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Early life body fatness, serum antimüllerian hormone and breast density in young adult women

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Abstract

Background—Emerging evidence suggests positive associations between serum antimüllerian hormone (AMH), a marker of ovarian function, and breast cancer risk. Body size at young ages may influence AMH levels, but few studies have examined this. Also, no studies have examined the relation of AMH levels with breast density, a strong predictor of breast cancer risk.

Methods—We examined associations of early life body fatness, AMH concentrations, and breast density among 172 women in the Dietary Intervention Study in Children (DISC). Height and weight were measured at baseline (ages 8–10) and throughout adolescence. Serum AMH concentrations and breast density were assessed at ages 25–29 at the DISC06 Follow-Up visit. We used linear mixed effects models to quantify associations of AMH (dependent variable) with quartiles of age-specific youth body mass index (BMI) Z-scores (independent variable). We

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assessed cross-sectional associations of breast density (dependent variable) with AMH concentration (independent variable).

Results—Neither early life BMI nor current adult BMI was associated with AMH concentrations. There were no associations between AMH and percent or absolute dense breast volume. In contrast, women with higher AMH concentrations had significantly lower absolute non-dense breast volume (p-trend <0.01).

Conclusions—We found no evidence that current or early life BMI influences AMH concentrations in later life. Women with higher concentrations of AMH had similar percent and absolute dense breast volume, but lower non-dense volume.

Impact—These results suggest that AMH may be associated with lower absolute non-dense breast volume; however, future prospective studies are needed to establish temporality.

Keywords

breast density; breast cancer; antimüllerian hormone; body mass index; childhood; adolescence; epidemiology

Introduction

Childhood and adolescent adiposity is inversely related to breast cancer risk across the life course (1–8). We previously reported strong inverse associations of body fatness during childhood and adolescence with percent breast density, a strong predictor of breast cancer risk, in young adulthood (9). Breast density refers to the proportion of fibroglandular tissue (vs. adipose tissue) in the breast (10). The biological mechanism by which body fatness in youth influences breast density and subsequent cancer risk is not well established. Since most breast development occurs during puberty, body fatness during this time period could have an important impact on breast morphology and breast density later in life, directly influencing breast cancer risk through this pathway.

Body fatness during childhood and adolescence could also decrease breast cancer risk via effects on ovarian function. Specifically, obesity suppresses ovarian function, leading to fewer ovulatory menstrual cycles and altered circulating levels of hormones in adolescent and premenopausal women (11, 12). Antimüllerian hormone (AMH) is an important marker of ovarian function; it is secreted by the ovaries starting in the prepubertal period and plays an important role in the recruitment and growth of follicles, and in the regulation of normal breast development and involution (13). In three recent prospective studies, strong positive associations between serum AMH concentrations and breast cancer risk were observed (14–16), supporting a role for AMH in breast cancer development.

Given the critical role of AMH in ovarian function, and previously demonstrated associations of AMH with breast cancer risk, it is plausible that body size at young ages may influence AMH levels. Inverse associations of AMH with adiposity have been reported in some studies (17–20), while others showed no clear associations (17, 18, 20–28). Further, few studies have examined the possible role of adiposity earlier in life. In addition, no prior studies have examined the relation of AMH levels with breast density. We hypothesized that

body fatness during childhood and adolescence would be inversely associated with AMH levels in young adult women and that AMH levels would be positively associated with high breast density. We examined these associations using prospectively-collected data from the Dietary Intervention Study in Children (DISC) and the DISC 2006 (DISC06) Follow-up Study. Recent evidence suggests that absolute dense and non-dense areas may have independent effects on cancer risk (29, 30); therefore, we also evaluated these phenotypes separately.

