BACKGROUND: Earlier age at menopause is widely considered to be associated with an increased risk of cardiovascular disease. However, the underlying mechanisms of this relationship remain undetermined. Indications suggest that anti-Müllerian hormone (AMH), an ovarian reserve marker, plays a physiological role outside of the reproductive system. Therefore, we investigated whether longitudinal AMH decline trajectories are associated with an increased risk of cardiovascular disease (CVD) occurrence.

METHODS: This study included 3108 female participants between 20 and 60 years of age at baseline of the population-based Doetinchem Cohort. Participants completed $\geq 1$ of 5 consecutive quinquennial visits between 1987 and 2010, resulting in a total follow-up time of 20 years. AMH was measured in 8507 stored plasma samples. Information on total CVD, stroke, and coronary heart disease was obtained through a hospital discharge registry linkage. The association of AMH trajectories with CVD was quantified with joint modeling, with adjustment for age, smoking, oral contraceptive use, body mass index, menopausal status, postmenopausal hormone therapy use, diastolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, and glucose levels.

RESULTS: By the end of follow-up, 8.2% of the women had suffered from CVD, 4.9% had suffered from coronary heart disease, and 2.6% had experienced a stroke. After adjustment, each ng/mL lower logAMH level was associated with a 21% higher risk of CVD (hazard ratio, 1.21; 95% confidence interval, 1.07–1.36) and a 26% higher risk of coronary heart disease (hazard ratio, 1.25; 95% confidence interval, 1.08–1.46). Each additional ng/mL/year decrease of logAMH was associated with a significantly higher risk of CVD (hazard ratio, 1.46; 95% confidence interval, 1.14–1.87) and coronary heart disease (hazard ratio, 1.56; 95% confidence interval, 1.15–2.12). No association between AMH and stroke was found.

CONCLUSIONS: These results indicate that AMH trajectories in women are independently associated with CVD risk. Therefore, we postulate that the decline of circulating AMH levels may be part of the pathophysiology of the increased cardiovascular risk of earlier menopause. Confirmation of this association and elucidation of its underlying mechanisms are needed to place these results in a clinical perspective.
Anti-Müllerian Hormone Decline in Relation to Cardiovascular Health

Clinical Perspective

What Is New?

- The mechanisms behind the relationship of ovarian reserve and cardiovascular disease are still undetermined.
- This study related individual trajectories of anti-Müllerian hormone to clinical outcomes of cardiovascular disease in 3108 women.
- Each unit lower level of anti-Müllerian hormone was associated with a 21% increased risk of cardiovascular disease and a 25% increased risk of coronary heart disease.
- Each unit of faster anti-Müllerian hormone decline was associated with a 53% increased risk of cardiovascular disease and a 54% increased risk of coronary heart disease.

What Are the Clinical Implications?

- This study supports the existence of a relationship between ovarian reserve and cardiovascular disease.
- The rate of ovarian reserve decline may be a risk factor for cardiovascular disease.
- Indications show that anti-Müllerian hormone can play a role in cardiovascular pathophysiology, which needs to be further clarified.
- This study identifies new research avenues that may enable the early detection of women at risk for cardiovascular disease.

Age at menopause is widely considered to be associated with risk of cardiovascular disease (CVD). Women with an earlier menopause have an increased risk of CVD compared with women with a later age at menopause, as summarized in a recent meta-analysis,1 and each postponed year of menopause decreases the risk of CVD mortality by 2%.2 Proposed mechanisms of this association include the sudden decrease of estrogen exposure, with putative effects on vasculature and cardiac function.3,4 Although this theory is often accepted as fact, the available evidence regarding the role of estrogens remains contradictory.5–7 It is therefore plausible that other factors contributing to the increased CVD risk associated with menopause are still unknown.

