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Relating smoking, obesity, insulin resistance, and ovarian biomarker changes to the final menstrual period

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To determine if smoking, obesity, and insulin resistance mediated age at final menstrual period (FMP), we examined anti-Müllerian hormone (AMH), inhibin B, and follicle-stimulating hormone (FSH) as biomarkers of changing follicle status and ovarian aging. We performed a longitudinal data analysis from a cohort of premenopausal women followed to their FMP. Our results found that smokers had an earlier age at FMP ($P < 0.003$) and a more rapid decline in their AMH slope relative to age at FMP ($P < 0.002$). Smokers had a lower baseline inhibin B level relative to age at the FMP than nonsmokers ($P = 0.002$). Increasing insulin resistance was associated with a shorter time to FMP ($P < 0.003$) and associations of obesity and time to FMP were observed ($P = 0.004$, in model with FSH). Change in ovarian biomarkers did not mediate the time to FMP. We found that smoking was associated with age at FMP and modified associations of AMH and inhibin B with age at FMP. Insulin resistance was associated with shorter time to FMP independent of the biomarkers. Interventions targeting smoking and insulin resistance could curtail the undue advancement of reproductive aging.

Keywords: obesity; insulin resistance; smoking; anti-Müllerian hormone; inhibin B; menopause

Introduction

Epidemiological studies have reported that cigarette smoking leads to reduced ovarian function and fertility and an earlier age at menopause,[1] and both body size and insulin resistance have been variably associated with measures of ovarian function.[2] The degree to which environmental factors, such as smoking, obesity, and insulin resistance, may impact follicle number and quality is an important question as the nature and quantity of ovarian reserve is indicative of reproductive capacity and the time remaining during which conception can occur prior to the menopause. Increasing evidence suggests that measured anti-Müllerian hormone (AMH) and inhibin B in conjunction with follicle-stimulating hormone (FSH) could reflect the shrinking ovarian reserve over time and provide a useful means of investigating the impact of environmental factors.

In women, AMH (Müllerian Inhibiting Substance [MIS]) from the granulosa cells of ovarian follicles reflects the transition of resting primordial follicles into growing primary and secondary follicles and the subsequent recruitment of FSH-sensitive follicles in the early antral stage.[3–5] Because AMH is produced only in growing ovarian follicles, serum AMH levels are regarded as a direct indicator of ovarian reserve, representing the quantity and quality of the recruitable ovarian follicle pool.[6] We have identified a linear decline of $_{\log}$AMH to low or nondetectable levels 5 years prior to the natural final menstrual period (FMP).[7]

Inhibin B is the primary inhibin produced by the small antral follicles; its levels have been interpreted as indicating growth of the antral follicle cohort.[8] Produced by granulosa cells, inhibin B suppresses FSH secretion through direct negative feedback to the pituitary.[9–11] There is a curvilinear decline of follicular-phase $_{\log}$inhibin B to

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undetectable levels 4–5 years prior to the natural FMP.[7]

Reproductive aging is also characterized by a progressive rise in serum FSH levels and reduced levels of ovarian steroids.[12–14] This FSH rise, a central endocrine feature of the perimenopause, was described by Sherman and Korenman in 1975,[15] and has been confirmed in subsequent epidemiological studies of the menopausal transition.[16–18] Globally, the FSH rise is associated with a progressive loss of ovulatory function.[19]

The degree to which environmental factors, such as smoking, obesity, and insulin resistance, impact the association of these ovarian markers in their relation to time to FMP and age at FMP is the subject of this research. The goal was to determine if smoking behavior, obesity, and HOMA-IR, an indicator of insulin resistance among nondiabetics, were associated with time to FMP independent of age and age at FMP. Further, we evaluated if women who smoked or were more insulin resistant had different AMH, inhibin B, and FSH profiles assuming that these biomarkers reflected ovarian aging.

