The Associations Between Body Mass Index, Smoking, and Alcohol Intake with Ovarian Volume in Midlife Women

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Abstract

Background: Despite the fact that ovarian volume is a marker of reproductive aging, there is little understanding of factors related to ovarian volume among aging women. The objective of this analysis was to examine the associations between body mass index (BMI), cigarette smoking, and alcohol intake with ovarian volume among midlife women.

Materials and Methods: Data were analyzed from 771 women (45–54 years of age at baseline) enrolled in the Midlife Women's Health Study, a cohort study that was initiated in 2006. At annual clinic visits, height and weight were measured, a transvaginal ultrasound was performed to measure ovarian volume, blood was drawn to measure hormone concentrations, and a comprehensive questionnaire was administered. Generalized linear models and repeated measures mixed models were conducted to examine the associations between BMI, cigarette smoking, and alcohol intake with ovarian volume, adjusting for age and race.

Results: Age was significantly and negatively associated with ovarian volume. However, BMI, smoking, and alcohol use were not associated with ovarian volume either when stratified by menopausal status or when adjusting for age and race. Estradiol, but not progesterone or testosterone, was significantly and positively associated with ovarian volume overall and among both white and black participants ($p < 0.05$).

Conclusions: This study provides insight into the associations between BMI, smoking, and alcohol use with ovarian volume among midlife women. The findings are somewhat consistent with the published literature and, thus, indicate that these factors may not be clinically important in terms of ovarian volume during the menopausal transition.

Introduction

AS A WOMAN AGES, the number of primordial follicles in her ovaries diminishes, leading to the loss of reproductive function and the onset of the menopausal transition.¹⁻³ During this time period, women experience changes in the hormonal milieu, including dramatic decreases in endogenous estrogen concentrations.⁴ These changes are accompanied by the appearance of a number of bothersome climacteric symptoms, such as hot flashes,^{5,6} as well as an increased risk of certain adverse health outcomes, such as osteoporosis.⁷

Although not used clinically to determine menopausal status, ovarian volume is a marker of reproductive aging.⁸ It

is associated with changes in hormone levels and loss of ovarian function.⁸ Several studies have shown that ovarian volume decreases across the menopausal transition and, further, with increasing age.⁸⁻¹¹

Despite the fact that ovarian volume is reflective and predictive of the impending changes that occur during the menopausal transition, there is little understanding of factors related to ovarian volume among midlife women. In the published literature, epidemiologic studies have shown that obese women have a smaller mean ovarian volume compared to nonobese women;^{12,13} however, this has not been observed in all studies investigating this association, including a study by Su et al.,¹⁴ which showed that obese women had a higher,

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although nonsignificant, mean ovarian volume than normal weight women. Cigarette smoking has also been hypothesized to be associated with smaller ovarian volume as smoking has been shown to reduce the number of oocytes in the ovary and diminish ovarian reserve.¹⁵ However, several studies have not shown an association.^{9,16} Other factors identified as possible risk factors for reduced ovarian volume include oral contraceptive use¹⁶ and alcohol intake.¹⁷ Most of these previously published studies have been small and have been conducted primarily among premenopausal women; thus, the results may not be applicable to what occurs among women during perimenopause.

The primary goal of this analysis was to examine the associations between body mass index (BMI), cigarette smoking, and alcohol intake with ovarian volume among 771 women aged 45–54 years at baseline participating in the Midlife Women's Health Study. A secondary goal was to analyze the association between ovarian volume and hormone concentrations, including estradiol, among women with these data at baseline. It was hypothesized that BMI, cigarette smoking, and alcohol intake would be negatively associated with ovarian volume and that ovarian volume would be positively associated with estrogen concentrations.