The onset of menopause is dictated by the depletion of the ovarian oocyte pool, also known as ovarian reserve.8 The decline of the ovarian reserve can be measured with proxy markers of ovarian reserve, such as anti-Müllerian hormone (AMH), which is produced by ovarian follicles in an early stage of their cyclic development.9 Although AMH is mainly thought to play a local role in ovarian follicle recruitment and development,10,11 it is also available in the peripheral circulation. The identification of the AMH receptor in tissues such as the neuronal system and lungs2,12,13 suggests a function of circulating AMH outside of the reproductive system. In particular, recent experimental and observational studies have identified AMH as a potential influencing factor of cardiovascular function or risk factors of CVD.14–18 To date, no studies have investigated a direct link between AMH and clinical CVD occurrence. Therefore, in this study, we aimed to determine whether an independent relationship exists between longitudinal trajectories of AMH and clinically manifest CVD in women from the general population.

METHODS

Study Population

Data from female participants of the Doetinchem Cohort Study were included in the current analysis, the details of which have been described elsewhere.19 In brief, the Doetinchem Cohort Study is an ongoing prospective cohort study in The Netherlands for which a sex- and age-stratified sample of participants (3641 men and 4128 women) was randomly recruited from the general population. The study commenced with the baseline visit (round 1) in 1987, after which participants were invited back for a follow-up visit every 5 years. At the time of the current study, data up until round 5 were available, resulting in a maximum follow-up time of 20 years. At each visit, participants answered an extensive questionnaire, anthropometric measurements were taken, and venous blood withdrawal occurred. Nonfasting blood was drawn, from which plasma and other fractions were stored for future use. All participants gave written informed consent, and ethical approval was granted by the Medical Ethics Committee of The Netherlands Institution of Applied Scientific Research. AMH levels were measured in all available stored plasma samples (details described later). Ethical approval for the AMH measurements was granted by the Ethical Committee for Biobank Studies of the University Medical Center Utrecht.

For the present study, all 4128 participating women of the Doetinchem Cohort Study were eligible. We excluded women without available plasma samples (n=802). We additionally excluded women with no AMH measurement before the occurrence of a CVD outcome or censoring (n=211 for stroke, n=214 for CHD, and n=218 for total CVD), leaving 3115 women for analysis of stroke, 3112 women for analysis of CHD, and 3108 women for analysis of total CVD.

AMH Measurement

AMH levels were measured with in all available stored plasma samples with the picoAMH assay (AnshLabs) after approval from the Ethical Committee for Biobank Studies of the University Medical Center Utrecht. The details of these measurements were previously described.20 The plasma samples from round 1 were stored at –30°C, and the samples from rounds 2 to 5 were stored at –80°C. Before the current study, samples were thawed once for additional measurements and immediately refrozen. In March 2015, the available samples of each participant were retrieved from storage and shipped on dry ice to AnshLabs, where they were temporarily stored at –20°C until the analyses were performed. The inter- and intraassay coefficients

ORIGINAL RESEARCH ARTICLE

of plate drift was observed; all coefficients of variation within plate columns and rows were <5%. For the purpose of log transformation, AMH levels <1.8 pg/mL, below the limit of detection, were censored at this level to avoid the use of null values. The analytic range of the picoAMH assay is 3 to 11 000 pg/mL, with a clinically reportable range up to 280 ng/mL. The total number of available AMH measurements before the occurrence of total CVD or censoring was 8507, with 2431 (28.9%) samples below the limit of detection. At baseline, women were divided into 4 categories based on their age-standardized AMH level. This process was performed by using the CG-LMS method (conjugate grading, lambda-mu-sigma), previously described by Doleman and de Kat.20,21 A model was made of the nonlinear distribution of AMH with standardization for age using smoothing splines for AMH and taking into account skewness, the median, and coefficient of variation. This fitted model was then applied to predict in which quartile the AMH level of a woman belonged given her age. The women who were predicted to have an AMH level in the lower 25% of their age were grouped into the lowest age-specific AMH category, and so on. As a result of the differing baseline ages, the number of women in each age-specific AMH category was not equal.

CVD Outcomes

Cause of death was ascertained through linkage with Statistics Netherlands, and morbidity data were collected through the Dutch Hospital Discharge Registry.22 In the current study, CVD end points included nonfatal and fatal occurrences of cerebrovascular events (stroke), coronary heart disease (CHD), and a combination of all manifestations of CVD (total CVD). Stroke comprised both hemorrhagic and ischemic stroke and was defined with the 9th (up to 1996) and 10th (from 1996 onward) editions of the International Classification of Diseases (ICD-9 and ICD-10, respectively) as follows: 430 to 438 (ICD-9) and I60-I67; I69; I70-I74; I50. Follow-up was complete until January 1, 2011.