Materials and Methods

Study population

The original DISC was a multicenter, randomized controlled trial to examine the safety and efficacy of a dietary intervention to reduce serum low-density lipoprotein cholesterol (LDL-C) in children (31–34). Briefly, between 1988 and 1990, 663 healthy, prepubertal 8–10 year old children, including 301 girls, with elevated LDL-C were recruited to six clinical centers and randomized to a behavioral dietary intervention or usual care control group. Children participated in annual clinic visits until the trial was terminated in 1997, when the average age of participants was 16.7 years, due to a lack of treatment effect on LDL-C. At the time of the original DISC, assent was obtained from participants; their parents/guardians provided informed consent before randomization. All female DISC participants were invited to participate in the DISC06 Follow-up Study, and 260 (86.4%) of the 301 females originally randomized in DISC participated (35). Participants were re-consented before the DISC06 Follow-Up Study, which took place in 2006–2008 when participants were 25 to 29 years old. The original DISC protocol was approved by an NHLBI-appointed independent data and safety monitoring committee and institutional review boards (IRBs) at all participating clinical centers and the data coordinating center. The DISC06 Follow-Up Study protocol was approved by IRBs at the Fox Chase Cancer Center, participating clinical centers, and the data coordinating center (please see Acknowledgments).

Participants in this analysis included 172 women who enrolled in the original DISC between 1988 and 1990, when they were ages 8–10 years, and also participated in the DISC06 Follow-up Study, when they were ages 25–29 years. Women who were pregnant or breastfeeding within 12 weeks (n=30) before their clinic visit, had breast implants or reduction surgery (n=16), or whose MRI was missing or of poor quality (n=32) were excluded. Ten women without AMH measurements were also excluded.

Data collection

During the original DISC, height and weight were measured at baseline and annual clinic visits by trained study staff blinded to treatment assignment. Specifically, height was measured using a stadiometer and weight was measured on an electronic or beam balance scale. Each measurement was made twice. A third measurement was taken if the first two measurements were not within allowable tolerances (0.5 cm for height and 0.2 kg for weight) and the two closest values were averaged. BMI was calculated as weight (kg)/height (m^2). Because the interpretation of BMI in children and adolescents is specific to age and sex, we expressed the age-specific BMI as a Z-score relative to CDC 2000 Growth Charts

for girls (36). Information on demographic and other characteristics, including medical history, reproductive factors, was ascertained on annual questionnaires while diet and physical activity were assessed at baseline and years 1, 3, 5 and last childhood visits (35, 37). DISC06 follow-up visits took place at the original 6 DISC clinics and were scheduled during the luteal phase of the menstrual cycle when possible (>85% of visits occurred within 14 days of onset of next menses). Medical history and other information was updated at the DISC06 follow-up visit.

Blood collection and laboratory assays

Blood samples were collected at the DISC06 follow-up visit in the morning after an overnight fast by venipuncture using standard procedures. Blood was allowed to stand at room temperature for 45 minutes to allow complete clotting. Blood was then centrifuged and serum was separated and pipetted in 0.5 mL aliquots into cryovials, which were labeled and stored at −80°C.

AMH was measured in serum using single lot of picoAMH ELISA kit (AL-124, Ansh Labs, Webster, TX) (38). The limit of detection (LOD) of the picoAMH ELISA is 1.2 pg/mL; no participant samples were below the LOD. The manufacturer-specified interassay coefficients of variation (CVs) are 4.5%, 2.2%, and 3.8% at 22.6, 86.5, and 373 pg/mL, respectively, for the picoAMH assays. Internal quality control samples included in the assay run resulted in CVs of 3.2% and 4.0% at mean AMH concentrations of 91.2 and 285 pg/mL, respectively. Finally, four participant samples were run in duplicate with mean CV of 4.7%.

Breast density assessment

At the DISC06 follow-up visit, breast density was assessed following a standardized protocol by non-contrast MRI. Equipment standards at each site were consistent with American College of Radiology guidelines for breast MRI (39) and required that imaging be performed using a whole-body MRI scanner of 1.5 Tesla or higher field strength and a dedicated breast imaging radiofrequency coil. A standard image-acquisition protocol was prescribed consisting of two pulse sequences performed in both the transaxial and coronal orientations with a 32 to 40 cm field of view for bilateral coverage: a three-dimensional fast gradient echo sequence without fat suppression, and a three-dimensional fast gradient echo sequence with fat suppression.

To ensure accuracy and uniformity of data acquisition at the different clinical centers, MRI technologists at the sites were trained (by C. Klifa) to recognize and correct failures due to incomplete fat suppression, motion artifacts, and inadequate breast coverage. In addition, acceptable image quality on three volunteers was required for site certification. Participant scans that were inaccurate due to artifacts, motion or technique were excluded (n = 21).