Methods and procedures

Population

This report is based on data from 50 Michigan Bone Health and Metabolism study (MBHMS) enrollees of a possible 629 women. The women were pre- and early perimenopausal at their baseline evaluation. Archival serum specimens from the initial six consecutive annual visits were assayed for AMH, inhibin B, and FSH. In addition, archival information from physical measurements and interviews were used to identify obesity and smoking behavior at these visits. MBHMS enrollees were followed annually, allowing the subsequent documentation of their FMP.

The organization of the population-based MBHMS cohort has been described.[20] It was organized in 1992 from two sampling sources including a list of the female offspring, aged 24–44 years, from the community-based Tecumseh (Michigan) Community Health Study (TCHS) enrollees and Kohl's Directory, a listing of community female residents (also aged 24–44) whose parents had not participated in the TCHS. This report includes data collected during the 15-year period from 1992/1993 through 2006/2007, excluding the 18- and 14-month lapses in funding in 1997 and 2003, respectively. Written informed consent has been obtained from all participants; this study has been approved by the University of Michigan Institutional Review Board. Since study inception, the annual cohort visits have included interviews about health status, menstrual bleeding patterns and health-related behaviors, and phlebotomy to provide serum and urine specimens for assay of hormones, metabolic measures, and repository storage.

To develop this substudy, which has been reported,[7] we selected and assayed repository specimens to correspond in time to 6 yearly measures, beginning in 1993, when regularly cycling (nine or more menstrual cycles per year) women were in their pre- and early menopause stages. This was to assure that we could examine both level and rate of change in the ovarian markers as women entered the menopause transition. Therefore, women were eligible for inclusion in this substudy only if their FSH values in 1992/1993 or 1993/1994 were $\leq$14 mIU/mL. Then, women were selected for study if they had a naturally occurring FMP by the 2006 annual visit (within the 13-year period after the baseline) and if their age at FMP was more than 41 years and therefore not reflective of factors thought to contribute to premature ovarian aging. Further, these women had no exposure to hormone therapy use or gynecological surgeries during the 13-year study period. It was important to be able to examine rates of change in the ovarian markers to address our hypotheses that smoking, insulin resistance and obesity altered ovarian aging and were thereby associated with menopause characteristics.

Data from 50 women meeting these eligibility criteria were selected. They were, on average, 4 years older than women not selected (a mean 1992 baseline age of 41 [SD = 2.6] years versus 38 years [SD = 5.0]). Initial body mass index (BMI), insulin resistance index, and smoking frequency were not statistically significantly different in selected versus nonselected women.

Specimens and assays

Specimens were collected fasting in days 2–7 of the follicular phase of the menstrual cycle, aliquoted and stored at –80° C without thaw until assay. A commercially available enzyme-linked immunosorbent assay (ELISA) from Diagnostic Systems Laboratories (Beckman Coulter, DSL,

Webster, TX) was used for the *in vitro* quantitative measurement of Müllerian Inhibiting Substance/Anti-Müllerian Hormone in human serum. This ELISA is a direct competitive immunoassay without sample extraction or hydrolysis. The detection system consisted of a biotinylated secondary antibody and streptavidin-labeled horseradish peroxidase. Samples were assayed in duplicate. There is no detectable cross-reactivity with closely related compounds. The assay measured AMH concentrations ranging from 0.017 to 10 ng/mL with an assay range (standard curve) of 0.05 to 10 ng/mL. Manufacturer-specified interassay coefficients of variation (CV) were 8.0% at 0.15 ng/mL, 4.8% at 0.85 ng/mL, and 6.7% at 4.28 ng/mL (mean = 6.5%); intra-assay CVs were 4.6% at 0.14 ng/mL, 2.4% at 0.84 ng/mL, and 3.3% at 4.41 ng/mL (mean = 4.0%). The level of assay detection was 0.05 ng/mL.