Covariates

Other information taken into account in the current study for each follow-up round included menopausal status, smoking, oral contraceptive (OC) use, postmenopausal hormone therapy use, body mass index, systolic and diastolic blood pressure, total cholesterol levels, high-density lipoprotein cholesterol (HDL-C), and glucose levels. Menopausal status was assessed through information on current cycle regularity, date of the last menstrual period, and reproductive surgery (for a more detailed description, see our study on AMH trajectories20). Current smoking status at each follow-up round was derived from the questionnaires, where participants were regarded as current smokers if they reported to smoke at least 1 cigarette per month. Current OC and hormone therapy users were identified by the question, “Are you currently using OC or estrogen for climacterial symptoms?” Body mass index was assessed using the standardized measurement of height and weight by trained staff. Systolic and diastolic blood pressure were measured twice in a seated position with a random zero sphygmonometer (Hawksley and Sons) by trained staff. Nonfasting total cholesterol and HDL-C levels were measured in ethylenediaminetetraacetic acid plasma (until 1998) or serum (from 1998 onward) at the Lipid Reference Laboratory using standardized enzymatic methods.22

Missing Data

At each round, a small proportion of missing information was found in the study population. For OC use, smoking, body mass index, systolic and diastolic blood pressure, total cholesterol, and HDL-C, this proportion was <0.1%. The proportion of missing information regarding hormone therapy use and nonfasting glucose was <5% per round (with the exception of round 1, where it was not included in the questionnaire or laboratory analyses). Missing cycle status information averaged 17% over all rounds because of the inclusion of women with a hysterectomy and missing information of the last menstrual period in rounds 2 and 3. Missing data were imputed through multiple imputation using 10 iterations and predictive mean matching. Multiple imputation was performed in SPSS Statistics (IBM), version 21.

Joint Modeling

First, we characterized decline trajectories of AMH with the use of a linear mixed model analysis, taking into account varying AMH levels and decline rates between individuals by adding a random intercept and slope, respectively, for each individual (details previously described20). In this way, a separate effect for an individual AMH level and slope at time point t could be modeled. The AMH was set as the outcome to be given an interpretation of declining rather than increasing AMH levels. The time axis of the linear mixed effects models was set as the follow-up time in years (t=0 at the baseline visit), and baseline age was added as a covariate in the model. The follow-up time of a participant either ended at the time of the event or diagnosis of CVD, CHD or stroke, or at the time of leaving the study (including censoring at the last follow-up visit). Natural splines were added to the time variable to take into account the individual nonlinear decline of AMH.20 Models were subsequently adjusted for the following time-varying covariates: current OC use, current smoking, menopausal status (pre- or postmenopausal based on cycle status), body mass index, diastolic blood pressure, total cholesterol, HDL-C, glucose, hormone therapy use, and use of blood pressure- or lipid-lowering medication. Diastolic blood pressure was chosen to represent blood pressure because it was normally distributed, whereas systolic blood pressure was slightly right-skewed, even after logarithmic transformation. Models were not adjusted for both systolic and diastolic blood pressure because of the strong, linear relationship (R=0.74) between the 2 variables in each participant. HDL-C was log transformed to achieve a normal distribution. Linear mixed model analyses were performed in R (http://www.R-project.org) using the lme4 library.

To relate the longitudinal decline trajectories of AMH to the CVD outcomes, the linear mixed effects models were
combined with a Cox proportional hazards model with a Weibull baseline hazard distribution (library survival, R) using a joint model analysis (library JM, R).23 The outcomes of the Cox proportional hazards models were total CVD, CHD, and stroke, with the corresponding follow-up times as a time variable. In accordance with the methods used for the linear mixed models, the Cox proportional hazards model was adjusted for baseline age with the inclusion of natural splines. The final joint models included time-varying AMH levels, a time-varying slope (ie, speed of AMH decline), or both time-varying AMH levels and a time-varying slope as potential predictors for the hazard of CVD.

