

# Age at Menarche and Risk of Cardiovascular Disease Outcomes: Findings From the National Heart Lung and Blood Institute-Sponsored Women's Ischemia Syndrome Evaluation

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**Background**—Previous studies have reported an association between the timing of menarche and cardiovascular disease (CVD). However, emerging studies have not examined the timing of menarche in relation to role of estrogen over a lifetime and major adverse cardiac events (MACE).

**Methods and Results**—A total of 648 women without surgical menopause undergoing coronary angiography for suspected ischemia in the WISE (Women's Ischemia Syndrome Evaluation) study were evaluated at baseline and followed for 6 years (median) to assess major adverse CVD outcomes. MACE was defined as the first occurrence of all-cause death, nonfatal myocardial infarction, nonfatal stroke, or heart failure hospitalization. Age at menarche was self-reported and categorized ( $\leq 10$ , 11, 12, 13, 14,  $\geq 15$  years) with age 12 as reference. Total estrogen time and supra-total estrogen time were calculated. Cox regression analysis was performed adjusting for CVD risk factors. Baseline age was  $57.9 \pm 12$  years (mean  $\pm$  SD), body mass index was  $29.5 \pm 6.5$  kg/m<sup>2</sup>, total estrogen time was  $32.2 \pm 8.9$  years, and supra-total estrogen time was  $41.4 \pm 8.8$  years. MACE occurred in 172 (27%), and its adjusted regression model was J-shaped. Compared with women with menarche at age 12 years, the adjusted MACE hazard ratio for menarche at  $\leq 10$  years was 4.53 (95% CI 2.13-9.63); and at  $\geq 15$  years risk for MACE was 2.58 (95% CI, 1.28-5.21).

**Conclusions**—History of early or late menarche was associated with a higher risk for adverse CVD outcomes. These findings highlight age at menarche as a potential screening tool for women at risk of adverse CVD events.

**Clinical Trial Registration**—URL: <http://www.clinicaltrials.gov>. Unique identifier: NCT00000554. (*J Am Heart Assoc.* 2019;8:e012406. DOI: 10.1161/JAHA.119.012406.)

**Key Words:** cardiovascular disease outcomes • estrogen • menarche • risk factors • women

Cardiovascular disease (CVD) continues to be the leading cause of mortality in women, causing nearly 1 in every 3 deaths<sup>1</sup> and about 400 000 deaths per year in the United States.<sup>2</sup> Despite advances in research and increases in clinical awareness, a gap still remains in understanding and effectively recognizing sex-specific risks of preventable CVD.<sup>3</sup> Specifically, women experience less obstructive coronary artery disease yet experience a greater burden

of ischemic symptoms and mortality compared with men.<sup>4</sup> The limited knowledge of the CVD risk factor profile specific to women contributes to management delays with underdiagnoses and undertreatment, resulting in poorer outcomes.<sup>5</sup> Identifying sex-specific risk factors for adverse CVD events in midlife could result in more timely primary prevention strategies and yield better clinical outcomes.<sup>6</sup>

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Accompanying Tables S1 through S3 and Figures S1 and S2 are available at <https://www.ahajournals.org/doi/suppl/10.1161/JAHA.119.012406>

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## Clinical Perspective

### What Is New?

- In the WISE (Women's Ischemia Syndrome Evaluation) study, women who experienced early or later age at menarche had a higher risk of developing major adverse cardiac events, defined as first occurrence of all-cause death, nonfatal myocardial infarction, nonfatal stroke, or heart failure hospitalization.
- This effect persisted after adjustment for traditional cardiovascular risk factors and lifetime total estrogen exposure, supporting the notion that age at menarche may be an independent risk factor for major adverse cardiac events.

### What Are the Clinical Implications?

- Specific to women, traditional cardiovascular disease risk factors do not fully capture major adverse cardiac event risk assessment, and it is important to further investigate sex-specific risk factors such as menarche to elucidate the different mechanisms in which cardiovascular disease may occur in women.

Age at menarche, defined as the age at first occurrence of menstruation, heralds the onset of cyclic ovarian function, which includes increased endogenous estradiol secretion and exposure. Menarche is a complex reproductive physiologic milestone that depends on a tightly orchestrated set of neurohormonal changes in the hypothalamus-pituitary-ovarian axis that influence further maturation of the female body. Menarche has also been associated with CVD risk factors.<sup>7-9</sup> Early menarche (before age 12 years) has been associated with adiposity, metabolic syndrome, and increased risk of breast cancer, suggested to be due, at least in part, to increased lifetime exposure to estrogen.<sup>10</sup> Both early and late age at menarche have been associated with increased risks of coronary heart disease.<sup>11</sup> Others have reported that early age at menarche is associated with increased risk of CVD events and all-cause mortality.<sup>12</sup> However, despite preliminary findings, the clinical significance of age at menarche as a sex-specific finding for major adverse cardiovascular events (MACE) in women represents an important knowledge gap.

To address this knowledge gap, we investigated the association between age at menarche and MACE among women participating in the original cohort of the National Heart Lung and Blood Institute WISE (Women's Ischemia Syndrome Evaluation) study. We further considered age of onset of menstruation and length of total estrogen exposure to control for the potential influence of reproductive hormones. We also evaluated biomarkers of inflammation to determine if there was an association between age at menarche and MACE.