All MRI image data were processed at the University of California, San Francisco by C. Klifa using customized software to identify the chest wall-breast tissue boundary and skin surface, and to separate breast fibroglandular and fatty tissue using a segmentation method based on fuzzy C-means clustering (40). Total volumes of fibroglandular and fatty tissue were computed separately for each breast and averaged for analysis. Outcomes of interest were percent dense breast tissue volume (ratio of fibroglandular volume to total volume of

the breast), absolute (total) volume of dense tissue (fibroglandular volume), and absolute (total) volume of non-dense tissue (fatty breast tissue volume).

Statistical analyses

We used linear mixed effects models to examine the association between body mass index (BMI) Z-scores at baseline (ages 8–10 years) (predictor) and AMH at ages 25–29 (outcome). The analysis was repeated, changing the predictor to BMI Z-score at each annual clinic visit during childhood and adolescence; for simplicity, we present results for baseline, Year 3, Year 5, and last childhood visits. For our predictor, body fatness during childhood, we created quartiles of age- and female-specific BMI Z-scores computed from CDC growth charts (36). We also assessed the cross-sectional association between BMI in kg/m^2 at the follow-up visit and AMH concentration at the same visit.

To improve normality of the outcome, we applied a natural log transformation to measured AMH concentration. We used the generalized extreme Studentized deviate many-outlier procedure (41) to formally identify potential outliers in AMH values; none were identified. Linear mixed effects models were fit by maximum likelihood with clinic included as a random effect and empirical (“robust”) standard errors to allow for correlated outcomes within clinics. The associations between quartiles of childhood BMI Z-scores and adult AMH concentration were quantified by adjusted least square means and 95% confidence intervals (CIs). Because of the log-transformation, we applied Duan’s “smearing estimate” to back-transform AMH estimates to original (untransformed) units of ng/mL; this method adjusts for bias arising in retransformation of estimates from nonparametric or generalized linear models (42). Tests for trend were based on models including childhood or adolescent BMI Z-score (or current BMI) as a continuous variable. All models were adjusted for clinic as a random effect and treatment assignment as a fixed effect. Subsequent models additionally controlled for current BMI and current BMI-squared – to allow for a possible curvilinear association with BMI – (Model 2) and age at menarche, duration of hormone use, number of live births, race, education, alcohol consumption, smoking status, and family history of breast cancer (Model 3). Categorical covariates were categorized as shown in Table 1a.

The same analytic approach (i.e., linear mixed effects models to estimate least square means and 95% confidence intervals) was used to examine the cross-sectional associations of quartiles of log-transformed AMH (now as the independent variable) concentrations with measures of breast density (i.e., percent dense breast volume, absolute dense breast volume, and absolute non-dense breast volume) (dependent variable). For the absolute measures of breast density, we applied natural log transformation and back-transformed estimates to original units of cm^3, as described above. Multivariable models were adjusted for the same covariates listed previously. Further adjustment for menstrual cycle day of blood collection or testosterone levels did not appreciably change the beta coefficients; therefore, these variables were not retained in the final model. All analyses were carried out using SAS 9.3 (Cary, NC).

Results

Women were on average 27 years old at the DISC06 follow-up visit, with mean BMI 25.4 kg/m2, mean percent dense breast volume 27.4%, and mean AMH concentration 4.2 ng/mL. The majority of women were white, educated, nulliparous, and were past or current users of hormonal contraceptives (Table 1). Participant characteristics were generally similar across quartiles of AMH levels; however, women in the lowest quartile of AMH had lower percent dense breast volume (23.5%) compared to those in the top quartile (29.1%), primarily reflecting greater non-dense breast volume in the lowest quartile group. Women in the lowest quartile of AMH were also somewhat more likely to be parous and to be current users of hormonal contraceptives compared to the other three quartiles (Table 1).

Neither early life BMI nor current adult BMI were associated with AMH concentrations in this population. In fully-adjusted multivariable models, adjusted mean AMH concentrations were 2.8, 3.1, 3.9, and 3.0 for successive quartiles of BMI Z-score at baseline clinic visit (ages 8–10) (p-trend, 0.62). Similarly null results were observed for BMI assessed at the other clinic visits during childhood and adolescence, as well as for current adult BMI at the DISC06 follow-up visit (Table 2).

