progestin, may alter levels of exposure to exogenous hormones in premenopausal women (24, 25). Taken together, the association between LOY and systemic inflammation remains equivocal, with plausible evidence supporting an association in either direction: Longer LOY may be associated with a lower level of inflammation, leading to reduced cardiometabolic disease risk, or a higher level of inflammation, leading to increased risk of gynecological cancer (see Web Figure 1, available at https://academic.oup.com/aje). However, to date, no study has systematically elucidated the associations of LOY and its determinants with inflammatory biomarkers, which would improve our understanding of the mechanisms linking reproductive factors to development of chronic disease.

To address this gap, we performed a secondary data analysis examining the associations of estimated LOY and its component reproductive factors with plasma levels of C-reactive protein (CRP), interleukin 6 (IL-6), and soluble tumor necrosis factor α receptor 2 (sTNFR2) in 2 US cohorts that represent women using older and newer generations of OCs, respectively. To estimate independent associations, we considered inflammation-related anthropometric factors, lifestyle, medication use, and comorbidity in the modeling. We further assessed the role of LOY in systemic inflammation by comparing the strengths of the associations with these established inflammatory factors.

METHODS

Study population

The Nurses' Health studies are 2 large prospective US cohort studies that enrolled 121,700 female registered nurses (Nurses' Health Study (NHS), ages 30–55 years) in 1976 and 116,429 nurses (Nurses' Health Study II (NHSII), ages 25–42 years) in 1989. All participants completed a baseline questionnaire regarding medical history and health-related information (including reproductive, hormonal, and lifestyle factors); information on these health-related factors was updated biennially through follow-up questionnaires. The study protocols were approved by the institutional review boards of Brigham and Women's Hospital (BWH) and Harvard T.H. Chan School of Public Health (Boston, Massachusetts).

In 1989–1990, 32,826 NHS participants (aged 43–70 years) provided a heparin blood sample and shipped it with an ice pack by overnight courier to the BWH/Harvard Cohorts Biorepository, where it was processed and separated into plasma, red blood cells, and white blood cells (26). In 1996–1999, 29,611 NHSII participants (aged 32–54 years) provided a blood sample, following the same protocol (27). All samples have been stored in liquid nitrogen freezers since collection. Researchers in nested case-control studies have utilized the blood samples to investigate associations between inflammatory biomarkers and multiple disease outcomes (28–34). We pooled the biomarker data from those studies, restricting the analysis to controls who were premenopausal or naturally postmenopausal with an intact uterus/ovaries at blood collection, which resulted in a sample of 7,308 women for CRP analysis, 5,435 women for IL-6 analysis, and 5,064 women for sTNFR2 analysis. Because of differential inflammatory profiles, as well as recency of exposure to reproductive factors by menopausal status, all analyses were conducted separately for premenopausal women and naturally postmenopausal women.

Assessment of reproductive factors

In the NHS, parity (defined as pregnancies lasting longer than 6 months) was reported biennially from 1976 until 1984, and this information was updated again in 1996. Information on duration of OC use was collected biennially until 1982, when use of OCs in the cohort became uncommon. Age at menarche was reported at baseline. Information on menopausal status, age at menopause, reason for menopause (surgery, natural, radiation/chemotherapy), and surgical removal of the uterus/ovaries was collected biennially throughout follow-up. In the NHSII cohort, which is younger, information on these reproductive factors has been updated on every biennial questionnaire since 1989, except age at menarche, which was assessed at baseline. Specifically, OC information in NHSII was collected every 2 years from 1989 to 2009, with participants reporting duration of OC use and brand of OC use starting at age 13 years onwards. The reported brands of OCs were used to calculate the cumulative estrogen and progestin dose for each NHSII participant from the age at which she first began OC use to the questionnaire cycle (1997) nearest her blood draw (35). The range of doses was 20–60 μg for estrogen and 1–5 mg for progestin, according to OC information reported in 1997, which is similar to the current low-dose OC formulation (24). LOY was estimated as the difference between age at menopause (or age at blood collection for premenopausal women) and age at menarche, subtracting duration of OC use and 1 year per pregnancy.

Biomarker assays

Plasma aliquots selected for the different projects were assayed for inflammatory biomarkers in the same laboratory, with consistently high performance over time. High-sensitivity CRP was measured via an immunoturbidimetric assay (Denka Seiken Company Ltd., Tokyo, Japan). IL-6 and sTNFR2 were measured by means of an ultrasensitive enzyme-linked immunosorbent assay from R&D Systems,

Inc. (Minneapolis, Minnesota). All projects included 10% blinded quality control samples. The coefficients of variation were generally less than 10% across projects for each biomarker.