## Methods

### Data Availability

The data that support the findings of this study are available from the BioLINCC data repository from the WISE data repository.<sup>13</sup>

### Study Participants

The original cohort of the WISE study is a National Heart Lung and Blood Institute–sponsored 4-center study of 936 women undergoing clinically indicated coronary angiography to further evaluate suspected myocardial ischemia. Additional details regarding the project methodologies are described elsewhere.<sup>14</sup> Briefly, women >18 years old underwent a detailed standardized symptom evaluation, risk assessment, and laboratory evaluation at baseline. Exclusion criteria included emergency referral, pregnancy, cardiomyopathy (as defined in the patients' medical records), New York Heart Association class IV congestive heart failure, acute ischemic syndrome, defined as acute myocardial infarction or unstable angina within 1 month before study entry, coronary revascularization within 6 months before study entry, conditions other than ischemic heart disease likely to be fatal or to require frequent hospitalization within 4 years (such as severe lung, renal, or hepatic disease, or surgically uncorrected significant congenital or valvular heart disease), contraindication to provocative myocardial stress or coronary reactivity testing, malignancy other than skin cancer, and any condition likely to affect study retention (alcoholism, drug abuse, or severe psychiatric illness). All women provided written informed consent in accordance with institutional guidelines, and the project was approved by the institutional review board at each site.

This study cohort included 648 women who reported age at menarche and had both ovaries to obtain the calculation for total estrogen time and supra-total estrogen time. Analyses of the biomarkers were added later during the recruitment period and included 505 women for serum amyloid A (SAA) and C-reactive protein (CRP), and 471 women for interleukin-6, and tumor necrosis factor- $\alpha$ .

### Baseline Evaluation

Women underwent a baseline evaluation that included collection of demographic information, age at menarche, reproductive history, exogenous hormone use, and history of other medical conditions from 1998 to 2002.<sup>14</sup> These measures also included relevant health behavior information such as smoking history. The WISE reproductive status questionnaire, a validated assessment of menopause status, included a detailed history of menarche, date of last

menstrual period, menstrual cycling patterns, parity, gynecological surgeries, hormone therapy, and oral contraceptive use. Exclusion criteria included surgical menopause as determined by the WISE hormone committee caused by abrupt loss of endogenous hormones. Coronary angiography for suspected ischemia was assessed by a core laboratory masked to other patient data as described elsewhere.<sup>15</sup> Blood was drawn at baseline after an overnight fast in close temporal proximity to the WISE testing.

### Measurement of Inflammatory Markers

Plasma sampled at baseline was frozen at  $-70^{\circ}\text{C}$  for measurement of the inflammatory markers. Levels of CRP and SAA were measured by a high-sensitivity method at a core laboratory using a BN II analyzer (Dade Behring, Deerfield, IL) by previously validated techniques.<sup>16</sup> Levels of interleukin-6 and tumor necrosis factor- $\alpha$  were measured with commercially available enzyme-linked immunoabsorbent assay kits (R&D systems, Minneapolis, MN).

### Menarche Status and Total Estrogen Time

Menarche history was obtained as part of the self-reported reproductive questionnaire at the baseline visit. Total estrogen time (TET) and supra-total estrogen time (sTET) were calculated with equations as previously published.<sup>17</sup> Briefly, TET included time of estimation of endogenous estrogen exposure during a woman's lifetime. sTET also included time of supra-physiologic levels of endogenous and exogenous hormone exposure such as pregnancy and oral contraceptive use during a woman's lifetime.

### Major Adverse Cardiovascular Events

MACE were collected as first occurrence among all-cause mortality, nonfatal myocardial infarction, nonfatal stroke, or heart failure hospitalization. Patients were contacted by telephone at 3 months, then annually, and asked about adverse CVD events or hospitalizations. All-cause mortality data were obtained from a search of the National Death Index and confirmed by death certificates, when available.

### Data Collection

#### Statistical Analyses

Women were grouped into categories by age at menarche ( $\leq 10$ , 11, 12, 13, 14,  $\geq 15$  years), with age 12 as reference, from the mean age at menarche for US women of 12.4 years.<sup>18</sup> Baseline data were reported as means $\pm$ SDs stratified by age at menarche. All *P*-values (with the exception

of menopause status and use of hormone replacement therapy) were adjusted for age by general linear model or, where appropriate, logistic models. Skewed variables were reported as medians (interquartile range) by age at menarche. Traditional CVD risk factors such as diabetes mellitus, hyperlipidemia, and smoking history were included in the descriptive analyses. Tests for clinical and demographic variables by age at menarche were chi-squared tests for categorical variables or nonparametric Kruskal-Wallis tests for continuous variables. Log transformation was performed where appropriate to reduce the effect of outliers in the distribution of inflammatory biomarkers.

For the analyses, women were examined by age at menarche to the first instance of MACE and all-cause mortality reported by frequency. For multivariate modeling, Cox proportional-hazards models were used to test the association of age at menarche with MACE adjusted for baseline demographics and CVD risk factors of age, body mass index, diabetes mellitus, dyslipidemia, hypertension, history of smoking, and log serum amyloid A. CRP was not included in the fully adjusted model because of its collinearity with SAA. Two different adjusted models were run in parallel—the first with TET and second model with sTET. These models were also run using all-cause mortality only. Models were assessed for proportional-hazards violations using plots of Schoenfeld residuals with a smoothed curve for the  $\beta$  estimates versus rank-transformed event times. Age at menarche was treated as a categorical variable to estimate the form of the relationship with relative hazard of MACE as well as all-cause mortality. A significance level of 0.05 was used for all tests. Analyses were conducted with SAS v9.3 (SAS Institute, Cary, NC).