Materials and Methods

Study sample

A cohort study of hot flashes among midlife women (45–54 years of age) was conducted starting in 2006 among residents of the Baltimore metropolitan region, which includes Baltimore City and its surrounding counties. Detailed methods of this study are described by Gallicchio et al.¹⁸ Briefly, women in the selected age range were recruited through mass mailings in the targeted region. Women who were interested in participating in this study, which was presented as a general "Midlife Health Study" to avoid reporting bias, were invited to call the clinic to obtain more information. During this call, the clinic staff determined whether the woman met the eligibility criteria. Women were eligible if they were between 45 and 54 years of age, had intact ovaries and uteri, and were either pre- or perimenopausal. Women were excluded if they were pregnant, had a history of cancer, or were postmenopausal. Women were also excluded if they were taking exogenous female hormones or herbal/plant substances so that we could study risk factors for hot flashes without the confounding effects of known treatments for hot flashes. Menopausal status was defined as follows: premenopausal women were those who experienced their last menstrual period within the past 3 months and reported 11 or more periods within the past year; perimenopausal women were those who experienced (1) their last menstrual period within the past year, but not within the past 3 months, or (2) their last menstrual period within the past 3 months and experienced 10 or fewer periods within the past year; and postmenopausal women were those women who had not experienced a menstrual period within the past year.

If a woman was eligible and interested in participating in the study, she was asked to make a clinic visit (the baseline visit) to a Johns Hopkins clinical site. Neither the baseline visit nor any follow-up visits were scheduled for a specific day or time period within the menstrual cycle as many of the women recruited into the study were perimenopausal and, thus, did

not have regular menstrual cycles. During the baseline clinic visit, the participant completed the detailed 26-page baseline study survey, donated blood and urine samples, was weighed and measured (height), had her blood pressure measured, and received a transvaginal ultrasound to measure ovarian volume. Each participant was then asked to visit the clinic once per week for the three weeks following the baseline visit so that the study staff could obtain additional blood and urine samples. Women also completed a brief questionnaire at the last of the three weekly visits following the baseline visit. These four consecutive weekly clinic visits were then repeated on a yearly basis throughout the woman's participation in the study. The first visit during each of the follow-up years was similar to the baseline visit described above. The remaining three visits were for collection of specimens and administration of the brief questionnaire (final weekly visit of each year only). If a woman missed a single visit or a year of visits, she was still asked to remain in the study, and data from those visits were considered missing. Questionnaire data for the baseline clinic visit and the first yearly visit for each follow-up year were analyzed in this study. Hormone data for each year were calculated by averaging values derived from all the blood samples collected in that year.

Through July 2014, 774 eligible women were enrolled in the study; 772 completed the baseline (year 1) clinic visit and the baseline questionnaire. As of July 2015, these 772 women had been followed for 1–7 years depending on their date of enrollment and whether they returned for their years 2 through 6 annual follow-up visits. Approximately 17% of the participants in the cohort dropped out after the first year of participation and ~5% dropped out after each subsequent year of participation (years 2 through 6). Some reasons for dropout included lack of time or a medical condition. A total of three participants were known to have died during the follow-up time period; these deaths were not study related.

The decision was also made to stop follow-up of women if they reported that they were on hormone therapy ($n=30$), had a hysterectomy and/or oophorectomy ($n=25$), or were diagnosed with cancer ($n=12$). Follow-up was also discontinued for women who were determined to be postmenopausal at the year 4 visit ($n=120$). Therefore, analyses were restricted to data collected from baseline through year 4. One woman was excluded from the analytic data set because she was determined to have premature ovarian failure at baseline.

Of the 771 women in the final analytic data set, 557 had year 2 data, 450 had year 3 data, and 388 had year 4 data. To note, not all 771 women included in the analysis had reached year 3 and year 4 of follow-up at the time of this analysis.

Ethical approval

All participants gave written informed consent according to procedures approved by the University of Illinois and Johns Hopkins University Institutional Review Boards.

Data collection

The transvaginal ultrasound examinations to collect data on ovarian volume were performed using the 7.5 MHz transvaginal probe on a GE transvaginal ultrasound machine without knowledge of the woman's age or menopausal status. Examination of the ovary was established by scanning from the outer to the inner margin.