Assessment of other inflammation-related factors

Date of birth and height were self-reported at baseline. Smoking status, weight, and medication use (aspirin, nonsteroidal antiinflammatory drugs, and hormone therapy) were reported biennially. For these covariates, we used the information obtained closest to blood collection (NHS: 1988/1990; NHSII: 1997/1999). Physical activity was assessed every 4 years from a validated questionnaire and was quantified as metabolic equivalent of task (MET) hours (36). A validated semiquantitative food frequency questionnaire was administered every 4 years, based on which an empirical inflammatory diet index was developed according to the food groups most predictive of inflammatory biomarker levels (37). Clinical diagnoses of diabetes and hypertension were self-reported biennially. For these covariates, we used all information collected prior to blood collection to derive cumulative average physical activity, inflammatory diet index, and histories of diabetes and hypertension.

Statistical analysis

Because the data were pooled across multiple projects, we recalibrated the biomarker levels to correct for potential laboratory variations across projects using the average batch method (38). Data for all biomarkers were log-transformed to normalize their right-skewed distributions. No outliers were detected on the natural logarithmic scale using the generalized extreme Studentized deviate many-outlier procedure (39). General linear regression (PROC GLM in SAS (SAS Institute Inc., Cary, North Carolina)) was used to evaluate the associations between LOY and inflammatory biomarkers with adjustment for other inflammation-related factors, including age at blood collection, smoking, body mass index (weight (kg)/height $(m)^2$), physical activity, hormone therapy (postmenopausal women only), current aspirin use, current use of nonsteroidal antiinflammatory drugs, inflammatory diet index (standardized to a z score), and histories of diabetes and hypertension. We categorized LOY into quartiles specific to premenopausal ($\leq$22, 23–26, 27–30, or $>$30 years) and postmenopausal ($\leq$29, 30–33, 34–36, or $>$36 years) women. Multivariable-adjusted least-squares geometric mean biomarker levels were estimated for each LOY category. We tested for linear trend by using LOY as a continuous variable and estimated the percent difference in biomarker levels for a 5-year increase in LOY. Using regression coefficients from the multivariable model, we further compared the associations between inflammatory biomarkers and LOY with associations with the other inflammation-related factors included in the multivariable model. To facilitate comparison of the associations across different inflammation-related factors, we estimated the percent difference in biomarker levels for every standard deviation (SD) increment of the continuous variable.

We performed similar analyses for individual components of LOY, including age at menopause (postmenopausal women only), duration of OC use, parity, and age at menarche. Given the weak-to-moderate correlations between individual components (Web Table 1), we mutually adjusted for them in the multivariable analysis, in addition to other inflammation-related covariates as described above. On the basis of additional details regarding OC use in NHSII (see above), we further evaluated the associations of the biomarkers with cumulative estrogen dose, cumulative progestin dose, time since last use, age at first use, and age at last use among premenopausal women with these data. We also repeated the main analysis among NHSII premenopausal women, since they were similar to premenopausal women who are using current OC formulations today (n = 2,371 after exclusion of NHS premenopausal women, who would have used the older generation of OCs). Sensitivity analyses restricted to postmenopausal women who had never used hormone therapy were conducted to evaluate the potential impact of exogenous hormones, which can increase inflammatory biomarker levels (40), on the associations. All analyses were performed in SAS 9.4.

RESULTS

As expected, women with longer LOY were older at blood collection (for premenopausal women) or at menopause (for postmenopausal women), younger at menarche, less likely to have ever used OCs, with a shorter duration of use among ever users, and less likely to be parous, with fewer children among parous women (Table 1). Both premenopausal and postmenopausal women with longer LOY had higher body mass index. Further, current aspirin use was more prevalent among premenopausal women with longer LOY, whereas postmenopausal women with longer LOY were more likely to have a history of hypertension but less likely to currently smoke or use hormone therapy.