Serum inhibin B concentrations were measured in duplicate with an α-ßB dimeric ELISA (DSL-10–84100, Diagnostic Systems Laboratories) and referenced to a standard of human inhibin B preparation isolated from human follicular fluid provided by Nigel Groome (Oxford Brookes University, Oxford, UK). The within- and between-assay variations were 11.7% and 15.6%, respectively. The assay lower limit of detection (lowest standard curve point) was 10.0 pg/mL. Assays for both AMH and inhibin B were measured in a single time period and kits came from single lots.

FSH concentrations were measured in annual batches across time using an in-house (CLASS laboratory, University of Michigan) two-site chemiluminoscence immunoassay directed to different regions on the beta subunit.[21] It has an interassay CV of 12.0% and 6.0% and a lower limit of detection of 1.05 mIU/mL. The intra-assay CV at five locations along the standard curve were as follows: 7.8% (3.3 mIU/mL), 3.2% (9.9 mIU/mL), 5.1% (18.2 mIU/mL), 4.4% (22 mIU/mL), and 3.3% (60.8 mIU/mL).

Serum insulin was measured using Radioimmuneassay (DPC Coat-a-count, Los Angeles, CA). Glucose was measured with a hexokinase-coupled reaction on a Hitachi 747–200 (Boehringer Mannheim Diagnostics, Indianapolis, IN). The hemostatic model-based insulin resistance index (HOMA-IR) was calculated as ([fasting insulin × fasting glucose]/22.5).[22] Serum glucose and insulin values were not available from the 1992/1993 assessment, but available at the subsequent five data points. HOMA-IR was treated as a time-varying covariate.

Other measures

Height (cm) and weight (kg) were measured at annual study visits with a calibrated stadiometer and balance-beam scale and these data used to calculate BMI as weight (in kilograms) divided by the square of height (in meters). Obesity was defined by dichotomizing BMI at 30 kg/m^2. Smoking status at study entry was included as an ever versus never dichotomous variable.

Statistical analysis

Variable distributions were examined for normality, the presence of nonplausible outliers, and/or changing variability over time. Scatter plots and box plots were used to determine whether transformations of outcome measures were necessary for satisfying model assumptions.

Biomarkers ($_{\log}$AMH, $_{\log}$inhibin B, and $_{\log}$FSH levels) as independent variables were related to time to FMP and to age at FMP as the outcomes of interest. First, the six annual biomarker values for each woman were decomposed into subject-specific intercept and slope. Then, these were incorporated as random independent variables and related to the outcome measure, age at FMP. When values below assay detection were present (for the biomarkers AMH and inhibin B), we used the nonlinear mixed model procedure, Proc NLMixed (in SAS) to address those below-detection values as in Thiébaut and Jacqmin-Gadda.[23] Otherwise, modeling was undertaken using Generalized Estimating Equations.

Baseline variables for age, smoking, or BMI were added to the basic models as independent main effects. In addition, and in separate models, HOMA-IR was treated as a continuous, time-varying covariate. Then, interaction terms were included in the models to test the hypotheses that in women who smoked, were obese or insulin resistant, these environmental factors modified the relationships of the measured biomarkers with FMP.

Appropriateness of model fitting was assessed both graphically and using residual analyses. SAS 9.1 and Macro facilities (SAS Institute, Cary, NC) were used to perform the statistical analyses.

Results

The baseline hormone biomarker characteristics of the 50 women included mean (SD) AMH of 0.66 ± 0.50 ng/mL; mean (SD) inhibin B of 72 ± 44 pg/mL; and mean (SD) FSH of 8.0 ± 2.4 mIU/mL. Table 1 shows the biomarker values across the six time points and the number of values that were below the AMH and inhibin B assay detections. The profiles of AMH and inhibin B changed significantly over the 6-year interval ($P < 0.0001$, respectively). Increasingly over time, the proportion of values below the levels of assay detection increased ($P < 0.0001$, respectively). The change in AMH and inhibin B profiles predated the rise in the follicular-phase FSH profile. The mean age in 1993 of the 50 women was 41.5 years and the mean (SD) BMI was 27.0 ± 5.9 kg/m^2. Twenty-four percent of women were obese and 18% of women smoked at baseline. Mean age at FMP was 51 years.