Height and weight measured at each clinic visit were used to calculate BMI, which was categorized as less than 25 kg/m², 25–29.9 kg/m², and 30 kg/m² or greater. Smoking status at each visit was categorized as current, former, and never using the questions: “Have you ever smoked cigarettes?” and “Do you still smoke cigarettes?” Alcohol intake was based on questions on consumption in the past year as well as the number of drinks per day on average that the woman consumed. Data on race, marital status, and education were collected at baseline (year 1) only.

Hormone assays

Serum concentrations of estradiol, testosterone, and progesterone were measured in all participant samples using enzyme-linked immunosorbent assays (ELISAs). ELISA kits were obtained from Diagnostic Systems Laboratories, Inc. (Webster, TX), and the assays were run using the manufacturer’s instructions.¹⁹ All assays were conducted in the same laboratory. All samples were run in duplicate, and mean values for each participant were used in the analysis. The laboratory personnel were blind with respect to any information concerning study subjects. In addition, positive controls containing known amounts of estradiol, testosterone, and progesterone were included in each batch. Furthermore, some samples were run in multiple assays to ensure that the assay values did not dramatically shift over time.

The minimum detection limits and intra-assay coefficients of variation were as follows: estradiol 7 pg/mL, 3.3% ± 0.17%; testosterone 0.04 ng/mL, 2.2% ± 0.56%; and progesterone 0.1 ng/mL, 2.1 ± 0.65. The average interassay coefficient of variation for all assays was less than 5%.

Statistical analysis

Ovarian volume was calculated using the following formula: length × height × width × 0.526.²⁰ Ovarian volumes greater than 30 cm³ were excluded from the analysis as it was assumed that a volume larger than this value was due to an ovarian cyst. Furthermore, if a cyst was observed on the ovary, volume for that ovary was not calculated. Among women with data on both the ovaries, the right and left ovarian volumes were averaged to create a mean ovarian volume measurement for the participant. If only one ovary was measured, the ovarian volume from this one ovary was used in the analysis. Ovarian volume values were log transformed because the data were not normally distributed; geometric means are reported.

Generalized linear models (GLMs) were conducted to examine the unadjusted associations between selected participant characteristics and ovarian volume at baseline and the year 4 follow-up time point. GLMs were also conducted to evaluate the age-adjusted associations between BMI, cigarette smoking, and alcohol intake category with ovarian volume at each follow-up time point among the three menopausal status subgroups. In addition, repeated measures mixed models with an autoregressive AR1 model were used to examine the associations accounting for within-woman correlation. Similar to the GLM analyses, the repeated measures mixed models were run for each of the three menopausal status subgroups separately and were conducted adjusting for participant age and race. These variables were

entered into the models as covariates because they have been shown to be significantly associated with ovarian volume in other published studies.^{11,16,21} Participant age was analyzed as a time-varying covariate in the repeated measures models. A sensitivity analysis was conducted for BMI using a four-category BMI variable (less than 25 kg/m², 25–29.9 kg/m², 30–34.9 kg/m², and 35.0 kg/m² or greater); however, estimates of ovarian volume in the analyses for the 35.0 kg/m² or greater category were similar to those in the 30–34.9 kg/m² category, and thus, the results using the original three-category BMI variable are reported.

An additional analysis was also conducted examining BMI, cigarette smoking, and alcohol intake at baseline and change in ovarian volume from baseline to year 4 among the women who had ovarian volume data at year 4. These analyses were conducted using GLMs, with baseline age entered as a covariate. The associations between BMI, cigarette smoking, and alcohol intake at baseline and change in ovarian volume were examined in the study sample overall and by baseline menopausal status.

Secondary analyses were conducted to examine the associations between baseline hormone concentrations and baseline ovarian volume using GLMs. The data for each hormone were categorized into tertiles, and the associations were examined for the study sample overall and by race. Baseline age was entered into the GLMs as a covariate as it has been shown in published studies to be associated with both hormone concentrations and ovarian volume.^{11,16}

For all analyses, a two-sided *p*-value of less than 0.05 was considered statistically significant. All analyses were performed using SAS Version 9.1 (Cary, NC).