After adjustment for multiple inflammation-related factors, LOY was inversely associated with CRP levels in both premenopausal and postmenopausal women (Table 2). In postmenopausal women, multivariable-adjusted geometric mean CRP levels were 2.14 mg/dL for LOY $\leq$29 years, 1.96 mg/dL for LOY 30–33 years, 1.89 mg/dL for LOY 34–36 years, and 1.76 mg/dL for LOY $>$36 years (P for trend $<$ 0.0001). The association was suggestively stronger for women with more recent menopause ($<$10 years since menopause) than for those with more distant menopause ($\geq$10 years since menopause) (P for interaction = 0.09). The difference in CRP level per 5-year increment of LOY was −11.1% (95% confidence interval (CI): −15.2, −7.1) for postmenopausal women with less than 10 years since menopause (P for trend $<$ 0.0001) and −4.0% (95% CI: −8.5, 0.6) for women with 10 or more years since menopause (P for trend = 0.09). In premenopausal women, adjusted CRP levels were 1.19 mg/dL for LOY $\leq$22 years, 0.99 mg/dL for LOY 23–26 years, 0.96 mg/dL for LOY 27–30 years, and 0.87 mg/dL for LOY $>$30 years (P for trend $<$ 0.0001). The associations were similar for premenopausal women aged 45 years or more (per 5-year increase in

Table 1. Age-Standardized Characteristics of Naturally Postmenopausal Women and Premenopausal Women With an Intact Uterus and Ovaries at Blood Collection, by Estimated Number of Lifetime Ovulatory Years, Nurses' Health Study (1989–1990) and Nurses' Health Study II (1996–1999)[a]

Characteristic	Lifetime Ovulatory Years (Naturally Postmenopausal Women)							
	≤29 (*n* = 1,003)		30–33 (*n* = 1,038)		34–36 (*n* = 1,002)		>36 (*n* = 872)	
	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%
Age at blood draw, years	57.5 (6.6)		59.6 (5.8)		60.4 (5.2)		61.7 (4.5)	
Age at menopause, years	47.3 (4.3)		49.1 (2.5)		50.8 (2.2)		52.7 (2.3)	
Age at menarche, years	13.0 (1.5)		12.9 (1.5)		12.6 (1.3)		11.9 (1.3)	
Ever use of OCs		72		41		28		16
Duration of OC use, years[b]	7.6 (4.4)		3.0 (2.6)		1.8 (1.7)		1.0 (0.9)	
Parous		96		96		93		84
No. of children[c]	3.9 (1.9)		3.6 (1.6)		3.2 (1.3)		2.6 (1.1)	
Current hormone therapy use		34		29		26		26
Current aspirin use		47		47		50		46
Current NSAID use		32		29		30		34
Current smoking		20		18		14		12
Body mass index[d]	24.8 (4.5)		24.9 (4.5)		25.5 (4.8)		25.8 (4.7)	
Inflammatory diet index[e]	−0.11 (0.30)		−0.10 (0.29)		−0.10 (0.30)		−0.09 (0.26)	
Physical activity, MET-hours/week	17.7 (17.7)		17.8 (18.3)		17.0 (15.5)		17.5 (16.7)	
History of hypertension		27		27		31		32
History of diabetes		2		3		2		3
	Lifetime Ovulatory Years (Premenopausal Women)							
	≤22 (*n* = 892)		23–26 (*n* = 716)		27–30 (*n* = 891)		>30 (*n* = 894)	
	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%	Mean (SD)	%
Age at blood draw, years	39.7 (3.7)		42.8 (3.5)		44.8 (3.0)		48.6 (2.9)	
Age at menarche, years	13.0 (1.5)		12.7 (1.4)		12.3 (1.4)		11.7 (1.2)	
Ever use of OCs		98		88		72		57
Duration of OC use, years[b]	9.8 (5.0)		4.9 (3.5)		2.8 (2.5)		1.4 (1.2)	
Parous		89		89		72		65
No. of children[c]	2.5 (1.0)		2.6 (1.2)		2.4 (1.0)		2.1 (0.9)	
Current aspirin use		18		24		24		28
Current NSAID use		43		47		43		44
Current smoking		14		9		11		10
Body mass index	24.1 (5.3)		24.9 (5.0)		25.5 (6.0)		26.6 (5.4)	
Inflammatory diet index	−0.06 (0.32)		−0.05 (0.30)		−0.04 (0.32)		−0.05 (0.29)	
Physical activity, MET-hours/week	18.8 (25.4)		16.2 (17.7)		16.7 (16.6)		17.3 (17.5)	
History of hypertension		12		10		13		12
History of diabetes		0		1		1		1

Abbreviations: MET, metabolic equivalent of task; NSAID, nonsteroidal antiinflammatory drug; OC, oral contraceptive; SD, standard deviation.
[a] Based on the sample size for C-reactive protein analyses.
[b] Among ever users.
[c] Among parous women.
[d] Weight (kg)/height $(m)^2$.
[e] Scores were standardized to *z* scores (quintiles: −1.93 to 0.41, −0.40 to −0.21, −0.20 to −0.06, −0.05 to 0.19, and 0.20 to 1.55).