Sensitivity Analysis
To exclude a potential effect of polycystic ovary syndrome (PCOS) or surgical menopause, the analyses of total CVD were separately repeated with exclusion of women who had never a regular menstrual cycle (n=647) and exclusion of women with a bilateral oophorectomy (n=50).

RESULTS
Population Characteristics
On average, participants completed 3.9 visits (including the baseline visit) with available AMH measurements. The baseline characteristics of participants per age-standardized baseline AMH category are listed in Table 1. Postmenopausal hormone therapy use was not assessed in the baseline questionnaire but declined from 4% in round 2 to 1.5% in round 5. In rounds 2 to 5, mean glucose levels were 5±1 mmol/L. The population characteristics stratified by round are presented in online-only Data Supplement Table I.

Table 1. Population Characteristics at Round 1* Compared Among Women in Different Baseline Age-Specific AMH Categories

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lowest Age-Specific AMH (1st Category)</th>
<th>Second Lowest Age-Specific AMH (2nd Category)</th>
<th>Second Highest Age-Specific AMH (3rd Category)</th>
<th>Highest Age-Specific AMH (4th Category)</th>
<th>P for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMH (ng/mL)</td>
<td>0.02 [0.00–0.34]</td>
<td>0.96 [0.02–2.12]</td>
<td>2.37 [0.63–3.63]</td>
<td>3.62 [1.84–6.06]</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Age (y)</td>
<td>47.9±8.1</td>
<td>40.8±9.5</td>
<td>36.0±7.8</td>
<td>33.1±6.7</td>
<td>0.02</td>
</tr>
<tr>
<td>OC use, % (n)</td>
<td>12 (130)</td>
<td>23 (136)</td>
<td>31 (92)</td>
<td>36 (364)</td>
<td>0.43</td>
</tr>
<tr>
<td>Current smoker, % (n)</td>
<td>29 (308)</td>
<td>34 (199)</td>
<td>39 (117)</td>
<td>36 (358)</td>
<td>0.20</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.6±3.9</td>
<td>24.9±3.9</td>
<td>23.8±3.6</td>
<td>23.6±3.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Premenopausal, % (n)</td>
<td>62 (658)</td>
<td>85 (450)</td>
<td>99 (283)</td>
<td>100 (969)</td>
<td>0.07</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>122±16</td>
<td>117±14</td>
<td>115±13</td>
<td>113±12</td>
<td>0.03</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77±11</td>
<td>76±10</td>
<td>74±10</td>
<td>74±9</td>
<td>0.05</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.7±1.1</td>
<td>5.4±1.0</td>
<td>5.1±0.9</td>
<td>5.1±0.9</td>
<td>0.06</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.4±0.3</td>
<td>1.3±0.3</td>
<td>1.4±0.3</td>
<td>1.4±0.3</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Values presented in mean±SD, median [IQR], or % (n).
AMH indicates anti-Müllerian hormone; BMI, body mass index; HDL, high-density lipoprotein; IQR, interquartile range; and OC, oral contraceptive.
*Total number of women with an available AMH measurement in round 1 (n=2940).

Outcome Characteristics
By the end of follow-up, 255 (8.2%) women had total CVD, 152 (4.9%) women had CHD, and 81 (2.6%) women had experienced a stroke. The incidence of total CVD was compared between age-standardized AMH categories, as shown in Table 2. The number of women with CVD decreased with the decline of age-standardized AMH.

AMH Trajectories in Relation to Total CVD
The results of the joint models with regard to total CVD are presented in Table 3. After adjustment for all covariates, each unit (ng/mL) lower logAMH level at any time point during the trajectory was associated with a 21% higher risk of total CVD during follow-up (hazard ratio [HR], 1.21; 95% confidence interval [CI], 1.07–1.36). Each additional unit (ng/mL/year) decrease in logAMH (ie, a steeper negative slope) was associated with a 46% higher risk of total CVD. When models were accounted for both time-varying AMH levels and time-varying slopes, both HRs [95% CI] were attenuated to 1.12 [0.98–1.28] and 1.42 [1.09–1.86], respectively. The fully adjusted relationship between AMH levels and the relative hazard of CVD was nonlinear and is provided in Figure 1.