### Results

Baseline demographic data, stratified by age at menarche, are summarized in Table 1. There were 55 women in the  $\leq 10$ -years group, 99 women in the 11-years group, 152 women in the 12-years group, 161 women in the 13-years group, 82 women in the 14-years group, and 99 women in the 15-years-or-older group. The mean ( $\pm$ SD) age in this cohort was  $57.9 \pm 12$  years. Women who entered menarche early ( $\leq 10$  years old) presented with signs and symptoms of ischemia at an earlier age, 55 years as opposed to women who entered menarche late ( $\geq 15$  years old), 59 years ( $P=0.037$ ). Overall, 496 (77%) were postmenopausal at baseline, and there were no age-adjusted differences among age-at-menarche groups ( $P=0.59$ ). Mean body mass index ( $\pm$ SD) was  $29.5 \pm 6.5$  kg/m<sup>2</sup> and not found to be significant after age adjustment ( $P=0.23$ ). Most (81%) of the women were non-Hispanic whites. Traditional CVD risk factors were

prevalent in this population, with 25% diagnosed with diabetes mellitus, 53% with dyslipidemia, and 57% with hypertension; 51% had ever smoked.

During follow-up at 5.8 years (median) for MACE and 9.2 years (median) for all-cause death, 172 (27%) women experienced MACE. Early age at menarche ( $\leq 10$  and 11 years) had the greatest burden of MACE (40% and 27%, respectively), as well as all-cause mortality (30% and 25%), respectively. After adjustment for age, the earliest age-at-menarche group had the highest rates of both MACE and all-cause mortality.

TET as estimated by our algorithm was  $32.2 \pm 8.9$  years, and sTET was  $41.4 \pm 8.8$  years (Table 1). As expected, mean TET and sTET were longer in women who had entered menarche earlier and shorter in those who entered menarche later ( $P < 0.0001$  for both, age-adjusted). Among postmenopausal women, age-adjusted endogenous sex hormone levels did not differ across age at menarche categories ( $P > 0.05$ , all variables, see Table S1).

Inflammatory biomarker levels by age at menarche are summarized in Table 2. Median SAA ranged from 0.45 to 0.74  $\mu\text{g/mL}$  and was significantly different among the groups ( $P = 0.017$ ). Median CRP ranged from 0.26 to 0.51  $\text{mg/L}$  and also was significantly different among the groups ( $P = 0.004$ ). For the inflammatory markers SAA and CRP, median ranks were higher in the early age-of-menarche groups than in the older age-at-menarche groups, particularly the age-11 and -12 groups, compared with those at age 15 and older. Tumor necrosis factor- $\alpha$  and interleukin-6 did not differ significantly among groups.

Adjusted hazard ratios from a Cox proportional-hazards model in Figure 1 show the relative hazard of developing MACE by age at menarche including the variable TET. The model demonstrated that both early and late menarche age were associated with higher adjusted hazard rates (HR) of MACE: menarche age  $\leq 10$ , HR 4.21 (95% CI 1.90-9.30); age 11, HR 2.27 (95% CI 1.09-4.75); age 13, HR 1.95 (95% CI 0.97-3.92); age 14, HR 2.74 (95% CI 1.21-6.20), and age  $\geq 15$ , HR 2.52 (95% CI 1.14-5.57). The highest risk for MACE was found in women with menarche  $\leq 10$  years of age (HR 4.21 [95% CI 1.90-9.30]) compared with women with menarche at age 12. In women with menarche at  $\geq 15$  years, there was also an elevated risk for MACE (HR 2.52 [95% CI 1.14-5.57]). Similarly, the adjusted Cox regression model using sTET shows a similar J-shaped curve for risk of MACE (see Figure S1). Furthermore, modeling with TET and sTET as well as without TET or sTET did not change the effect size (see Tables S2 and S3) for either CVD or time to death. The association of TET with time to MACE was not found to be different, adjusted for age, body mass index, diabetes mellitus, dyslipidemia, hypertension, smoking status, log serum amyloid A, and age at menarche ( $P = 0.64$ , HR 0.99, 95% CI 0.97-1.02). The adjusted association of sTET with time to MACE was also not different ( $P = 0.10$ , HR 0.98, 95% CI 0.95, 1.01).