Table 2. Associations Between Number of Lifetime Ovulatory Years and Levels of Circulating Inflammatory Biomarkers Among Naturally Postmenopausal Women and Premenopausal Women With an Intact Uterus and Ovaries, Nurses' Health Study (1989–1990) and Nurses' Health Study II (1996–1999)[a]

Inflammatory Biomarker	Lifetime Ovulatory Years (Naturally Postmenopausal Women)				Difference per 5 Years, %	95% CI	*P* for Trend
	≤29	30–33	34–36	>36			
CRP, mg/L							
Overall	2.14	1.96	1.89	1.76	−7.2	−10.0, −4.3	<0.0001
<10 years since menopause	2.22	1.86	1.87	1.67	−11.1	−15.2, −7.1	<0.0001
≥10 years since menopause	2.09	2.08	1.91	1.84	−4.0	−8.5, 0.6	0.09
IL-6, pg/mL							
Overall	1.30	1.36	1.25	1.20	−2.6	−4.9, −0.2	0.03
<10 years since menopause	1.26	1.32	1.24	1.16	−2.9	−6.3, 0.6	0.11
≥10 years since menopause	1.36	1.44	1.27	1.30	−1.3	−5.1, 2.6	0.51
sTNFR2, pg/mL							
Overall	2,679	2,656	2,643	2,645	0.1	−0.8, 1.0	0.86
<10 years since menopause	2,516	2,535	2,510	2,545	0.8	−0.5, 2.2	0.24
≥10 years since menopause	2,794	2,751	2,763	2,767	0.4	−0.9, 1.8	0.52
	Lifetime Ovulatory Years (Premenopausal Women)						
	≤22	23–26	27–30	>30			
CRP, mg/L							
Overall	1.19	0.99	0.96	0.87	−11.5	−15.0, −8.0	<0.0001
Age <45 years at blood draw	1.06	0.88	0.90	0.77	−11.3	−15.7, −6.6	<0.0001
Age ≥45 years at blood draw	1.33	1.28	1.13	1.04	−11.5	−16.8, −5.8	0.0001
IL-6, pg/mL							
Overall	0.98	0.97	0.99	0.97	0.1	−2.5, 2.8	0.95
Age <45 years at blood draw	0.77	0.77	0.81	0.75	1.2	−2.2, 4.6	0.49
Age ≥45 years at blood draw	1.17	1.14	1.17	1.13	−1.4	−5.7, 3.0	0.52
sTNFR2, pg/mL							
Overall	2,373	2,380	2,368	2,416	0.8	−0.3, 1.9	0.16
Age <45 years at blood draw	2,253	2,219	2,258	2,213	0.6	−0.8, 2.0	0.41
Age ≥45 years at blood draw	2,354	2,604	2,454	2,534	1.0	−0.7, 2.8	0.23

Abbreviations: CI, confidence interval; CRP, C-reactive protein; IL-6, interleukin 6; MET, metabolic equivalent of task; sTNFR2, soluble tumor necrosis factor α receptor 2.

[a] Multivariable-adjusted geometric mean values are shown. Results were adjusted for age at blood collection (years; continuous), smoking status (current, past, or never smoker), body mass index (weight (kg)/height (m)^2; continuous), physical activity (<3.0, 3.0–8.9, 9.0–17.9, 18.0–26.9, or ≥27.0 MET-hours/week), hormone therapy use (current, past, or never use; postmenopausal women only), current aspirin use (yes, no), current use of nonsteroidal antiinflammatory drugs (yes, no), inflammatory diet index (*z* score; in quintiles), history of diabetes (yes, no), and history of hypertension (yes, no).

LOY, CRP difference = –11.5% (95% CI: –16.8, −5.8); *P* for trend = 0.0001) and women aged less than 45 years (difference = –11.3% (95% CI: −15.7, −6.6); *P* for trend < 0.0001). IL-6 was moderately inversely associated with LOY in postmenopausal women (per 5-year increase in LOY, difference = –2.6% (95% CI: –4.9, −0.2); *P* for trend = 0.03), with similar differences observed by time since menopause, but was not associated in premenopausal women (*P* for trend = 0.95). We observed no association between LOY and sTNFR2 in either premenopausal or postmenopausal women. Restricting the analysis to postmenopausal women who had never used hormone therapy resulted in somewhat stronger inverse associations, particularly for IL-6 (Web Table 2).