Age at FMP, smoking, and ovarian biomarkers

As seen in Table 2, there were statistically significant and important associations between the ovarian markers and age at FMP, but the nature of the association varied according to smoking status. The rate of change of AMH is related to age at FMP. However, women who smoked were likely to have an earlier age at FMP and the slope of their AMH levels in relation to age at FMP was steeper (that is, AMH levels declined faster in women who smoked, which was, in turn, associated with an earlier age at FMP). As seen in the second model of Table 2, smoking was associated with an earlier age at FMP and smoking modified the level at which inhibin B was associated with the earlier age at menopause in that it happened at a higher level of baseline inhibin B ($P = 0.002$). Obesity and HOMA-IR were not associated with age at FMP. FSH not associated with age at FMP and there was no interaction between FSH and smoking in relation to age at FMP.

Time to FMP, AMH, and lifestyle modifiers

$_{\log}$AMH was significantly associated with time to FMP, with a decline of one unit in observed $_{\log}$AMH being associated with 1.75 earlier years to FMP ($P < 0.0001$). When AMH became nondetectable, it was also significantly associated with time to FMP. An increasingly higher HOMA-IR was significantly associated with a closer time to FMP ($P = 0.009$), apart from AMH. However, neither baseline smoking nor obesity measures were significantly associated with time to FMP (see Table 3). There were no statistically significant interactions between AMH and smoking, obesity, or HOMA-IR in relation to time to FMP, indicating that the relationship between AMH and the time to FMP was not significantly different in women who smoked, were obese, or had greater insulin resistance (models not shown).

Table 1. Sample characteristics from 50 pre- and perimenopausal women at six points across time—MBHMS

	1993/1994 Mean ± SD	1994/1995 Mean ± SD	1995/1996 Mean ± SD	1997/1998 Mean ± SD	1998/1999 Mean ± SD	1999/2000 Mean ± SD
Age	41.5 ± 2.67	42.5 ± 2.67	43.4 ± 2.61	46.3 ± 2.60	47.3 ± 2.67	48.3 ± 2.56
AMH (ng/mL)						
Observed values[a]	0.66 ± 0.50	0.51 ± 0.36	0.39 ± 0.30	0.16 ± 0.11	0.19 ± 0.11	0.15 ± 0.10
% ↓ detection	3 (6%)	5 (10%)	8 (16%)	26 (52%)	27 (54%)	32 (64%)
Inhibin B (pg/mL)						
Observed values[a]	72 ± 44	56 ± 43.5	59 ± 35	38 ± 21	47 ± 72	34 ± 30
% ↓ detection	2 (4%)	2 (4%)	9 (18%)	18 (36%)	23 (46%)	23 (46%)
FSH (mIU/mL)	8.0 ± 2.4	7.6 ± 3.7	7.7 ± 4.9	16.4 ± 15.7	18.3 ± 15.2	21.5 ± 18.3
HOMA-IR	N/A[b]	1.68 ± 1.05	1.84 ± 1.74	2.48 ± 2.36	2.35 ± 2.10	2.60 ± 2.97
BMI (kg/m^2)	27.0 ± 5.9	27.6 ± 6.1	27.6 ± 5.9	28.3 ± 6.5	28.5 ± 6.3	28.8 ± 6.4

[a]Data are for AMH and inhibin B that are observed above level of assay detection.
[b]HOMA-IR data are not available from this annual visit.