The adjusted hazard ratios from a Cox proportional hazards model in Figure 2 show the relative hazard of all-cause mortality by age at menarche including the variable TET. The model demonstrates similar results to MACE in that both early and late menarche ages were associated with higher adjusted

**Table 1.** Baseline Characteristics by Age at Menarche Category

	$\leq 10$ y (n=55)	11 y (n=99)	12 y (n=152)	13 y (n=161)	14 y (n=82)	$\geq 15$ y (n=99)	Overall (n=648)	P Value
Age, mean (SD), y	55 (10.5)	58 (11.3)	56 (12.7)	59 (11.0)	59 (12.4)	59 (12.7)	58 (12.0)	0.037
BMI, mean (SD), $\text{kg/m}^2$	31.6 (6.2)	30.0 (5.4)	29.8 (6.9)	28.9 (6.3)	29.3 (7.0)	28.8 (6.7)	29.5 (6.5)	0.23
White race, n (%)	42 (76)	80 (81)	124 (82)	134 (83)	66 (80)	76 (77)	522 (81)	0.74
Ever smoker, n (%)	31 (56)	55 (56)	79 (52)	89 (55)	38 (46)	40 (40)	332 (51)	0.16
Hypertension, n (%)	32 (58)	67 (68)	80 (53)	98 (61)	41 (51)	51 (52)	369 (57)	0.06
Diabetes mellitus, n (%)	19 (35)	31 (31)	33 (22)	36 (22)	35 (30)	18 (18)	162 (25)	0.08
Dyslipidemia, n (%)	24 (46)	55 (63)	69 (49)	80 (54)	45 (58)	43 (48)	316 (53)	0.20
Postmenopausal, n (%)	45 (82)	76 (77)	110 (72)	128 (80)	60 (73)	77 (78)	496 (77)	0.59
HRT, n (%)	18 (33)	33 (34)	48 (32)	53 (33)	25 (31)	30 (31)	207 (32)	0.99
TET, mean (SD), y	35.0 (6.3)	33.6 (9.3)	32.2 (9.4)	32.2 (8.4)	30.9 (8.2)	30.1 (9.5)	32.2 (8.9)	$< 0.0001$
sTET, mean (SD), y	43.3 (7.8)	43.8 (8.2)	41.3 (8.8)	41.5 (8.6)	40.4 (8.6)	38.6 (9.6)	41.4 (8.8)	$< 0.0001$
MACE, n (%)	22 (40)	27 (27)	23 (15)	36 (22)	19 (23)	22 (22)	149 (23)	0.0009
Death, n (%)	16 (30)	25 (25)	20 (13)	28 (17)	15 (18)	18 (19)	122 (19)	0.016

BMI indicates body mass index; HRT, hormone replacement therapy; MACE, major adverse cardiovascular events; sTET, supra-TET; TET, total estrogen time.

**Table 2.** Inflammatory Biomarkers by Age at Menarche

	≤10 y (n=55)	11 y (n=99)	12 y (n=152)	13 y (n=161)	14 y (n=82)	≥15 y (n=99)	Overall (n=648)	P Value
SAA, median (IQR)* μg/mL	0.45 (0.27, 0.89)	0.74 (0.35, 1.26)	0.56 (0.35, 1.26)	0.56 (0.31, 1.08)	0.56 (0.30, 0.84)	0.44 (0.27, 0.71)	0.54 (0.31, 0.98)	0.017
CRP, median (IQR)* mg/L	0.39 (0.13, 0.82)	0.51 (0.19, 0.87)	0.39 (0.15, 1.06)	0.29 (0.15, 0.81)	0.26 (0.11, 0.66)	0.27 (0.11, 0.58)	0.35 (0.14, 0.79)	0.004
IL-6, median (IQR) <sup>†</sup> pg/mL	3.2 (2.1, 5.8)	3.4 (2.1, 5.8)	3.1 (1.7, 5.8)	2.8 (1.6, 6.5)	3.0 (1.7, 5.7)	2.4 (1.5, 3.8)	3.0 (1.8, 5.6)	0.12
TNF-α, median (IQR) <sup>†</sup> pg/mL	3.7 (2.5, 6.6)	3.2 (2.0, 5.0)	3.2 (1.8, 4.7)	2.9 (2.1, 5.2)	3.3 (1.9, 4.9)	3.2 (2.2, 4.6)	3.2 (2.0, 5.0)	0.29

CRP indicates C-reactive protein; IL-6, interleukin-6; IQR, interquartile range; SAA, serum amyloid A; TNF-α, tumor necrosis factor-α.

\*n=505.

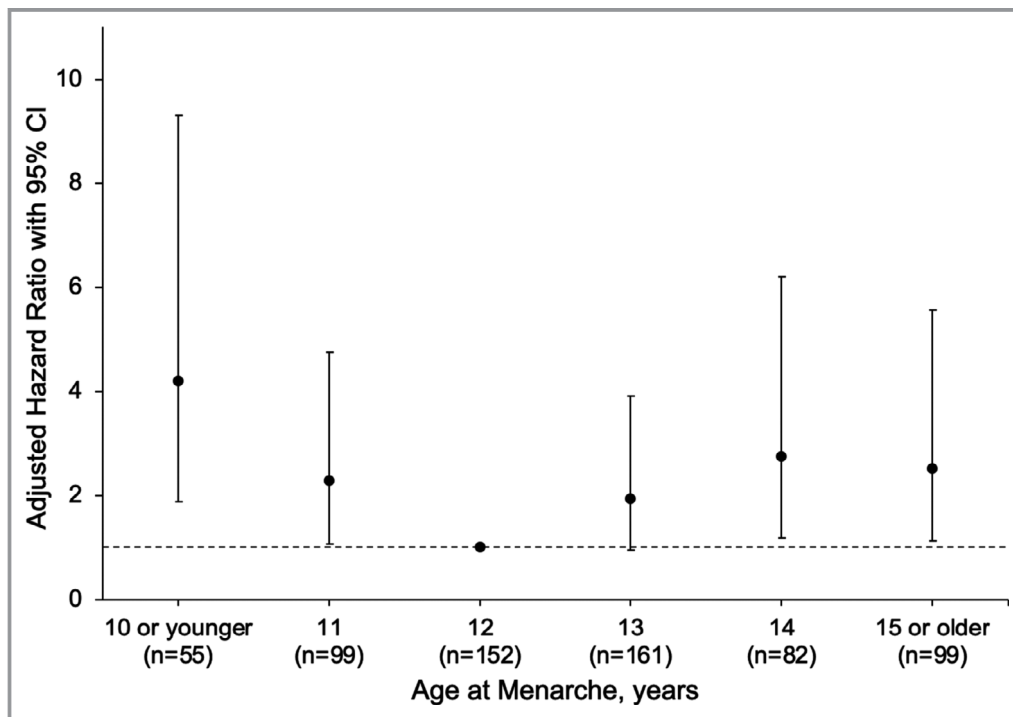
<sup>†</sup>n=471.