AMH Trajectories in Relation to CHD and Stroke
The results of the joint models with regard to CHD and stroke are presented in Table 3. After adjustment for all covariates, each ng/mL lower logAMH level at any time point during the trajectory was associated with a 26% higher risk of CHD during follow-up (hazard ratio, 1.25;
95% CI, 1.08–1.46). Each additional ng/mL/year decrease in logAMH (ie, a steeper negative slope) was associated with a 56% higher risk of CHD (HR, 1.55; 95% CI, 1.14–2.10) by the end of follow-up. When models were accounted for both time-varying AMH levels and time-varying slopes, both HRs [95% CI] were attenuated to 1.16 [0.98–1.37] and 1.49 [1.07–2.07], respectively. Both AMH levels and decline rates were not associated with the occurrence of nonfatal or fatal stroke in this study. When the models were run with the inclusion of both time-varying AMH levels and time-varying slopes, the HRs [95% CI] of logAMH levels and slopes were 1.00 [0.78–1.25] and 1.14 [0.94–1.37], respectively.

Sensitivity Analysis

Of the 2461 women who had ever had a regular cycle, 182 (7.4%) developed a manifestation of CVD by the end of follow-up. The results of the joint model analyses in this group of women are presented in Table 4. After full adjustment, each ng/mL lower level of logAMH was associated with a 17% higher risk of total CVD (HR, 1.17; 95% CI, 1.02–1.35). Each additional ng/mL/year decline was associated with a 38% increased risk of total CVD (HR, 1.38; 95% CI, 1.03–1.85). Of the 3054 women without a bilateral oophorectomy, 246 (8.1%) developed a manifestation of CVD. The coefficient estimates of AMH levels and rate of decline approximated those in the whole study group (Table 4).

DISCUSSION

In the present study, we observed an association between AMH levels and the rate of AMH decline with the incidence of CHD and total CVD in women, independent of menopausal status and metabolic risk factors. No

<table>
<thead>
<tr>
<th>Variable</th>
<th>↓AMH Level HR (95% CI)</th>
<th>P Value</th>
<th>AMH Decline Rate HR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cardiovascular disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only time-varying AMH level</td>
<td>1.21 (1.07–1.37)</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only time-varying decline rate</td>
<td>1.46 (1.14–1.87)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time-varying AMH level and decline rate</td>
<td>1.12 (0.98–1.28)</td>
<td>0.09</td>
<td>1.42 (1.09–1.86)</td>
<td>0.01</td>
</tr>
<tr>
<td>Coronary heart disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only time-varying AMH level</td>
<td>1.26 (1.08–1.46)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only time-varying decline rate</td>
<td>1.56 (1.15–2.12)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time-varying AMH level and decline rate</td>
<td>1.16 (0.98–1.37)</td>
<td>0.09</td>
<td>1.49 (1.07–2.07)</td>
<td>0.02</td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only time-varying AMH level</td>
<td>1.03 (0.82–1.30)</td>
<td>0.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only time-varying decline rate</td>
<td>1.17 (0.94–1.45)</td>
<td>0.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time-varying AMH level and decline rate</td>
<td>1.00 (0.78–1.25)</td>
<td>0.93</td>
<td>1.14 (0.94–1.37)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Models are adjusted for age, OC use, smoking, BMI, menopausal status, TC, DBP, HDL-C, HT, glucose, lipid-lowering medication, and blood pressure-lowering medication.

AMH indicates anti-Müllerian hormone; BMI, body mass index; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; HT, hormone replacement therapy; OC, oral contraceptive; and TC, total cholesterol.
clear evidence was found for an association between AMH level and stroke, although an inverse relationship was discovered between the speed of AMH decline and the risk of stroke. Both a lower AMH level and swifter rate of decline were individually associated with an increased hazard of CHD and total CVD, but the corresponding effect estimates were attenuated when models were mutually adjusted for time-varying levels and slopes.