Table 2. Association of age at final menstrual period with $_{log}$AMH and $_{log}$inhibin B profiles according to smoking status

Covariates	Beta ± SE	*P*-value
	Model of AMH and smoking	
$_{log}$AMH intercept	0.12 ± 0.39	0.76
$_{log}$AMH slope	**11.49 ± 4.14**	**0.008**
Smoked at baseline	**−7.31 ± 2.36**	**0.003**
$_{log}$AMHintcpt*smoking at baseline	**1.92 ± 0.65**	**0.005**
$_{log}$AMHslope*smoking at baseline	**−19.27 ± 5.84**	**0.002**
	Model of inhibin B and smoking	
$_{log}$Inhibin B intercept	−2.75 ± 2.19	0.22
$_{log}$Inhibin B slope	3.92 ± 4.02	0.33
Smoked at baseline	**−44.32 ± 12.69**	**0.001**
$_{log}$Inhibin B intcpt*smoking at baseline	**10.20 ± 3.12**	**0.002**
$_{log}$Inhibin B slope*smoking at baseline	−7.99 ± 6.23	0.21

Bolded variables are statistically significant.

Time to FMP, $_{log}$inhibin B, and lifestyle modifiers

A decline of one unit in observed $_{log}$inhibin B was associated with 1.58 earlier years to FMP. Inhibin B below detection was also associated with time to FMP. As seen in Table 4, insulin resistance, expressed with HOMA-IR, was significantly independently associated with time to FMP ($P = 0.003$); the association with obesity, defined as a BMI >30 kg/m^2, was of borderline statistical significance ($P = 0.06$). Women who smoked did not have a statistically different time to FMP than women who did not smoke. There were no statistically significant interactions with smoking behavior, obesity classification, or HOMA-IR and inhibin B in relation to time to FMP (data not shown).

Time to FMP and FSH

Though the linear component of $_{log}$FSH was not associated with time to the FMP ($P = 0.13$), the curvilinear rate of change in $_{log}$FSH was associated with the time to the FMP ($P = 0.0005$). As reported in Table 5, smoking was not associated with time to FMP ($P = 0.46$). In addition to the curvilinear rise in FSH, obesity ($P = 0.004$) and HOMA-IR ($P = 0.0003$) were independently associated with time to FMP in separate models. There were no significant interactions with smoking, obesity, or HOMA-IR and FSH in relation to time to FMP (data not shown).

Discussion

The ovarian aging concept incorporates the timing of reproductive events, including the beginning of subfertility, the transition from menstrual cycle regularity to irregularity, absolute infertility, and the final menstrual period.[24] Identifying lifestyle factors that influence the rate of ovarian aging is of interest, both clinically and from a public health perspective. After including 6-year levels and changes in AMH, inhibin B, and FSH as biomarkers of the progressive decline in functional ovarian cells, we considered whether three factors, smoking, obesity, and insulin resistance, might be associated with the rate of ovarian aging in their relation to both age at FMP and time to FMP independent of age.

Role of smoking

We identified a marked difference between smokers and nonsmokers in the relation of both baseline $_{log}$AMH and change in $_{log}$AMH with age at FMP. There was an earlier age at FMP and a more rapid decline in AMH levels among women who were smokers, suggesting that smoking behavior may lead to either fewer oocytes or an earlier decline in oocyte number. In addition, we found that smoking was associated with a lower initial level of inhibin B but not associated with the rate of change in inhibin B levels. This led us to conclude that smoking might be associated with an earlier onset of processes associated

Table 3. The associations of time to FMP and AMH incorporating smoking, obesity (defined at baseline), or HOMA-IR (time-varying) as main effects

	Beta ± SE	*P*-value
	Model of AMH and smoking	
$_{log}$observed AMH	**−1.75 ± 0.14**	**<0.0001**
AMH below detection	**6.57 ± 0.31**	**<0.0001**
Smoked at baseline (dummy variable)	−0.22 ± 0.38	0.57
	Model of AMH and obesity (dichotomized)	
$_{log}$observed AMH	**−1.75 ± 0.14**	**<0.0001**
AMH below detection	**6.57 ± 0.32**	**<0.0001**
Obesity > 30 kg/m^2	0.03 ± 0.44	0.94
	Model of AMH with time-varying HOMA-IR	
$_{log}$observed AMH	**−1.65 ± 0.16**	**<0.0001**
AMH below detection	**6.06 ± 0.36**	**<0.0001**
HOMA-IR (time-varying)	**0.16 ± 0.06**	**0.009**