HRs for all-cause mortality: menarche age ≤10 HR 2.67 (95% CI 1.38-9.72); age 11, HR 2.62 (95% CI 1.12-6.11); age 13, HR 1.32 (95% CI 0.58-3.02); age 14, HR 2.54 (95% CI 0.97-6.66), age ≥15, HR 2.63 (95% CI 1.11-6.26). Results using sTET in the model showed no differences from the model with TET (see Figure S2).

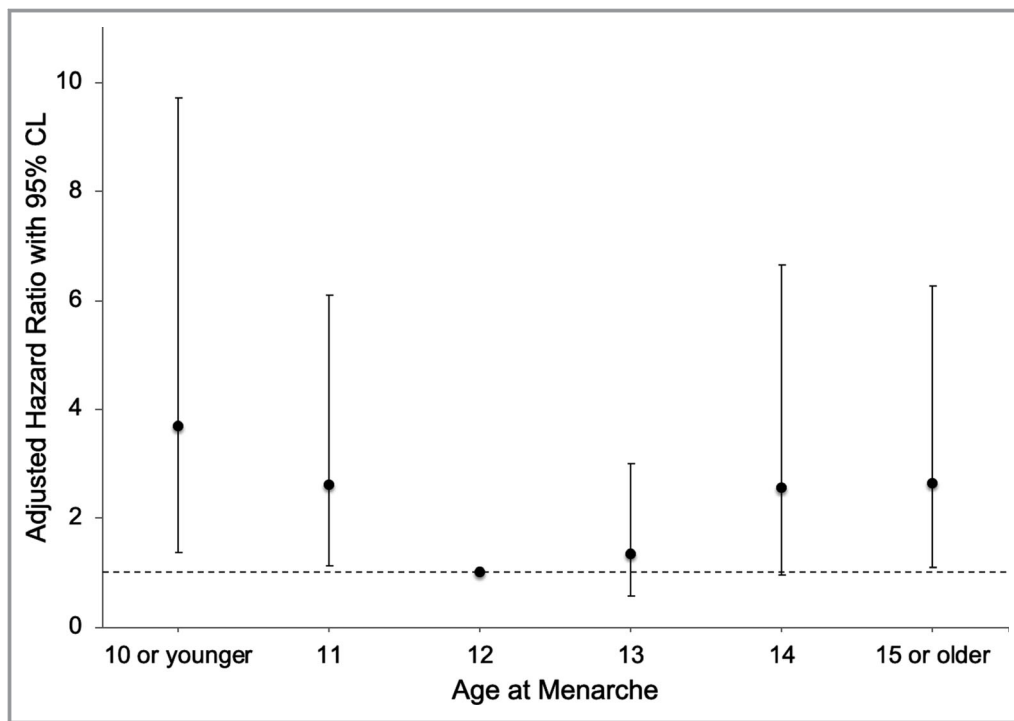
## Discussion

We observed that, among women with symptoms and/or signs of ischemic heart disease referred for coronary angiography, both early and late age at menarche predicted higher risk of developing MACE over intermediate-term follow-up.

This effect persisted after adjustment for multiple CVD risks, estrogen exposure, and inflammatory markers. Overall, the adjusted model illustrated that the relationship of age at menarche to the risk of MACE was J-shaped, with higher MACE risk observed in those with earlier age at menarche. Additionally, MACE was not associated with TET or sTET, suggesting that effects of estrogen exposure did not account for the differences observed by age at menarche. Inflammatory markers such as SAA and CRP were also different among women by age at menarche. In prior WISE analyses, SAA levels were found to be independently predictive of obstructive coronary artery disease and related MACE, further supporting the notion that state of systemic inflammation



**Figure 1.** Adjusted relative hazard (with TET) and 95% CI of major adverse cardiac events (MACE) by age at menarche. TET indicates total estrogen time.



**Figure 2.** Adjusted relative hazard (with TET) and 95% CI of mortality by age at menarche. TET indicates total estrogen time.

may contribute to atherosclerotic plaque destabilization and lead to MACE in women.<sup>19</sup> All-cause mortality with a longer follow-up also had a J-shaped relationship after adjustment for the same covariates. This finding further supports the notion that age at menarche may be an independent risk factor for MACE and all-cause mortality.