Before interpreting our findings, the strengths and limitations of the present study should be addressed. Strengths of the present study include the availability of AMH data of up to 5 study visits over a 20-year period, enabling the modeling of longitudinal AMH trajectories. Furthermore, with the use of national morbidity and mortality registries, we were able to associate AMH to clinically relevant outcomes rather than just risk factors. The population-based design and long follow-up time are uniquely suited to study this association. Moreover, the adjustment for relevant, time-varying confounders strengthens the observation of an independent relationship. The fact that this relationship was upheld after the exclusion of women with an irregular cycle or bilateral oophorectomy affirms that the results presented here are likely generalizable to the general female population.

An observational design is suitable to detect associations but does not allow for conclusions to be drawn on causality. It also does not rule out residual confounding. Because of the low incidence of stroke, our study had limited power of 35% to detect an association with AMH, also impeding the distinction between ischemic and hemorrhagic stroke. We have no data on the presence of PCOS in our cohort, and therefore we chose to repeat our analyses only in women who had ever had a regular cycle. Therefore, it is possible that we misclassified non-PCOS women as PCOS and vice versa. Because we excluded ≈20% of the women in the study population with an irregular cycle, more than the expected prevalence of PCOS,24 and adjusted for cardiometabolic risk factors, it is likely that we captured an association between AMH and CVD combined.

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**Figure 1. Relative hazard of cardiovascular disease by anti-Müllerian hormone (AMH) levels after full adjustment.**

The relationship between adjusted AMH and hazard of cardiovascular disease is nonlinear. An adjusted AMH level of 38.4 ng/mL is associated with the lowest risk of cardiovascular disease and is therefore the reference value.

**Table 4. Association of Fully Adjusted AMH Decline Trajectories With the Risk of Total CVD After Exclusion of Women With an Irregular Menstrual Cycle or Surgical Menopause**

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (95% CI) AMH Level</th>
<th>P Value</th>
<th>HR (95% CI) AMH Decline Rate</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>After exclusion of irregular menstrual cycle (n=2461)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only time-varying AMH level</td>
<td>1.17 (1.02–1.34)</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only time-varying decline rate</td>
<td></td>
<td></td>
<td>1.39 (1.04–1.86)</td>
<td>0.03</td>
</tr>
<tr>
<td>Time-varying AMH level and decline rate</td>
<td>1.09 (0.9–1.27)</td>
<td>0.28</td>
<td>1.43 (1.04–1.95)</td>
<td>0.03</td>
</tr>
<tr>
<td>After exclusion of surgical menopause (n=3054)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only time-varying AMH level</td>
<td>1.21 (1.07–1.36)</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only time-varying decline rate</td>
<td></td>
<td></td>
<td>1.55 (1.19–2.03)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Time-varying AMH level and decline rate</td>
<td>1.22 (0.98–1.28)</td>
<td>0.09</td>
<td>1.48 (1.11–1.97)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Models are adjusted for age, OC, smoking, BMI, menopausal status, TC, DBP, logHDL-C, HT, glucose, lipid-lowering medication, and blood pressure-lowering medication.

AMH indicates anti-Müllerian hormone; BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; HT, hormone replacement therapy; OC, oral contraceptive; and TC, total cholesterol.
and CVD independently from PCOS. Moreover, women with missing AMH measurements smoked more often and had a higher body mass index at baseline. Therefore, it is possible that our results are based on a relatively healthier selection of the general population. However, because no differences in incidence of CVD were found, this is not likely to influence the association between AMH and CVD. Furthermore, it would have been interesting to take into account other hormones such as estrogen in addition to cycle status; because these measurements were not performed in the Doetinchem Cohort Study, this approach was not possible for the current study. The lack of an association between endogenous sex steroid concentrations and CVD in prospective studies suggests that this would not influence our results.25 Last, the joint models assume that study censoring is independent of the random effects. Although study participation in each round remained >75%, the reasons for loss to follow-up were not fully known and could therefore not be taken into account in the analyses, which could have caused a slight overestimation of our results.