Results

The final analytic sample comprised 771 women at baseline and 388 women with year 4 follow-up data (Table 1). At baseline, approximately two thirds of the participants were 45–49 years of age (65.1%) and categorized as being of premenopausal status (66.0%). The majority of women were of white race (65.2%), were married or living with partner (64.9%), and had at least some college education (90.1%). Approximately 10% of the women were current smokers, and 33.7% were categorized as being obese (BMI ≥30 kg/m²). Of the women with year 4 follow-up data, about three quarters were 50 years or older (73.7%) and 30.9% were postmenopausal. Similar to baseline, the majority were of white race (67.3%), were married or living with partner (63.7%), and had at least some college education (89.7%). Approximately 9% were current smokers, and 37.1% were categorized as obese.

Of the characteristics examined at baseline and the 4-year follow-up time point, only age and menopausal status were significantly related to ovarian volume (Table 1). Specifically, ovarian volume declined with older age and throughout the menopausal transition. In age-adjusted models, BMI was not significantly associated with ovarian volume among any of the subgroups defined by menopausal status, overall or at any of the follow-up time points (data not shown). In addition, BMI was not significantly associated with ovarian volume among the menopausal status subgroups in repeated measures models adjusted for age and race (Table 2). Similar patterns of finding results were observed for smoking status

TABLE 1. CHARACTERISTICS AND ASSOCIATION OF OVARIAN VOLUME WITH CHARACTERISTICS AT BASELINE AND THE 4-YEAR FOLLOW-UP

Characteristic	Baseline (n = 771)				Four-year follow-up (n = 388)			
	n	%	GM (cm ³)	p	n	%	GM (cm ³)	p
Age, years				<0.0001				0.02
45–49	502	65.1	4.5		102	26.3	3.3	
≥50	269	34.9	3.2		286	73.7	2.6	
Race				0.2				0.1
White	503	65.2	3.9		261	67.3	2.8	
Black	236	30.6	4.3		114	29.4	2.9	
Other	30	3.9	3.5		13	3.4	1.8	
Educational level				0.9				0.9
<College	73	9.5	3.8		38	9.8	3.0	
Some college/technical	205	26.6	4.0		100	25.8	2.6	
Graduated college	223	28.9	4.1		116	29.9	2.8	
Graduate level	267	34.6	4.0		132	34.0	2.8	
Marital status				0.8				0.5
Single	142	18.4	3.9		76	19.6	3.1	
Married/living with partner	500	64.9	4.0		247	63.7	2.7	
Widowed/divorced/separated	126	16.3	4.1		64	16.5	3.0	
Smoking status				0.2				0.6
Current	79	10.2	4.2		34	8.8	2.8	
Former	269	34.9	3.8		147	37.9	2.6	
Never	422	54.7	4.1		207	53.4	2.9	
Menopausal status				<0.0001				<0.0001
Premenopausal	509	66.0	4.6		143	36.9	3.9	
Perimenopausal	262	34.0	3.0		125	32.2	2.8	
Postmenopausal	—				120	30.9	1.7	
BMI, kg/m ²				0.6				0.1
<25	302	39.2	3.9		132	34.0	2.8	
25–29.9	209	27.1	3.9		110	28.4	2.4	
≥30	260	33.7	4.2		144	37.1	3.1	
Alcohol intake in past year				0.8				0.1
Yes, >1 drink/day	285	37.0	3.9		166	42.8	2.5	
Yes, ≤1 drink/day	215	27.9	4.1		92	23.7	2.9	
None	267	34.6	4.1		129	33.2	3.1	

For some characteristics, percentages do not add up to 100% due to missing values.
 BMI, body mass index; GM, geometric mean.

(Table 3) and alcohol intake (Table 4). No statistically significant associations between baseline BMI, smoking status, alcohol intake, and change in ovarian volume from baseline to year 4 were observed, overall or by baseline menopausal status (data not shown).