Our findings are similar to those from the United Kingdom population-based Million Women Study that evaluated post-menopausal women without prior CVD. They found that the relation of age at menarche to CVD was U-shaped, with both early and late menarche being associated with increased risk in that women with early menarche ( $\leq 10$  years) had a relative risk of 1.25 (95% CI 1.22-1.31), and those with later menarche ( $\geq 17$  years) had a relative risk of 1.23 (95% CI 1.16-1.30).<sup>11</sup> Another UK study found that early menarche ( $< 12$  years) and early menopause ( $< 47$  years) were independently associated with a higher CVD risk later in life (adjusted HRs 1.10 and 1.33, respectively).<sup>20</sup> The association between age at menarche and CVD risk has been also found outside of Western populations. In a study on Chinese women, early and later age at menarche and higher CVD risks were similarly present.<sup>21</sup> Early age at menarche was associated with increased risk of ischemic heart disease in a Shanghai cohort.<sup>22</sup> A Singaporean study found increased CVD mortality risk with decreasing age at menarche among nonsmoking women.<sup>23</sup> Early age at menarche was also associated with risk of coronary heart disease in a Korean cohort.<sup>24</sup> These

international studies suggest that there are specific factors that trigger early menarche, such as stress, that also accelerate onset of CVD.

Findings for association between age at menarche and mortality were varied. In a Japanese study there was a J-shaped association of all-cause mortality and age at menarche that was independent of lifestyle and reproductive factors.<sup>25</sup> A study on Norwegian women found a similar J-shaped association with total mortality.<sup>26</sup> However, a prospective cohort study in Europe reported lower all-cause mortality with a later age at menarche.<sup>27</sup> The Japan Collaborative Cohort study found that a later age at menarche was associated with a higher mortality due to stroke.<sup>28</sup> It is unclear what factors that trigger later menarche may affect risk for mortality from any causes.

Although increased estrogen exposure from earlier age at menarche has been correlated with increased rates of breast cancer<sup>29</sup> and later age at menarche with lower bone mineral density,<sup>30</sup> we did not find estrogen exposure linked with MACE and all-cause mortality. In premenopausal women disruption of ovulatory cycling characterized by hypothalamic hypogonadism has been associated with increased risk of obstructive coronary artery disease.<sup>31</sup> Despite the notion that exposure to estrogens may be cardioprotective, we did not find this to be the case based on age at menarche.<sup>32</sup> Factors linked to earlier menarche include psychosocial stress and overnutrition. Factors linked to later menarche

include polycystic ovary syndrome, excessive exercise, and undernutrition; later menarche may be linked to relative hypercortisolism and hypoestrogenism, whereas earlier menarche may be linked to metabolic syndrome. Our study did not demonstrate a significant link between estrogen exposure and MACE. It is possible that some other mechanism, such as the oxidative damage to vessels caused by states of chronic inflammation as mediated by understudied biomarkers, may predispose women to a higher CVD risk.<sup>33-35</sup> Glucocorticoids play a significant role not just in the stress-induced reproduction response but also in the establishment and maintenance of fertility.<sup>36</sup> Hypercortisolemia interferes with estrogen action at the cellular level, so chronic stress may not only lower endogenous estrogen exposure, it may also counteract the benefits of estrogen. States of inflammation have been linked to the pathology of formation and disruption of plaques. Further studies to specifically measure vascular damage and other markers of inflammation may be necessary to delineate possible mechanisms.

The strengths of the present study are many. The original WISE cohort is a longitudinal, prospective, and validated study ongoing since 1998. Reproductive health has been comprehensively assessed both by collecting clinical data and by measuring salient reproductive hormones. It is the first study of menarche and MACE in women that incorporates calculation of lifetime estrogen exposure by means of TET and sTET. The lack of a robust association between estrogen exposure and MACE suggests that the precursors of MACE are multifactorial. CVD risk factors and MACE were assessed comprehensively and prospectively. In contrast to studies that examined links between *International Classification of Diseases (ICD)* codes or deaths, the WISE repository contained valuable patient data such as blood work, inflammatory biomarkers, and years of hospital admission, data that permitted a more nuanced examination of cytokines and oxidative stress. The WISE prospectively collected clinical data using standardized methods and biochemical data to test again the hypothesis that inflammatory states promote CVD.

One limitation of this study includes the historical study variables used such as age at menarche, pregnancy, and contraceptive use, which were self-reported and may be subject to recall bias. The WISE sample, which consisted of women who were recruited on the clinical suspicion of myocardial ischemia, may not be representative of a general population of symptomatic women in the United States. Furthermore, the women were mostly white, so the findings may not apply to minority ethnic groups, particularly Hispanic and black populations, who present at an earlier age at menarche and are more likely to present with traditional CVD risk factors.<sup>2</sup>

## Conclusions

Using data from the original WISE prospective cohort, we observed that women with early or later menarche had a relatively higher risk of MACE and all-cause mortality. These associations were not explained by previously known CVD risk factors or estrogen exposure or supra-physiologic states of estrogen exposure. Inflammatory biomarkers such as SAA have shown a connection with angiographic coronary artery disease and thus could play a role in mediating atherosclerotic plaque destabilization. Women who experience earlier and later menarche may differ in fundamental ways from women whose menarche occurred on time. To date, traditional CVD risk factors do not appear to fully capture MACE risk assessment in women. Future studies should focus on female-specific risk factors that include menarche and mechanisms by which inflammatory biomarkers increase risk of CVD events in women.