In cross-sectional analyses adjusting for clinic and treatment assignment only, there was a suggestive, but non-significant, positive association between AMH and percent dense breast volume. Mean percent dense breast volume was 23.5% (95% CI: 16.3, 30.7) in the lowest quartile of AMH vs. 29.3% (95% CI: 19.6, 30.0) in the highest quartile (p-trend, 0.24). However, the apparent association was substantially attenuated upon adjustment for current BMI, a strong negative predictor of percent dense breast volume. In the fully-adjusted multivariable model, the corresponding means for percent breast density were 25.8% (95% CI: 20.1, 31.5) and 25.8% (95% CI: 20.0, 31.6) (p-trend, 0.54). Similarly, there was no apparent association of AMH concentration with absolute dense breast volume. Women with higher AMH concentrations, however, had significantly lower absolute non-dense breast volume after controlling for predictors of breast density and absolute dense volume. Specifically, mean absolute non-dense breast volume was 328.7 cm^3 (95% CI: 275.8, 391.7) among women in the lowest quartile of AMH compared to 280.6 cm^3 (95% CI: 231.8, 339.6) for the highest quartile (p-trend <0.01) (Table 3).

Results were similar among nulliparous women (n=122) and parous women (n=50) and among ever/former hormonal contraceptive users (n=161) (data not shown). There were too few women (n=11) reporting never using hormonal contraceptives for meaningful subanalysis in this group. Finally, there was no significant interaction between AMH and treatment group assignment for any of the density phenotypes evaluated (p-interaction >0.05).

Discussion

In summary, we found no evidence that earlier life BMI influences AMH concentrations in young adulthood. Similarly, current BMI was not associated with AMH concentrations. We also found no association between AMH and percent or absolute dense breast volume

measured concurrently; however, AMH was inversely associated with absolute non-dense breast volume.

Early life, including childhood and adolescence, is hypothesized to be a critical time window for breast carcinogenesis (43). This is a time of rapid growth and development, with especially high rates of mammary gland cell proliferation during puberty, which could increase vulnerability to molecular damage and explain why exposures during this time period might be important for breast density and breast cancer risk later in life (43–45). An inverse association between early life adiposity and breast cancer risk is well established (1–8). Our previous analyses in this population demonstrated an inverse association between youth adiposity and dense breast volume (9), in agreement with several other studies of this association (46–51). Given that obesity is known to influence ovarian function (11, 12), body fatness during childhood and adolescence could also decrease breast cancer risk by influencing AMH levels. However, our null results do not support this hypothesis. Similarly, in a birth cohort study of >1300 adolescent girls (mean age 15.5), neither birth weight nor current BMI were associated with AMH levels (52, 53). Cross-sectional analyses in Chinese girls also reported no associations of BMI in childhood (ages 0–10 years) or adolescence (ages 11–18 years) with AMH measured concurrently (54). In contrast, in a study of adolescent girls who were normal (n=43), oligomenorrheic (n=27), or had polycystic ovarian syndrome (n=150), AMH levels were significantly lower in obese compared to non-obese girls within each group (55). Another study of 10 normal weight and 10 obese ovulatory young women between ages 18 and 35, AMH levels were 34% lower in the obese group (17). AMH levels peak in the mid-20s and subsequently decline with age, becoming non-detectable by menopause (15, 19, 56, 57). Since participants in the DISC06 Follow-up Study were ages 25–29 and consequently had relatively high AMH concentrations, there may have been insufficient variation at low concentrations in AMH levels to observe associations with current or earlier life BMI.

Epidemiological evidence for associations between current adult BMI and serum AMH levels is mixed. Similar to our findings, many studies have not found an association between current BMI and serum AMH levels (21–26); however, some studies have reported lower AMH levels among obese women (17–20). In light of these conflicting results, some researchers have suggested that inverse associations between BMI and AMH in some studies may reflect residual confounding by age (which is inversely related to AMH) (22) or the phenomenon of serum hormone dilution due to increased blood volume among larger women (21).