The results of the present study are in accordance with prior observations of a relationship between AMH and subclinical CVD. In a longitudinal study, female premenopausal cynomolgus macaques with the lowest AMH levels at baseline had the largest atherosclerotic plaques after 2 years of follow-up.26 A cross-sectional evaluation of both healthy and HIV-infected women concurred that premenopausal women with undetectable AMH levels had larger atherosclerotic plaques than premenopausal women with detectable AMH levels.27 Although these observations could potentially be explained by a relationship between either ovarian reserve status or AMH levels and lipid measures,18,28–30 the results of the current study add to evidence suggesting that the association of AMH with CVD occurrence is independent from cholesterol levels.26,27 It is interesting to note that a previous study did not find any association between age-specific AMH levels and silent coronary artery disease, based on a composite factor of various electrocardiography-based ischemic changes, in a 10-year follow-up period.31 The high reported rate of silent coronary artery disease in this population (14%, compared to 4.8%2 and 3.6/10,000 person-years33 in a Dutch population) suggests that the used classification of silent coronary artery disease may not be entirely able to distinguish the women with clinically relevant coronary-based ischemia from this population.31 A recent study reported an association between lower AMH levels and higher all-cause mortality in a population-based sample of 989 men,34 suggesting that this effect may not be limited to women alone, although the study in question was underpowered to assess a relationship with CVD-related mortality.

Because no previous studies have studied the association between AMH trajectories and CVD risk or outcomes, it is challenging to interpret the meaning of the rate of AMH decline. We previously found the AMH level to be associated with the rate of AMH decline rate,20 which explains the attenuation of the coefficients when mutually adjusted. Processes that cause a swifter decrease of AMH, such as accelerated general aging35,36 likely also influence CVD risk. However, it can be speculated that the faster decline of AMH could also be involved in the pathophysiology of CVD. This theory is potentially represented by the increased CVD risk of women with surgical menopause compared with women experiencing natural menopause.37 In our study, the small number of women with surgical menopause precluded separate analysis of this group.

It is relevant to consider variation in AMH levels to place AMH decline into perspective. To date, few studies have studied longitudinal and short-term variation of AMH. Two recent reports found AMH levels to remain stable throughout the menstrual cycle.38,39 whereas others reported absolute differences ≤0.5 ng/mL10 and 0.8 ng/mL.41 The average yearly decline of AMH, also measured with an AnshLabs assay, was estimated to be 6% to 8%.40 Given that in the current study AMH measurements were spaced 5 years apart, the absolute AMH decline would likely be greater than the observed short-term variation. Another potential source of AMH variation arises from the storage of samples. Two-week sample storage of serum aliquots at −20°C and −80°C was previously associated with a difference in detected AMH levels in the magnitude of 0.1 ng/mL.42 A previous study did not find any effects of freeze–thaw cycles on AMH measurements, albeit with a different assay.43 Although no long-term data are found on the stability of specimen storage, it is likely that some variation is caused by the storage effects in our study. However, because samples were treated the same way within each round and we found no significant variations in between-round age groups and a correlation >90% with prior measurements in round 2 (data not shown), it is unlikely that this variation greatly affects our results of relative AMH change. Last, the recent emergence of high-sensitive assays, such as the picoAMH assay used in this study, has enabled measurement of AMH in women in and after the menopausal transition.20,44 However, the relevance of AMH detection and variation in low ranges still remains a subject of speculation and requires further research.

To date, there are some indications that AMH may be directly involved in cardiovascular physiology. The AMH molecule is part of the same family as bone morphogenetic proteins, which are thought to play an important role in vascular homeostasis.17,45 Moreover, cardiac tissue from neonates with hypoplastic left heart syndrome exhibited fewer AMH receptors than controls, suggesting a role for AMH in (early) myocardial development.14 Women with hypertensive pregnancy disorders, which
are thought to originate from impaired vascularization and cause increased cardiovascular risk later in life,\textsuperscript{46} were previously shown to have lower AMH levels than controls.\textsuperscript{16,47,48} Taking these results together, we speculate that AMH may exert an influence on cardiovascular tissue function throughout reproductive life, with decreasing AMH levels leaving the vasculature more prone to injury and atherosclerosis. This notion requires further investigation in experimental and epidemiological studies before being considered as a likely hypothesis.