Table 3. Comparison of Associations Between Lifetime Ovulatory Years and Inflammatory Biomarkers With Associations Between Lifetime Ovulatory Years and Other Inflammation-Related Factors, Nurses' Health Study (1989–1990) and Nurses' Health Study II (1996–1999)

Inflammation-Related Factor[a]	Inflammatory Biomarker					
	CRP		IL-6		sTNFR2	
	PD	95% CI	PD	95% CI	PD	95% CI
Postmenopausal women						
LOY, per SD increase (5.5 years)	−8.1	−11.2, −5.0	−2.7	−5.3, 0.0	0.1	−0.8, 1.1
Age, per SD increase (5.8 years)	12.1	8.3, 16.1	8.7	5.6, 11.8	4.9	3.8, 5.9
Body mass index[b], per SD increase (4.6 units)	53.7	48.5, 59.1	16.2	13.0, 19.5	4.8	3.8, 5.9
Smoking (current smoker vs. never smoker)	47.4	34.2, 61.9	18.2	9.6, 27.5	2.0	−0.7, 4.9
Physical activity, per SD increase (17.3 MET-hours/week)	−6.6	−9.6, −3.6	−4.8	−7.2, −2.3	−0.5	−1.4, 0.5
Inflammatory diet index, per SD increase (*z* score: 0.29)	12.1	8.5, 15.9	7.3	4.4, 10.2	3.0	2.0, 4.0
Hormone therapy (current use vs. never use)	40.1	29.9, 51.2	−7.3	−12.7, −1.7	−3.8	−5.8, −1.7
Current aspirin use (yes vs. no)	−3.0	−9.1, 3.5	−1.5	−6.4, 3.8	0.5	−1.4, 2.3
Current NSAID use (yes vs. no)	3.3	−3.7, 10.8	1.1	−4.4, 6.9	1.8	−0.2, 3.9
History of diabetes (yes vs. no)	24.8	1.5, 53.4	4.9	−11.2, 24.0	4.4	−1.8, 10.9
History of hypertension (yes vs. no)	19.1	10.7, 28.2	7.7	1.5, 14.3	3.7	1.6, 6.0
Premenopausal women						
LOY, per SD increase (6.3 years)	−14.5	−18.7, −10.1	0.2	−3.1, 3.7	1.0	−0.4, 2.3
Age, per SD increase (4.6 years)	23.6	17.4, 30.0	2.8	−0.6, 6.3	1.1	−0.3, 2.5
Body mass index, per SD increase (5.3 units)	84.6	78.2, 91.4	27.4	24.4, 30.5	5.8	4.8, 6.8
Smoking (current smoker vs. never smoker)	8.7	−3.2, 22.0	12.9	4.4, 22.0	4.4	1.3, 7.6
Physical activity, per SD increase (19.8 MET-hours/week)	−4.7	−7.8, −1.4	−0.7	−2.9, 1.6	0.4	−0.5, 1.3
Inflammatory diet index, per SD increase (*z* score: 0.32)	4.6	1.1, 8.3	2.9	0.6, 5.3	2.5	1.6, 3.5
Current aspirin use (yes vs. no)	−2.3	−10.0, 6.1	2.6	−3.0, 8.5	0.4	−1.7, 2.7
Current NSAID use (yes vs. no)	6.2	−0.7, 13.7	−3.1	−7.4, 1.4	1.1	−0.7, 2.9
History of diabetes (yes vs. no)	31.4	−11.9, 96.0	−8.3	−32.1, 23.7	9.2	−1.5, 21.1
History of hypertension (yes vs. no)	14.3	2.0, 28.1	6.1	−1.7, 14.5	2.7	−0.3, 5.8

Abbreviations: CI, confidence interval; CRP, C-reactive protein; IL-6, interleukin 6; LOY, lifetime ovulatory years; MET, metabolic equivalent of task; NSAID, nonsteroidal antiinflammatory drug; PD, percent difference; SD, standard deviation; sTNFR2, soluble tumor necrosis factor α receptor 2.

[a] These factors were simultaneously adjusted for in the same multivariable model.

[b] Weight (kg)/height $(m)^2$.