To date, only five studies have evaluated AMH and breast cancer risk. Of 30 women undergoing breast biopsy, 22 were determined to have cancer or precancer and these women had significantly lower AMH levels than those with benign breast disease ($p<0.001$) (58). However, in a case-control study among women ages 28–44 (108 cases, 99 controls), there was no significant difference in AMH levels by case status (59). Neither of these studies measured AMH prior to breast cancer diagnosis, however, thus the influence of disease could not be ruled out. The first prospective analysis to evaluate this association (n=105 cases, 204 controls) showed a strong positive association between serum AMH levels and breast cancer risk (p-trend <0.001) (14). These findings were recently supported by two

case-control studies nested within the Sister Study cohort (n=452 cases, 902 controls) (15) and the Nurses' Health Study II (n=539 cases, 471 controls) (16), both of which also demonstrated a significant positive association between AMH concentrations and breast cancer risk, with a more than 2-fold increased risk among women with the highest AMH concentrations compared to those with AMH <LOD.

To our knowledge, no previous studies have evaluated possible associations of AMH with breast density. An important limitation of this analysis is its cross-sectional design: AMH and breast density were measured concurrently, limiting inferences about a possible temporal association. Also, because the original DISC study population excluded children whose weight-for-height was greater than the 90th percentile or lower than the 5th percentile at baseline (31), our findings may not be generalizable to very lean or very obese children. Major strengths of this study include the objective and repeated measures of childhood and adolescent weight and height. In addition, while confounding by unmeasured factors cannot be ruled out, we had detailed questionnaire information and the ability to adjust for many potential confounders. We used an ultra-sensitive AMH assay with demonstrated reproducibility. Similarly, systematic measurement of breast density via MRI afforded us the ability to consider dense vs. non-dense breast volume separately.

While we observed no apparent association between AMH concentrations and percent or absolute dense breast volume in cross-sectional analyses, our results suggest that higher AMH levels may be associated with lower absolute non-dense breast volume. Considering recent evidence that suggests higher amounts of non-dense breast tissue may be inversely associated with breast cancer risk, independent of absolute dense breast tissue amount (29, 30), our findings, if confirmed in future studies, could be consistent with the hypothesis that AMH is associated with increased breast cancer risk. However, future prospective studies are needed to establish temporality of associations of AMH with breast density.

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Table 1

Participant characteristics at the DISC06 follow-up visit, overall and by quartile of AMH

Descriptive characteristic	n	Overall Mean (SD) or %	Q1 n=43	Q2 n=43	Q3 n=43	Q4 n=43
Age (y)	172	27.2 (1.0)	27.2 (1.0)	27.1 (1.1)	27.2 (1.0)	27.2 (1.0)
Percent dense breast volume (%)	172	27.4 (20.0)	23.5 (19.8)	26.7 (19.9)	30.0 (19.6)	29.1 (20.7)
Absolute dense breast volume (cm^3)	172	104.0 (70.3)	101.0 (73.0)	110.5 (69.2)	110.1 (83.0)	94.4 (51.0)
Absolute non-dense breast volume (cm^3)	172	418.7 (369.3)	465.6 (348.9)	459.4 (426.6)	362.7 (327.2)	387.0 (369.3)
AMH (ng/mL)	172	4.21 (3.28)	1.2 (0.4)	2.6 (0.5)	4.3 (0.6)	8.7 (3.2)
BMI (kg/m^2)	172	25.4 (5.4)	25.8 (5.0)	26.0 (6.2)	25.1 (4.6)	24.9 (5.6)
Height (cm)	172	165.4 (6.4)	163.6 (5.8)	166.6 (6.1)	165.0 (7.0)	166.2 (6.3)
BMI z-score at 8–10 years old	172	0.23 (0.90)	0.28 (1.0)	0.35 (0.9)	0.15 (0.8)	0.15 (0.9)
Age at menarche (y)	172	12.9 (1.3)	12.6 (1.1)	13.0 (1.3)	12.9 (1.3)	13.1 (1.4)
Duration of hormonal contraceptive use (y)*	161	5.6 (3.5)	6.7 (3.1)	5.6 (3.9)	4.9 (3.6)	5.3 (3.1)
Menstrual cycle length (days)**	71	27.9 (2.6)	26.1 (3.9)	27.7 (2.1)	28.9 (1.6)	28.2 (2.6)
Race						
White	155	90.1%	95.3%	93.0%	86.0%	86.0%
Non-white	17	9.9%	4.7%	7.0%	14.0%	14.0%
Education						
High school, vocational, or technical school	17	9.9%	9.3%	9.3%	9.3%	11.6%
Some college	41	23.8%	30.2%	14.0%	30.2%	20.9%
College / Bachelor's	92	53.5%	53.5%	72.1%	39.5%	48.8%
Graduate school	22	12.8%	7.0%	4.7%	20.9%	18.6%
Number of live births						
0	122	70.9%	60.5%	76.7%	72.1%	74.4%
1	29	16.9%	23.3%	18.6%	9.3%	16.3%
2+	21	12.2%	16.3%	4.7%	18.6%	9.3%
Ever breast fed (among parous)						
Yes	38	74.5%	88.2%	60.0%	66.7%	81.8%