Much debate has occurred regarding what governs the association between menopause and increased CVD risk. It was long believed that the reduction of estrogen as a result of the ovarian reserve depletion was responsible for the rise in CVD. However, this theory may be “stronger than the evidence”\textsuperscript{5} because no conclusive proof has been found that estrogen therapy can eliminate the increased CVD risk of menopause, despite an improvement in lipid parameters.\textsuperscript{5,6} Another hypothesis is the aging soma theory. Processes that influence general aging, such as impaired DNA repair,\textsuperscript{35,49} may lead to both earlier menopause and aging of the cardiovascular system.\textsuperscript{50} The reverse may also be true. Transplanting ovaries of young mice into older mice significantly increased their lifespan, suggesting that ovarian function dictates general aging.\textsuperscript{51} Within this context, it is possible that lower AMH levels and a swifter rate of AMH decline are illustrative of a deterioration of ovarian function, with potential effects on cardiovascular aging. Whether this finding could be mediated through AMH requires further research. We are the first to report on the association between AMH and CVD and must therefore wait for others to confirm our results.

CONCLUSIONS

This study is the first to identify an independent relationship between AMH levels and AMH decline rate with CVD in women. The potential role of AMH in cardiovascular health may provide a missing link to the CVD risk associated with menopause, for which an unequivocal explanation is still lacking. We eagerly await future studies to confirm and elaborate on our results for the transition to clinical practice.

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DISCLOSURES

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FOOTNOTES

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## SUPPLEMENTAL MATERIAL

**Supplementary Table 1. Population characteristics per follow-up round.**

<table>
<thead>
<tr>
<th></th>
<th>Round 1 (N=2,940)</th>
<th>Round 2 (N=2,848)</th>
<th>Round 3 (N=2,330)</th>
<th>Round 4 (N=2,195)</th>
<th>Round 5 (N=1,944)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AMH (ng/mL)</strong></td>
<td>0.99 [0.04-3.07]</td>
<td>0.18 [0.00-1.47]</td>
<td>0.00 [0.00-0.52]</td>
<td>0.00 [0.00-0.05]</td>
<td>0.00 [0.00-0.00]</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>40.2 ± 10.1</td>
<td>46.1 ± 10.0</td>
<td>50.7 ± 9.9</td>
<td>55.2 ± 9.8</td>
<td>59.6 ± 9.6</td>
</tr>
<tr>
<td><strong>OC use (% (n))</strong></td>
<td>25 (722)</td>
<td>20 (580)</td>
<td>16 (380)</td>
<td>9 (205)</td>
<td>6 (112)</td>
</tr>
<tr>
<td><strong>Current smoker (% (n))</strong></td>
<td>33 (982)</td>
<td>31 (875)</td>
<td>26 (601)</td>
<td>22 (479)</td>
<td>18 (339)</td>
</tr>
<tr>
<td><strong>BMI (kg/m^2)</strong></td>
<td>24.6 ± 3.8</td>
<td>25.6 ± 4.2</td>
<td>26.2 ± 4.3</td>
<td>26.6 ± 4.6</td>
<td>27.0 ± 4.8</td>
</tr>
<tr>
<td><strong>Premenopausal (% (n))</strong></td>
<td>84 (2,260)</td>
<td>70 (1,472)</td>
<td>59 (1,046)</td>
<td>32 (532)</td>
<td>18 (278)</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mm Hg)</strong></td>
<td>117 ± 15</td>
<td>122 ± 17</td>
<td>126 ± 18</td>
<td>128 ± 19</td>
<td>129 ± 18</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mm Hg)</strong></td>
<td>75 ± 10</td>
<td>78 ± 11</td>
<td>80 ± 11</td>
<td>80 ± 10</td>
<td>79 ± 10</td>
</tr>
<tr>
<td><strong>Total cholesterol (mmol/L)</strong></td>
<td>5.4 ± 1.0</td>
<td>5.5 ± 1.0</td>
<td>5.7 ± 1.1</td>
<td>5.7 ± 1.0</td>
<td>5.7 ± 1.1</td>
</tr>
<tr>
<td><strong>HDL cholesterol (mmol/L)</strong></td>
<td>1.4 ± 0.3</td>
<td>1.5 ± 0.4</td>
<td>1.5 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>1.6 ± 0.4</td>
</tr>
</tbody>
</table>

*Values presented in mean ± SD, median [IQR] or % (n).*

*AMH = anti-Müllerian hormone; OC = oral contraceptive; BMI = body mass index; HDL=high-density lipoprotein*