Bolded variables are statistically significant. Smokers were designated 1 while nonsmokers were designated with 0.

with ovarian aging, but not necessarily a disruption in the sequence of activities and that inhibin B and FSH are better indicators of the sequence of activities leading to the selection of a dominant follicle as compared to being indicators of the size of the follicle pool.

Multiple epidemiological studies have reported that cigarette smoking leads to reduced ovarian function and fertility and an earlier age at menopause,[25] suggesting that smoking impairs ovarian function. The mechanism of tobacco's toxic effect on the ovary is unclear but may be due to effects on oocyte quantity,[26] oocyte quality, or disruption of endocrine function.[27,28] Cotinine, a long-lived metabolite of nicotine, can be detected in ovarian follicular fluid, indicating that toxic constituents of cigarette smoke including nicotine and cadmium have access to the follicular environment and could affect ovarian function.[29–32]

The sample size of this report, limited to data from 50 women, precluded our investigation of important related public health questions, such as whether quitting smoking altered these associations and whether the patterns varied according to the time of initiation, duration, or total exposure to tobacco use.

Role of obesity and HOMA-IR

We identified that there were stronger and more frequent associations of insulin resistance with the biomarkers of ovarian aging or their change with time than with obesity when using a widely used cutpoint for obesity (or with BMI treated as a continuous variable, data not reported). Data on the association of AMH and obesity are scarce. A recent study reported that AMH levels were significantly lower in obese women as compared to nonobese women in the late reproductive years; however, that study did not report measures of insulin resistance.[33] There are reports of an inverse association of obesity with inhibin B levels in women with polycystic ovary syndrome (PCOS) and in anovulatory premenopausal women.[34,35] Thus, there is limited data in healthy reproductively aged women suggesting a significant association between decreasing inhibin B levels and greater BMI but mechanisms for the association have not been established.[36]

We identified that AMH and insulin resistance were predictive of time to FMP. Importantly, obesity and insulin resistance should not be treated as synonymous as there are obese women without insulin resistance and lean insulin resistant women.[37] Published studies of insulin resistance and ovarian biomarkers are largely limited to investigations of PCOS.[38] Several studies in women with PCOS have identified that elevated circulating levels of AMH are correlated with increased numbers of small antral follicles in the ovary,[39] potentially reflecting alterations granulosa cell function and a role not necessarily associated with body size.[40,41] If insulin

Table 4. The associations of time to FMP with $_{log}$inhibin B incorporating smoking, obesity (defined at baseline), or HOMA-IR (time-varying) as main effects

Covariates	Beta ± SE	*P*-value
	Model of inhibin B with smoking	
$_{log}$Inhibin B	**−1.58 ± 0.28**	**<0.0001**
Inhibin B below assay detection	**−2.52 ± 1.08**	**0.02**
Smoked at baseline	0.33 ± 1.81	0.86
	Model of inhibin B with obesity	
$_{log}$Inhibin B	**−1.58 ± 0.22**	**<0.0001**
Inhibin B below detection	**−2.43 ± 0.88**	**0.0065**
Obesity > 30 kg/m^2	0.77 ± 0.41	0.06
	Model of inhibin B with time-varying HOMA-IR	
$_{log}$Inhibin B	**−1.27 ± 0.24**	**<0.0001**
Inhibin B below detection	−1.81 ± 0.92	0.052
HOMA-IR (time-varying)	**0.22 ± 0.07**	**0.003**

Bolded variables are statistically significant.