In the multivariable model that mutually adjusted for inflammation-related factors, including LOY, body mass index was most strongly associated with inflammatory biomarkers in both premenopausal and postmenopausal women (Table 3). Every SD increase in body mass index was associated with 84.6% higher CRP levels (95% CI: 78.2, 91.4) in premenopausal women and 53.7% higher levels (95% CI: 48.5, 59.1) in postmenopausal women. By contrast, every SD increment of LOY was associated with 14.5% lower CRP levels (95% CI: −18.7, −10.1) in premenopausal women (SD, 6.3 years) and 8.1% lower levels (95% CI: −11.2, −5.0) in postmenopausal women (SD, 5.5 years). The magnitudes of the associations between LOY and CRP were similar to or stronger than those for hypertension (yes vs. no: 14.3%), smoking (current vs. never: 8.7%), physical activity (per SD increase: −4.7%), and inflammatory diet (per SD increase: 4.6%) in premenopausal women and were comparable to those for age (per SD increase: 12.1%), inflammatory diet (per SD increase: 12.1%), and physical activity (per SD increase: −6.6%) in postmenopausal women. Notably, while age, body mass index, and smoking were also consistently associated with IL-6 and sTNFR2, LOY were only associated with IL-6 in postmenopausal women, as described above.

When evaluating the associations with the individual components of the LOY calculation (Table 4), older age at natural menopause was associated with lower levels of CRP (per 1-year increase, difference = –1.3%, 95% CI: −2.2, −0.3), IL-6 (difference = −0.9%, 95% CI: −1.6, −0.2), and sTNFR2 (difference = −0.4%, 95% CI: −0.6, −0.1). Duration of past OC use was positively associated with CRP levels in both premenopausal (difference = 3.0%, 95% CI:

Table 4. Associations of Inflammatory Biomarkers With Individual Determinants of Number of Lifetime Ovulatory Years, Nurses' Health Study (1989–1990) and Nurses' Health Study II (1996–1999)[a]

LOY Determinant	Inflammatory Biomarker and Menopausal Status					
	CRP, mg/L		IL-6, pg/mL		sTNFR2, pg/mL	
	Premenopausal	Postmenopausal	Premenopausal	Postmenopausal	Premenopausal	Postmenopausal
Age at menopause, years						
<45		2.28		1.32		2,746
45–49		2.07		1.28		2,624
50–54		1.94		1.25		2,610
≥55		2.01		1.14		2,557
P for trend		0.007		0.01		0.004
Duration of OC use, years						
0 (no use)	0.96	1.97	0.96	1.23	2,352	2,693
<1.0	0.89	1.98	1.00	1.32	2,335	2,614
1.0–4.9	0.93	1.90	1.00	1.26	2,354	2,696
5.0–9.9	1.08	2.21	0.99	1.25	2,326	2,577
≥10.0	1.32	2.33	0.99	1.17	2,295	2,590
P for trend	<0.0001	0.002	0.81	0.60	0.11	0.006
Age at menarche, years						
≤11	1.07	1.91	1.00	1.21	2,327	2,623
12	1.02	2.03	0.96	1.22	2,289	2,634
13	1.04	2.08	0.98	1.26	2,349	2,625
14	1.02	2.13	0.98	1.26	2,311	2,680
≥15	0.98	2.22	1.01	1.28	2,386	2,606
P for trend	0.44	0.007	0.51	0.06	0.23	0.82
Parity						
0	1.00	1.97	1.03	1.18	2,363	2,611
1	1.09	2.09	0.94	1.16	2,360	2,619
2	0.99	2.08	0.96	1.31	2,320	2,660
3	1.02	2.05	1.00	1.30	2,333	2,662
≥4	1.02	2.17	1.00	1.29	2,285	2,614
P for trend	0.49	0.09	0.74	0.09	0.12	0.33

Abbreviations: CRP, C-reactive protein; IL-6, interleukin 6; LOY, lifetime ovulatory years; MET, metabolic equivalent of task; OC, oral contraceptive; sTNFR2, soluble tumor necrosis factor α receptor 2.

[a] Multivariable-adjusted geometric mean values are shown. Results were adjusted for age at blood collection (years), smoking status (current, past, or never smoker), body mass index (weight (kg)/height (m)^2), physical activity (<3.0, 3.0–8.9, 9.0–17.9, 18.0–26.9, or ≥27.0 MET-hours/week), hormone therapy use (current, past, or never use; postmenopausal women only), current aspirin use (yes, no), current use of nonsteroidal antiinflammatory drugs (yes, no), inflammatory diet index (*z* score; in quintiles), history of diabetes (yes, no), and history of hypertension (yes, no). Results were mutually adjusted for age at menopause (years; postmenopausal women only), duration of OC use (years), age at menarche (years), and parity (0, 1, 2, 3 or ≥4 pregnancies of >6 months).