Descriptive characteristic	n	Overall Mean (SD) or %	Q1 n=43	Q2 n=43	Q3 n=43	Q4 n=43
No	13	25.5%	11.8%	40.0%	33.3%	18.2%
Hormonal contraceptive use						
Never	11	6.4%	4.7%	4.7%	9.3%	7.0%
Former	62	36.1%	20.9%	39.5%	37.2%	46.5%
Current	99	57.6%	74.4%	55.8%	53.5%	46.5%
Family history of breast cancer						
Yes	7	4.1%	0.0%	7.1%	7.1%	2.4%
No	161	95.9%	100.0%	92.9%	92.9%	97.6%
Alcohol consumption						
Never/former	15	8.7%	11.6%	9.3%	4.7%	9.3%
Current, <3 per week	67	39.0%	32.6%	51.2%	39.5%	32.6%
Current, 3–<6 per week	32	18.6%	27.9%	20.9%	14.0%	11.6%
Current, 6–<10 per week	37	21.5%	14.0%	14.0%	32.6%	25.6%
Current, 10+ per week	21	12.2%	14.0%	4.7%	9.3%	20.9%
Smoking history						
Never	94	54.7%	51.2%	62.8%	53.5%	51.2%
Former	36	20.9%	25.6%	11.6%	23.3%	23.3%
Current	42	24.4%	23.3%	25.6%	23.3%	23.3%
Treatment assignment						
Intervention	86	50.0%	51.2%	44.2%	60.5%	44.2%
Usual care	86	50.0%	48.8%	55.8%	39.5%	55.8%

*Among current or former hormonal contraceptive users

**Among women not using hormonal contraceptives

Table 2

Mean and 95 % confidence interval (CI) for AMH concentration (ng/mL) at the DISC06 follow-up visit according to quartile of age-specific BMI z-score

Quartile of age-specific BMI z-score:		Q1	Q2	Q3	Q4	p-value*
BMI at baseline visit (ages 8–10)	Model 1	3.33 (2.48, 4.45)	2.93 (2.38, 3.60)	3.39 (2.58, 4.46)	2.90 (2.33, 3.61)	0.43
n=172	Model 2	3.14 (2.46, 4.02)	2.90 (2.41, 3.47)	3.48 (2.52, 4.82)	3.02 (2.31, 3.96)	0.90
	Model 3	2.82 (2.18, 3.64)	3.06 (2.74, 3.43)	3.85 (2.62, 5.70)	2.98 (2.46, 3.61)	0.62
BMI at Year 3 visit (ages 11–13)	Model 1	3.57 (2.67, 4.77)	3.10 (2.60, 3.69)	2.76 (2.01, 3.80)	2.93 (2.39, 3.56)	0.52
n=161	Model 2	3.53 (2.70, 4.62)	3.13 (2.65, 3.69)	2.78 (1.95, 4.00)	2.90 (2.25, 3.73)	0.78
	Model 3	3.32 (2.44, 4.52)	3.28 (2.87, 3.74)	2.88 (2.01, 4.13)	2.87 (2.24, 3.67)	0.80
BMI at Year 5 visit (ages 13–15)	Model 1	3.40 (2.82, 4.10)	3.21 (2.25, 4.59)	2.68 (2.03, 3.54)	3.13 (2.41, 4.06)	0.44
n=146	Model 2	3.22 (2.44, 4.26)	3.11 (2.25, 4.30)	2.81 (1.92, 4.13)	3.23 (2.27, 4.60)	0.80
	Model 3	2.68 (1.98, 3.62)	3.28 (2.48, 5.09)	3.27 (2.10, 5.09)	3.22 (2.19, 4.75)	0.62
BMI at last visit (ages 15–17)	Model 1	3.78 (2.62, 4.43)	2.57 (2.02, 3.26)	2.68 (2.11, 3.41)	3.12 (2.23, 4.37)	0.09
n=149	Model 2	3.42 (2.43, 4.81)	2.52 (2.00, 3.17)	2.85 (2.17, 3.74)	3.30 (2.18, 4.98)	0.06
	Model 3	2.98 (2.13, 4.17)	2.62 (2.07, 3.31)	3.29 (2.55, 4.25)	3.14 (2.03, 4.85)	0.77
BMI† at follow-up visit (ages 25–29)	Model 1	3.50 (2.50, 4.91)	2.84 (2.20, 3.66)	3.38 (3.11, 3.67)	2.85 (2.27, 3.56)	0.47
n=172						
	Model 3	3.53 (2.43, 5.11)	3.12 (2.51, 3.89)	3.48 (3.04, 3.98)	2.58 (1.86, 3.58)	0.48