resistance has an impact on granulosa or theca cell functioning, this would be consistent with our findings. As the perception of PCOS changes from that of a reproductive disorder to one of a metabolic syndrome with reproductive implications, greater focus is being placed on dysfunction of the hypothalamic–pituitary axis and primary defects of insulin activity as contributory causes to the syndrome.[42] Although the underlying cause of the ovulatory dysfunction associated with PCOS is unknown, it is thought to be associated with the dysregulation of thecal steroidogenesis.[43] The resulting hyperandrogenism plays a role in the arrest of folliculogenesis,[44,45] a mechanism that has not been evaluated in normal ovarian aging. Alternatively, studies in knockout models suggest considering other mechanisms including insulin receptor insufficiency or GLUT4 dysregulation.[46]

This report includes strengths and limitations. The data reflect appropriately collected specimens without thaw-refreeze prior to assay. Data were obtained from a substudy nested in a population-based study that includes documentation of the natural progression of women through stages of the menopause transition to the FMP and subsequent postmenopause. This allowed the selection of archival specimens for assay that represented the late reproductive period. Simultaneously, information about smoking behavior, obesity status, and insulin resistance were obtained concurrently with the specimens assayed for AMH, inhibin B, and FSH and did not require women to engage in interviews that required long-term recall. The study design allowed us to consider change in biomarkers of ovarian aging and how environmental factors were related to these changes in relation to menopause characteristics. However, this is an epidemiological study without access to certain measures of follicle status, such as antral follicle count. The population is Caucasian so findings may not be generalizable to non-Caucasian women, although the study addresses factors that are often disproportionately associated with non-Caucasian populations including smoking, insulin resistance, and obesity. The major deficit is that the size of the sample evaluated may be too small to detect important interactions in relation to the biomarkers and time to FMP. The sample size was too limited to detect three-way interaction patterns (insulin resistance in smokers who were obese versus insulin resistance in smokers who were not obese) that may characterize important subgroups that could be targeted for intervention.

In summary, smoking was not only associated with age at FMP but also modified the associations of two biomarkers, AMH and inhibin B, with age at FMP. These biochemical markers for oocyte quality and quantity may provide an indication of the potential mechanisms whereby smoking is associated

Table 5. The associations of time to FMP with $_{log}$FSH and considering smoking, obesity (defined at baseline), or HOMA-IR (time-varying) as main effects

Covariates	Beta ± SE	*P*-value
	Model of FSH with smoking	
$_{log}$FSH	−1.61 ± 1.05	0.13
$_{log}$FSH * $_{log}$FSH	**0.72 ± 0.20**	**0.0005**
Smoked at baseline	−0.29 ± 0.40	0.46
	Model of FSH with obesity (dichotomized)	
$_{log}$FSH	−1.62 ± 1.04	0.12
$_{log}$FSH * $_{log}$FSH	**0.73 ± 0.20**	**0.0004**
Obesity (dichotomized) at baseline	**1.22 ± 0.42**	**0.004**
	Model of FSH with time-varying HOMA-IR	
$_{log}$FSH	−0.37 ± 0.99	0.71
$_{log}$FSH * $_{log}$FSH	**0.43 ± 0.19**	**0.03**
HOMA-IR (time-varying)	**0.27 ± 0.07**	**0.0003**

Bolded variables are statistically significant.

with the earlier age at FMP, but does not necessarily disrupt progression in the timing of events leading to the FMP. The absence of significant interactions between insulin resistance with AMH and inhibin B in relation to time to FMP may indicate that other mechanisms in ovarian and adrenal steroidogenesis should be considered that are not limited to antral follicle recruitment. Although future studies will more fully reveal these relationships, both smoking behavior and greater insulin resistance are likely to accelerate reproductive aging. This report contributes additional evidence that targeting smoking and insulin resistance would be important practices to moderate in women as they appear to generate undue advancement in reproductive aging.

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Conflicts of interest

The authors declare no conflicts of interest.

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