2.2, 3.9) and postmenopausal (difference = 1.4%, 95% CI: 0.5, 2.3) women, was not associated with IL-6 levels in either premenopausal or postmenopausal women, and was inversely associated with sTNFR2 levels in postmenopausal women (difference = −0.4%, 95% CI: −0.6, −0.1). Additional analyses in NHSII participants showed that cumulative OC estrogen dose, OC progestin dose, and age at last OC use were strongly positively associated with CRP levels (*P* for trend < 0.0001 for all; Web Table 3); cumulative progestin dose was moderately inversely associated with sTNFR2 levels (*P* for trend = 0.05). Older age at menarche was associated with higher CRP levels only in

postmenopausal women (difference = 3.2%, 95% CI: 0.9, 5.5) and was not associated with IL-6 or sTNFR2 in either group. No significant associations were observed for parity. The results restricted to postmenopausal women who had never used hormone therapy were largely similar (Web Table 2), except that parity was positively associated with CRP (P for trend = 0.003) and IL-6 (P for trend = 0.04) and the associations between OC use and sTNFR2, as well as age at menarche and CRP, were no longer significant. The associations were similar to the main results when the analysis was restricted to NHSII premenopausal women exposed to newer formulations of OCs (Web Table 4).

DISCUSSION

We found that increased years of ovulation were associated with lower CRP levels in both premenopausal and postmenopausal women. The association did not differ by age in premenopausal women, and it persisted for many years after menopause, although the association waned after more than 10 years since menopause. The strength of the association between LOY and CRP was comparable to that for other well-established inflammatory exposures, including physical activity and inflammatory diet, though weaker than the association for body mass index, suggesting that LOY may be a potential marker for systemic inflammation in women. LOY was associated with lower IL-6 levels among postmenopausal women. Younger age at menopause was the individual component factor most consistently associated with unfavorable inflammatory profiles. Our results suggest that longer LOY may be associated with lower chronic systemic inflammation independently of other known factors that modulate inflammation.

We initially hypothesized that longer LOY, which is characterized by more ovulatory/menstrual cycles and thus more local acute inflammatory responses (15), may lead to increased long-term chronic systemic inflammation. Contrary to this, we observed that women with a high estimated lifetime number of ovulations had lower chronic systemic inflammation, as measured by CRP, both before and after menopause. Interestingly, most of the component predictors of estimated LOY were related to CRP, with the strongest associations being observed for age at menopause and duration of OC use. Few studies have examined the long-term associations between reproductive factors and markers of systemic inflammation, particularly taking into account their combined association. For example, in a study of parity and diabetes risk (10), women with more children had higher levels of fibrinogen, a marker of systemic inflammation; no significant difference was observed for leukocyte count. In another small study of 25 fertile Polish women, age at menarche was inversely associated with urinary CRP levels (41); such an inverse association was also noted in the Women's Health Study (42) but was not observed in our study. Further, several studies have shown that current OC use acutely and markedly increases CRP levels (43–46). Similar to increased CRP levels with hormone therapy use (17), this elevation is thought to reflect estrogen-mediated hepatic synthesis of CRP but not necessarily to indicate physiological responses to inflammation (17, 45). Thus, most notable is our finding that premenopausal OC use may have a long-lasting impact on circulating CRP levels among premenopausal women even after use ceases and into the postmenopausal years, decades after stopping the medication. Our results suggest that premenopausal women exposed to newer low-dose OCs had similarly increased CRP levels, but whether this association also persisted into postmenopausal years requires further research. Additional investigation is warranted to understand both short-term and long-term pathogenic implications of OC-modulated CRP elevation.

Older age at natural menopause was consistently associated with lower levels of CRP, IL-6, and sTNFR2. Interestingly, multiple genetic variants identified for age at natural menopause in a meta-analysis of 22 genome-wide association studies were located at genes involved in DNA repair and immune pathways (47). Several of these genes, namely NOD-like receptor family pyrin domain containing 11 (*NLRP11*), interleukin 11 (*IL11*), and proline-rich coiled-coil 2A (*PRRC2A*), play key roles in the activation of proinflammatory responses that may lead to oocyte depletion and early menopause (47, 48). Chronic inflammation throughout reproductive years may also limit the number of ovulatory cycles. Therefore, there may be a complex reciprocal relationship between LOY/age at natural menopause and inflammatory profiles that needs to be elucidated in future studies (Web Figure 1). By contrast, genetic loci identified for age at menarche in genome-wide association studies are implicated in adipogenesis, energy homeostasis, and hormonal regulation, with less direct relevance to inflammation (49). This may explain less consistent associations between age at menarche and inflammatory biomarkers observed in the current study.