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## Disclosures

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Randomized, Placebo-Controlled Study Evaluating Three Doses of Subcutaneous Pulsatile GnRH Administered via OmniPod Pump for Ovulation Induction in Female Subjects with Primary Amenorrhea with Hypogonadotropic Hypogonadism,” 2014-2018. Dr Stanczyk would like to report consultantships with the following: Agile Therapeutics, Therapeutics MD, Mithra Pharmaceuticals, Dr Reddy's Laboratories. The remaining authors have no disclosures to report.

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# **Supplemental Material**

**Table S1. Sex Hormone Levels by Age at Menarche Among Post-Menopausal women.**

	≤10 years (n=55)	11 years (n=99)	12 years (n=152)	13 years (n=161)	14 years (n=82)	≥15 years (n=99)	Overall (n=648)	P- Value
<b>Testosterone, median, (IQR) ng/dl, n=403</b>	24.6 (15.9, 33.4)	23.7 (17.7, 32.1)	25.4 (17, 33.7)	22.15 (15.1, 30.1)	24.5 (18.8, 32)	25.2 (17.9, 34.9)	24.0 (17.1, 32.2)	0.52
<b>Estradiol, median, (IQR) pg/ml, n=453</b>	18 (11, 34)	18 (11, 39)	16 (8, 42)	16 (9.5, 28.5)	15 (9, 36)	15 (10, 33)	16 (10, 33)	0.89
<b>Testosterone: Estradiol Ratio, median (IQR), n=400</b>	1.18 (0.7, 1.92)	1.25 (0.62, 2.18)	1.64 (0.69, 2.94)	1.51 (0.7, 2.57)	1.64 (0.54, 2.65)	1.57 (0.85, 2.64)	1.5 (0.7, 2.64)	0.40
<b>FSH*, median, (IQR) MLU/ml, n=453</b>	30.4 (9.7, 52.2)	33.2 (18.6, 50.9)	37.9 (20.7, 54.6)	40.95 (22.35, 57.65)	40.9 (23.4, 60.15)	41.1 (21.4, 57.6)	37.2 (21.2, 55.7)	0.57
<b>LH<sup>†</sup>, median (IQR) MLU/ml, n=452</b>	12 (3.6, 18.7)	12.8 (6.5, 19.1)	14.35 (8.2, 22.5)	15.1 (8.4, 23.8)	14 (9.9, 19.15)	17.1 (9.2, 21.1)	14.5 (8.4, 21.55)	0.40
<b>Sex Hormone Binding Globulin (SHBG), median (IQR), n=403</b>	38.1 (28.2, 65.4)	46.6 (23.55, 70.85)	51.3 (34.5, 90.8)	45.5 (31.5, 82.6)	49.5 (34.9, 84.7)	40.9 (25.8, 76)	46 (29.5, 82.6)	0.14
<b>Androstenedione, median (IQR) pg/ml, n=403</b>	560 (407, 802)	487.5 (339.5, 777.5)	456 (315, 716)	479 (302, 699)	555 (309, 677)	579.5 (416, 861)	519 (327, 732)	0.37
<b>DHEA-S<sup>‡</sup>, median (IQR) µg/dl, n=382</b>	46.2 (29, 65.4)	38.9 (22.35, 69.8)	36.85 (20.6, 63.25)	34.8 (15.6, 61.3)	36.4 (20.6, 65.5)	36.9 (15.2, 81.7)	37.55 (19.3, 65.5)	0.59

\*Follicle stimulating hormone <sup>†</sup>Luteinizing hormone <sup>‡</sup>Dehydroepiandrosterone sulfate

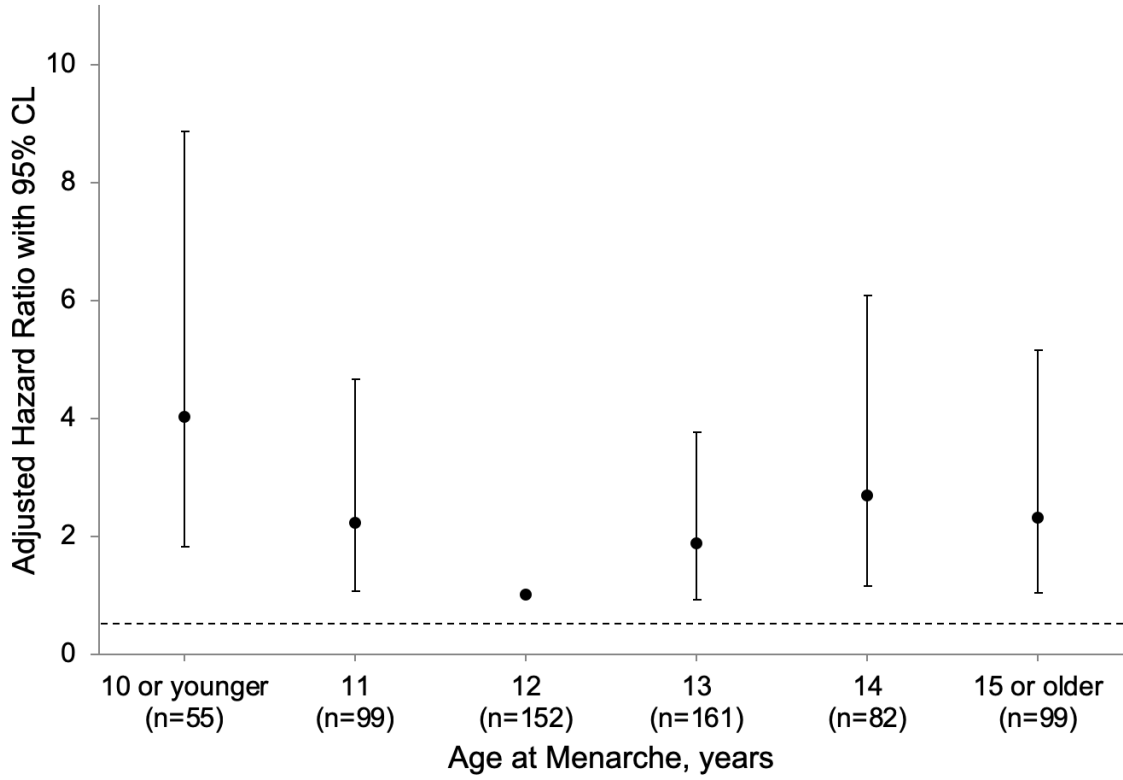
**Table S2. Models with TET and sTET for time to CVD event.**