Model 1 Means estimated from linear mixed effects models including clinic as a random effect and treatment group as a fixed effect.

Model 2 Means estimated from linear mixed effects models including clinic as a random effect and adjusted for treatment group and current BMI (continuous, kg/m^2 and squared BMI) as fixed effects.

Model 3 Means estimated from linear mixed effects models including clinic as a random effect and adjusted for treatment group, current BMI (continuous, kg/m^2 and squared BMI), number of live births, duration of hormone use, age at menarche, race, education, alcohol consumption, smoking status, and family history of breast cancer, as fixed effects (4 missing).

*test for trend

†Quartiles of BMI in kg/m^2 (untransformed); cut-points are Q1: <21.4, Q2: 21.5–24.1, Q3: 24.1–28.7, Q4: >28.7

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Table 3

Mean and 95 % confidence interval (CI) for three breast density phenotypes at the DISC06 follow-up visit according to quartile of AMH (n=172)

Quartile of AMH:	Q1	Q2	Q3	Q4	p-value*
Percent dense breast volume					
Model 1	23.5 (16.3, 30.7)	26.9 (23.0, 30.9)	29.7 (22.8, 36.6)	29.3 (19.6, 39.0)	0.24
Model 2	25.5 (18.4, 32.5)	27.5 (24.8, 30.2)	29.8 (25.2, 34.3)	26.6 (25.2, 34.3)	0.36
Model 3	25.8 (20.1, 31.5)	27.9 (25.4, 31.3)	29.6 (25.7, 33.4)	25.8 (20.0, 31.6)	0.54
Absolute dense breast volume (cm^3)					
Model 1	69.1 (54.2, 88.0)	88.9 (81.2, 97.3)	84.6 (68.3, 104.7)	76.3 (50.7, 114.9)	0.46
Model 2	72.0 (56.2, 92.1)	90.0 (77.6, 104.5)	85.1 (69.0, 104.9)	73.2 (50.2, 106.7)	0.68
Model 3†	70.3 (53.9, 91.7)	90.0 (73.0, 111.1)	89.2 (70.4, 113.0)	72.0 (55.0, 94.2)	0.55
Absolute non-dense breast volume (cm^3)					
Model 1	356.8 (287.4, 442.9)	323.4 (256.7, 407.4)	258.8 (256.7, 407.4)	247.2 (188.7, 323.9)	0.03
Model 2	335.2 (275.3, 408.3)	307.2 (282.6, 334.0)	263.2 (220.4, 314.3)	272.9 (232.3, 320.6)	<0.01
Model 3≠	328.7 (275.8, 391.7)	303.7 (278.4, 331.2)	267.4 (234.2, 305.3)	280.6 (231.8, 339.6)	<0.01

Model 1 Least square means estimated from linear mixed effects models including clinic as a random effect and treatment group as a fixed effect.

Model 2 Least square means estimated from linear mixed effects models including clinic as a random effect and adjusted for treatment group and current adult BMI (continuous, kg/m^2) as fixed effects.

Model 3 Least square means estimated from linear mixed effects models including clinic as a random effect and adjusted for treatment group, current adult BMI (continuous, kg/m^2), number of live births, duration of hormone use, age at menarche, race, education, alcohol consumption, smoking status, and family history of breast cancer, as fixed effects (4 missing).

*test for trend;

†additionally adjusted for log-non-dense volume;

≠additionally adjusted for log-dense volume.