Reproductive factors that lead to fewer estimated LOY, such as shorter reproductive life span (mainly driven by early menopause) (1–4), longer duration of OC use (5), and more pregnancies (7–10), have been studied in relation to higher risk of cardiometabolic disease or death. Our findings suggest that inflammatory pathways may be a key mechanism underlying these observations, in addition to hormonal and sociodemographic factors. The inverse associations of LOY with CRP and IL-6 were also consistent with prior findings for several inflammation-related diseases, including increased risk of inflammatory bowel disease associated with OC use (50, 51) and increased risk of rheumatoid arthritis associated with early menopause (52, 53). However, although inflammation is a critical mechanism promoting carcinogenesis (54), our results of lower systemic inflammation with increasing LOY are not consistent with the strong positive associations of LOY with ovarian and endometrial cancers (11–14). This suggests that the repeated local acute inflammation induced by ovulation and menstruation may outweigh the long-term reduction of systemic inflammation for these cancers. It may also explain why age at menopause has been a relatively weak predictor of these cancers despite being a key driver of LOY. Of particular interest in the context of ovarian cancer, the marked risk reduction associated with OC use is attenuated with increasing time since last OC use (55), which may be due to the long-lasting increase in CRP after OC use and the positive association

of CRP with ovarian cancer risk (56). Additional studies are required to understand other mechanisms (e.g., increased genetic instability after ovulation (57)) through which higher LOY may increase gynecological cancer risk.

Our study had several notable strengths. First, the large sample spanning a wide age range during both reproductive and menopausal periods provided a unique opportunity to characterize long-term associations separately in premenopausal and postmenopausal women. On the basis of the 2 birth cohorts, we were able to differentiate between women exposed to different generations of OC formulations, observing similar positive associations between use of newer generations of OCs and CRP that are applicable to women today. Second, given the detailed information on multiple important inflammation-related factors, we were able to identify the independent associations of LOY with inflammatory biomarkers, as well as to compare the associations for LOY with those for other, more established inflammatory factors. Third, the accuracy of self-reported information on reproductive factors has been proven previously, increasing the validity of our results (58). Repeated assessment of these factors through biennial questionnaires further reduced potential measurement error. Finally, although this study was a secondary data analysis, the consistency of biomarker assays and the average batch correction approach enhanced the quality of the pooled data by minimizing extraneous variations across different projects.

However, it should be noted that LOY was only a crude estimate proportional to the number of lifetime ovulatory cycles. Other unconsidered factors, such as menstrual irregularity and variable duration of pregnancy and lactation, may influence the accuracy of LOY estimates and may have led to misclassification that biased the association (11, 12). Another limitation was the homogeneity of the study population, which included predominantly white registered nurses. Future studies are needed to confirm whether our findings can be extrapolated to other populations.

In summary, longer LOY, particularly older age at menopause, was associated with lower levels of chronic systemic inflammation. Our results suggest that reproductive history may be an important source of inflammation in women that is comparable to diet and physical activity. Future studies are needed to clarify the potentially pathogenic role of inflammation linking reproductive factors to chronic disease risk, as well as to evaluate whether assessment of LOY may help inform long-term inflammatory profiles and future disease risk in women.

ACKNOWLEDGMENTS

Author affiliations: Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts (Tianyi Huang, A. Heather Eliassen); Boston Center for Endometriosis, Boston Children's Hospital and Brigham and Women's Hospital, Boston, Massachusetts (Amy L. Shafrir); Division of Adolescent and Young Adult Medicine, Department of Medicine, Boston Children's Hospital and Harvard Medical School, Boston, Massachusetts (Amy L. Shafrir); Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts (A. Heather Eliassen, Shelley S. Tworoger); Division of Women's Health, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts (Kathryn M. Rexrode); Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts (Kathryn M. Rexrode); and Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida (Shelley S. Tworoger).

This work was supported by National Institutes of Health grants UM1 CA186107, UM1 CA176726, R01 CA49449, R01 CA67262, and P01 CA87969. T.H. was supported by National Institutes of Health grant K01HL143034.

Conflict of interest: none declared.

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