HR's for time to CVD event	HR (95% CI) for model with TET	HR (95% CI) for model with sTET	HR (95% CI) for model without TET or sTET
Age at menarche $\leq 10$ vs 12	4.21 (1.90, 9.30)	4.03 (1.83, 8.87)	4.0 (1.84, 8.69)
Age at menarche 11 vs 12	2.27 (1.09, 4.75)	2.24 (1.07, 4.67)	2.14 (1.04, 4.40)
Age at menarche 13 vs 12	1.95 (0.97, 3.92)	1.88 (0.93, 3.79)	1.80 (0.91, 3.55)
Age at menarche 14 vs 12	2.74 (1.21, 6.20)	2.69 (1.18, 6.09)	2.25 (1.02, 4.97)
Age at menarche $\geq 15$ vs 12	2.52 (1.14, 5.57)	2.32 (1.04, 5.18)	2.53 (1.18, 5.42)
age	1.03 (1.003, 1.05)	1.03 (1.01, 1.05)	1.02 (1.003, 1.04)
BMI	0.98 (0.95, 1.02)	0.98 (0.95, 1.02)	0.98 (0.94, 1.01)
Diabetes No vs Yes	0.38 (0.24, 0.61)	0.39 (0.25, 0.63)	0.41 (0.26, 0.64)
Ever smoker No vs Yes	0.45 (0.28, 0.70)	0.42 (0.27, 0.67)	0.49 (0.32, 0.76)
Dyslipidemia No vs Yes	0.63 (0.39, 1.02)	0.61 (0.37, 0.98)	0.62 (0.39, 0.99)
Hypertension No vs Yes	0.57 (0.35, 0.94)	0.56 (0.34, 0.93)	0.57 (0.35, 0.93)
logSAA	1.26 (1.05, 1.52)	1.27 (1.05, 1.53)	1.26 (1.05, 1.52)
TET/sTET/-	0.99 (0.97, 1.02)	0.98 (0.95, 1.01)	-

**Table S3. Models with TET and sTET for time to death.**

HR's for time to death	HR (95% CI) for model with TET	HR (95% CI) for model with sTET	HR (95% CI) for model without TET
Age at menarche $\leq 10$ vs 12	3.67 (1.38, 9.72)	3.41 (1.29, 9.05)	3.42 (1.32, 8.87)
Age at menarche 11 vs 12	2.62 (1.12, 6.11)	2.51 (1.08, 5.84)	2.42 (1.06, 5.49)
Age at menarche 13 vs 12	1.32 (0.58, 3.02)	1.26 (0.55, 2.89)	1.23 (0.55, 2.74)
Age at menarche 14 vs 12	2.54 (0.97, 6.66)	2.46 (0.94, 6.48)	2.30 (0.91, 5.82)
Age at menarche $\geq 15$ vs 12	2.63 (1.11, 6.26)	2.43 (1.02, 5.83)	2.43 (1.05, 5.61)
age	1.03 (1.01, 1.06)	1.03 (1.01, 1.05)	1.03 (1.01, 1.05)
BMI	0.92 (0.88, 0.97)	0.92 (0.88, 0.97)	0.92 (0.88, 0.97)
Diabetes No vs Yes	0.35 (0.21, 0.59)	0.36 (0.22, 0.60)	0.36 (0.22, 0.59)
Ever smoker No vs Yes	0.67 (0.41, 1.09)	0.64 (0.39, 1.05)	0.68 (0.42, 1.11)
Dyslipidemia No vs Yes	0.64 (0.37, 1.11)	0.64 (0.37, 1.10)	0.65 (0.38, 1.11)
Hypertension No vs Yes	0.28 (0.15, 0.53)	0.28 (0.15, 0.53)	0.28 (0.15, 0.52)
logSAA	1.31 (1.05, 1.63)	1.31 (1.05, 1.64)	1.31 (1.05, 1.63)
TET/sTET/-	0.99 (0.96, 1.02)	0.98 (0.95, 1.01)	-

**Figure S1. Adjusted relative hazard (with sTET) and 95% confidence interval (CI) of major adverse cardiac events (MACE) by age at menarche.**



**Figure S2. Adjusted relative hazard (with sTET) and 95% confidence interval (CI) of mortality by age at menarche.**