The age- and smoking-adjusted associations between baseline hormone concentrations and ovarian volume overall

and by race are shown in Table 5. Estradiol concentration was significantly and positively associated with ovarian volume overall and among both white and black participants ($p < 0.05$). In addition, progesterone concentration was significantly and positively associated with ovarian volume among white ($p = 0.02$), but not black participants ($p = 0.5$). When the estradiol and progesterone variables were entered

TABLE 2. ASSOCIATIONS BETWEEN OBESITY AND OVARIAN VOLUME BY MENOPAUSAL STATUS IN MULTIVARIABLE-ADJUSTED^a REPEATED MEASURES MODELS

	BMI ≤25	BMI 25 to <30	BMI ≥30	p-Trend
	Ovarian volume (cm ³) GM (95% CI)	Ovarian volume (cm ³) GM (95% CI)	Ovarian volume (cm ³) GM (95% CI)	
Premenopausal	4.4 (4.2–4.7)	4.6 (4.3–5.0)	4.6 (4.2–4.9)	0.5
Perimenopausal	2.9 (2.6–3.3)	2.9 (2.6–3.2)	3.3 (3.0–3.7)	0.1
Postmenopausal	2.2 (1.8–2.6)	1.8 (1.5–2.2)	2.2 (1.8–2.5)	0.9

^aAdjusted for age and race.

95% CI, 95% confidence interval.

TABLE 3. ASSOCIATIONS BETWEEN CIGARETTE SMOKING AND OVARIAN VOLUME BY MENOPAUSAL STATUS IN MULTIVARIABLE-ADJUSTED^a REPEATED MEASURES MODELS

	<i>Never smoker</i>	<i>Former smoker</i>	<i>Current smoker</i>	
	<i>Ovarian volume (cm³)</i> <i>GM (95% CI)</i>	<i>Ovarian volume (cm³)</i> <i>GM (95% CI)</i>	<i>Ovarian volume (cm³)</i> <i>GM (95% CI)</i>	<i>p-Trend</i>
Premenopausal	4.6 (4.4–4.9)	4.3 (4.1–4.6)	4.7 (4.1–5.4)	0.5
Perimenopausal	3.0 (2.7–3.3)	3.0 (2.7–3.3)	3.4 (2.9–3.9)	0.4
Postmenopausal	2.2 (1.9–2.6)	1.8 (1.5–2.0)	2.6 (2.1–3.4)	1.0

^aAdjusted for age and race.

into the same model, estradiol concentration, but not progesterone concentration, was significantly associated with ovarian volume among the white participants. In contrast, testosterone concentration was not significantly associated with ovarian volume in the study sample overall or among either black or white participants.

Discussion

The results of this study indicate that BMI, smoking, and alcohol intake are not associated with ovarian volume among women undergoing the menopausal transition. These associations were not statistically significant when stratified by menopausal status or after adjusting for age and race. Our results conflict with our initial hypotheses that BMI, cigarette smoking, and alcohol intake are associated with ovarian volume among midlife women.

Previously published studies have reported conflicting results regarding the association between BMI and ovarian volume;^{9,11–14} however, it should be noted that most of these published studies showing statistically significant negative or positive associations for BMI and ovarian volume have been of premenopausal, and not peri- or postmenopausal women. For example, Zaidi et al.¹³ showed that BMI was negatively and significantly correlated with ovarian volume in a sample of 40 fertile women aged 20–39 years; interestingly, this association was not observed among 40 subfertile women of the same ages. Similarly, Halawaty et al.¹² reported that among women in the early transition phase of the late premenopause, the 50 obese study participants had a smaller mean ovarian volume (3.7 mL) compared to the 50 nonobese study participants (6.6 mL) ($p=0.03$), although BMI did not affect other parameters, such as antral follicle count, anti-Müllerian hormone, or follicle-stimulating hormone. Importantly, the two studies by Su et al.¹⁴ and Oppermann et al.¹¹ with samples that are the closest in age range to the present

study found no statistically significant association between BMI and ovarian volume among samples of 36 women aged 40–52 years and 98 women aged 35–55 years, respectively. Thus, BMI appears not to be clinically relevant in terms of ovarian volume during the midlife.

Although it has been hypothesized that the chemicals found in cigarette smoking and the ethanol in alcohol beverages may adversely affect reproductive function, very few studies have examined the relationship between cigarette smoking and alcohol intake with ovarian volume. Consistent with the findings from the present study, both Flaws et al.¹⁶ and Bastos et al.⁹ showed no statistically significant association between cigarette smoking and ovarian volume in cross-sectional studies of 527 pre- and postmenopausal women participating in a uterine fibroid study and 273 women aged 36–62 years living in Southern Brazil, respectively. In contrast, two studies conducted among women undergoing assisted reproductive care showed that smoking was associated with a reduction in ovarian volume²² and a lower mean number of retrieved oocytes.¹⁵ As suggested by Flaws et al.,¹⁶ it is possible that the associations between smoking and ovarian volume differ among healthy women compared to infertile women and that the ovaries from infertile women may be more susceptible to the toxic effects of cigarette smoke than ovaries from healthy women. This hypothesis, however, has not been tested in a research study.

To our knowledge, only one study has been published on the association between alcohol intake and ovarian volume. In that study, Li et al.¹⁷ showed that the amount of alcohol consumed was negatively associated with ovarian volume among 36 Chinese women between the ages of 18 and 29 years. In the study by Li et al.,¹⁷ the sample was recruited specifically based on alcohol consumption; half had moderate alcohol consumption, defined as daily alcohol consumption of more than 15 g, but less than 55 g; alcohol consumption of least 2 days a week, but no more than 5 days a week; and

TABLE 4. ASSOCIATIONS BETWEEN ALCOHOL INTAKE OVER THE PAST YEAR AND OVARIAN VOLUME BY MENOPAUSAL STATUS IN MULTIVARIABLE-ADJUSTED^a REPEATED MEASURES MODELS

	<i>None</i>	<i>≤1 drink/day</i>	<i>>1 drink/day</i>	
	<i>Ovarian volume (cm³)</i> <i>GM (95% CI)</i>	<i>Ovarian volume (cm³)</i> <i>GM (95% CI)</i>	<i>Ovarian volume (cm³)</i> <i>GM (95% CI)</i>	<i>p-Trend</i>
Premenopausal	4.6 (4.3–5.0)	4.5 (4.2–4.8)	4.5 (4.2–4.8)	0.5
Perimenopausal	3.0 (2.6–3.4)	3.1 (2.8–3.5)	3.1 (2.8–3.4)	0.7
Postmenopausal	2.3 (1.8–2.8)	2.1 (1.7–2.7)	1.8 (1.6–2.1)	0.1

^aAdjusted for age and race.

TABLE 5. MULTIVARIABLE-ADJUSTED^a ASSOCIATIONS BETWEEN HORMONE CONCENTRATIONS^b AND OVARIAN VOLUME AT BASELINE, OVERALL AND STRATIFIED BY RACE

	<i>All participants: ovarian volume (cm³)</i>		<i>White women: ovarian volume (cm³)</i>		<i>African American women: ovarian volume (cm³)</i>	
	<i>GM (95% CI)</i>	<i>p</i>	<i>GM (95% CI)</i>	<i>p</i>	<i>GM (95% CI)</i>	<i>p</i>
Estradiol (pg/mL)						
Tertile 1 (5.6 to <43)	3.3 (3.1–3.6)	<0.0001	3.2 (2.8–3.6)	<0.0001	3.6 (3.1–4.2)	0.004
Tertile 2 (43 to <69)	4.0 (3.7–4.4)		4.0 (3.6–4.5)		4.1 (3.6–4.8)	
Tertile 3 (≥69 to 1120)	4.7 (4.3–5.0)		4.5 (4.1–5.0)		5.2 (4.5–6.0)	
Progesterone (ng/mL)		0.0007		<0.0001		0.6
Tertile 1 (0.05 to <0.62)	3.5 (3.2–3.8)		3.1 (2.7–3.5)		4.2 (3.6–4.8)	
Tertile 2 (0.62 to <2.3)	4.2 (3.8–4.6)		4.0 (3.6–4.5)		4.6 (3.9–5.4)	
Tertile 3 (≥2.3 to 32.9)	4.4 (4.0–4.8)		4.5 (4.1–4.9)		4.2 (3.5–4.9)	
Testosterone (ng/mL)		0.3		0.5		0.4
Tertile 1 (0.08 to <0.2)	4.3 (3.9–4.7)		4.2 (3.7–4.7)		4.6 (4.0–5.5)	
Tertile 2 (0.2 to <0.34)	4.0 (3.7–4.4)		3.9 (3.5–4.3)		4.3 (3.7–5.1)	
Tertile 3 (≥0.34 to 41.9)	3.9 (3.6–4.2)		3.8 (3.5–4.2)		4.1 (3.5–4.6)	

^aAdjusted for age.^bBlood was not drawn on a specific menstrual cycle day or within a specific menstrual cycle phase.

consumption of alcohol for at least 3 years, but not longer than 5 years. The difference in the amount of alcohol consumption at the higher levels between the study by Li et al.¹⁷ and the present study, as well as the age difference in study populations, may explain the discrepancy in the findings between alcohol consumption and ovarian volume. It may be that only very high levels of alcohol intake reduce ovarian volume and, thus, reproductive function.

As expected, in the secondary analyses, ovarian volume was positively and significantly associated with estradiol concentrations among both white and black women. This finding shows the internal validity of the data as the association of estradiol with ovarian volume is consistent with what is known about estrogens and ovarian function during the aging process. Estradiol is produced by the ovarian follicles, and ovarian volume is directly reflective of the number of follicles in a woman's ovary. Thus, as a woman ages, and the number of follicles declines due to atresia, the amount of estradiol produced declines with a concomitant reduction in ovarian volume.²³ We also found a statistically significant positive association between ovarian volume and progesterone levels among white women only. Given that ovarian follicles and corpora lutea produce progesterone, it is likely that the decline in the number of ovarian follicles and corpora lutea leads to a decline in progesterone concentration. However, it is unclear why we only observed this association in white women and not in black women. We can only speculate that it is due to the smaller sample size of black women compared to white women.

Several limitations of this study should be noted. First, we did not count the number of ovarian follicles in this study, and it has been shown that the number of follicles is a better marker for ovarian reserve than ovarian volume. Second, there were, overall, a small number of current cigarette smokers (9% at baseline) as well as women of black and other races compared to nonsmokers and women of white race, respectively. The small number may have resulted in inadequate statistical power to observe certain real associations. Furthermore, it is well known that there are limitations in using traditional ELISAs to measure testosterone concen-

trations in women, whose testosterone concentrations are, overall, low because of nonspecific antibody interactions, inconsistent reproducibility, and inadequate sensitivity.^{24,25} However, the ELISA and data analysis methods we used in our study increase our confidence in the reported results; for example, we reran the samples in duplicate as well as in different assays over time to make sure that results were consistent. In addition, we analyzed the data using tertile categories and not the specific values, and while the assays may not yield precise data at very low levels, the categories created would accurately rank the women in terms of high, average, and low concentrations. Finally, the transvaginal ultrasounds were not all conducted by the same physician; thus, there may have been some variability in terms of the ovarian volume data introduced because of operator differences. It should be noted that all transvaginal ultrasounds for this study were conducted on the same instrument.

In addition to the limitations, the study had several strengths. First, this study was one of the largest in terms of sample size to examine BMI, cigarette smoking, and alcohol intake in relation to ovarian volume in any population of women. This allowed for greater statistical power to examine these associations overall and within strata defined by menopausal status. Second, this study is the first, to our knowledge, to examine the relationships between BMI, cigarette smoking, and alcohol intake with ovarian volume longitudinally, assessing associations over the menopausal transition. The longitudinal data allowed for the modeling of associations over time and enabled us to stratify by menopausal status. Third, women taking hormone therapy were excluded from this study, and few women who enrolled reported ever taking hormone therapy (1.6%). In addition, women were discontinued in the study if they reported receiving hormone therapy. Thus, hormone therapy use is unlikely to bias the results of this study (in terms of either confounding or effect modification).

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Author Disclosure Statement

No competing financial interests exist.

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